CAPITAL UNIVERSITY OF SCIENCE AND TECHNOLOGY, ISLAMABAD

Prevalence of Potential Pathogens Present in Biomedical Waste and Analysis of Efficacy of Routinely Used Disinfectants

by

Sermad Bilal

A thesis submitted in partial fulfillment for the degree of Master of Science

in the

Faculty of Health and Life Sciences
Department of Bioinformatics and Biosciences

2020

Copyright © 2020 by Sermad Bilal

All rights reserved. No part of this thesis may be reproduced, distributed, or transmitted in any form or by any means, including photocopying, recording, or other electronic or mechanical methods, by any information storage and retrieval system without the prior written permission of the author.

I dedicate this thesis to my parents and my teachers.

CERTIFICATE OF APPROVAL

Prevalence of Potential Pathogens Present in Biomedical Waste and Analysis of Efficacy of Routinely Used Disinfectants

by

Sermad Bilal

(MBS183012)

THESIS EXAMINING COMMITTEE

S. No.	Examiner	Name	Organization
(a)	External Examiner	Dr. Iftikhar Ahmed	NARC, Islamabad
(b)	Internal Examiner	Dr. Sahar Fazal	CUST, Islamabad
(c)	Supervisor	Dr. Arshia Amin Butt	CUST, Islamabad

Dr. Arshia Amin Butt
Thesis Supervisor
October, 2020

Dr. Sahar Fazal
Head
Dept. of Biosciences & Bioinformatics
October, 2020

Dr. Muhammad Abdul Qadir
Dean
Faculty of Health & Life Sciences
October, 2020

Author's Declaration

I, **Sermad Bilal** hereby state that my MS thesis titled "**Prevalence of Potential Pathogens Present in Biomedical Waste and Analysis of Efficacy of Routinely Used Disinfectants** " is my own work and has not been submitted previously by me for taking any degree from Capital University of Science and Technology, Islamabad or anywhere else in the country/abroad.

At any time if my statement is found to be incorrect even after my graduation, the University has the right to withdraw my MS Degree.

(Sermad Bilal)

Registration No: MBS183012

Plagiarism Undertaking

I solemnly declare that research work presented in this thesis titled "**Prevalence of Potential Pathogens Present in Biomedical Waste and Analysis of Efficacy of Routinely Used Disinfectants** " is solely my research work with no significant contribution from any other person. Small contribution/help wherever taken has been dully acknowledged and that complete thesis has been written by me.

I understand the zero tolerance policy of the HEC and Capital University of Science and Technology towards plagiarism. Therefore, I as an author of the above titled thesis declare that no portion of my thesis has been plagiarized and any material used as reference is properly referred/cited.

I undertake that if I am found guilty of any formal plagiarism in the above titled thesis even after award of MS Degree, the University reserves the right to withdraw/revoke my MS degree and that HEC and the University have the right to publish my name on the HEC/University website on which names of students are placed who submitted plagiarized work.

(Sermad Bilal)

Registration No: MBS183012

Acknowledgements

To start with the greatest name of *Almighty Allah* the most gracious the most merciful, whose blessings are limitless, whose benignant is everlasting, whose being is eternal, whose mercy is unlimited, whose provisions are un-ending, and whose most loving, whose worship is our faith. Words seem too little to express my gratitude to Allah Almighty who has bestowed me with more than I deserve.

Foremost, I would like to express my sincere gratitude to my supervisor **Dr. Arshia Amin Butt** for her kind support and guidance thorough out my research. Her patience and motivation to help her students and immense knowledge helped me a lot during the practical work as well as during the write up of my research. I cannot thank her for her unconditional support and guidance.

I would like to say thanks to Dr. Sahar Fazal (HOD Biosciences department) for her courteous support and guidance started from my first day in the university. She is such a loveable and humble personality. I would also like to thank Dr. Shaukat Iqbal Malik the way he indoctrinated us genetics especially cancer genetics was quite amazing. As a matter of teaching his teaching skills are incredible. I express my profound gratitude to my mother for her utter support and absolute trust in me allowed me to reach at this level. This accomplishment has not been possible without her prayers. I would like to express my heartiest gratitude to my elder brothers who supported me and guided me after my father's demise. They allowed me to fulfill my dreams. At last but not the least I would acknowledge one of my best friend in the university who supported me in difficult times Mr. Sayed Shah Ali. He is such a nice personality.

Thanks to all

(Sermad Bilal)

Registration No: MBS183012

Abstract

My present work was on the bacteriological analysis of BMW (Biomedical Waste). BMW is a kind of waste generated by healthcare establishments as a result of diagnosis, prognosis, treatment and vaccination of the patients. There are different categories of BMW according to the level of hazard they pose but the two major categories of BMW are Non- hazardous and Biohazardous waste. Four most common bacterial species found in the BMW were selected based on the literature review of articles on BMW. These bacterial species were *E.coli, S.aureus, P.aeruginosa* and *S.typhi*. Samples of BMW were collected from different Healthcare Establishments. Bacteria were first isolated from the samples of BMW and then were identified by using selective culture media and biochemical tests. After analysis it was found that *E.coli* was the most predominant bacteria in the BMW. S.aureus was the 2nd most common bacteria while *P.aeruginosa* and *S.typhi* were at 3rd and 4th respectively. These pathogens are associated with multiple diseases in the humans. Three (3) most commonly used disinfectants in the hospitals (Dettol, Isopropanol and Phenol) were tested against these isolates. Standard concentrations of these disinfectants were tested. After analysis of these disinfectants against bacterial isolates it was found that Chloroxylenol 20% (Dettol) was the most effective disinfectant against all four isolates while Phenol was the least effective disinfectant. IPA showed intermediate effectiveness. *P.aeruginosa* showed resistance against Phenol while *S.typhi* was the most sensitive against all three disinfectants tested. Standard concentrations of these disinfectants that are commonly used in hospital settings were tested. After analysis of these disinfectants against bacterial isolates it was found that Chloroxylenol in a concentration of 20% (or Dettol) was the most effective disinfectant against all four isolates (short contact time was enough to kill pathogens) while Phenol was the least effective disinfectant (long contact time was required to kill pathogens was required). IPA showed intermediate effectiveness. For *P.aeruginosa* long contact time (>30 minutes) was required for phenol disinfectant while for *S.typhi* short contact time (<15 minutes wasenough).

Contents

List of Figures

List of Tables

Abbreviations

3Rs	Reduce, Reuse, Recycle
BMW	Biomedical Waste
BMWM	Biomedical Waste Management
CDC	Centers for Disease Controland Prevention
DHQ Hospital	District Headquarters Hospital
HBV	Hepatitis B Virus
HCE	Health Care Establishments
HCV	Hepatitis C Virus
HIV	Human Immunodeficiency Virus
MSA	Mannitol Salt Agar
NIH	National Institute of Health
THQ Hospital	Tehsil Headquarters Hospital
TSA	Tryptic Soya Agar
UV	Ultraviolet
VRB	Violet Red Bile Agar
WHO	World Health Organization
XLD	Xylose Lysine Deoxycholate Agar

Chapter 1

Introduction

1.1 Bacterial Profile of Potential Pathogens Present in Bio-Medical Waste

Biomedical waste [BMW] is any kind of waste generated as a result of diagnosis, treatment, prognosis or immunization of patients which may be harmful for the patients and their attendants visiting the Healthcare Establishments [HCEs], Health workers, personnel handling it and also for the environment if not treated carefully. Waste which is produced in the research laboratory is also categorized as medical or BMW [1]. We can categorize biomedical waste as solid or liquid waste according to the consistency of the waste [2].

BMW generated in the HCEs can be categorized into 2 major categories according to the level of infectiousness as; nonhazardous and bio-hazardous. Nonhazardous waste is noninfectious waste and includes plastic, cardboards, paper and packaging material etc. This kind of waste does not pose any health issues for the healthcare workers, patients and environment. Bio-hazardous waste may either be infectious i.e. pathological agents, stock cultures, culture medias, used blood collection containers, blood and blood products, animal carcasses in the research labs, pharmaceutical waste, amputated organs and tissues i.e. placenta etc. or noninfectious waste i.e. Radioactive waste, chemical waste and cytotoxic waste.

Bio-hazardous waste may pose serious kind of health problems i.e. bacterial infections, chemical injury and even cancers [radioactive waste] if not handled carefully [3].

BMW is categorized into ten defined categories with their codes as 1 to 10.

1. Human anatomical waste i.e. amputated tissues, organs etc.
2. Animal waste i.e. waste from veterinary hospitals
3. Microbiological waste i.e. culture bottles, vaccines etc.
4. Sharps i.e. needles, surgical blades, syringes etc.
5. Medicines and drugs i.e. expired medicines.
6. Soiled waste i.e. cotton, bandages contaminated with blood and body fluids
7. Solid waste i.e. tubes, cardboard, papers etc.
8. Liquid waste i.e. chemicals and waste generated from cleaning of bed sheets, linen etc.
9. Incinerated waste i.e. waste generated from incineration of waste
10. Chemical waste i.e. disinfectants, antiseptics etc[4].

The potential sources of biomedical waste generation may include hospitals, nursing homes, dental clinics, medical laboratories, veterinary clinics, research centers, pharmaceutical companies, HCE's etc [5]. HCEs are complex institutions which are frequented by people from all walks of life, age and gender. Patients are potential source of generation of BMW, which if not handled carefully may cause serious health and environmental consequences [6].

Improper handling and processing of BMW may lead to the severe health issues to the medical workers, patients and on the environment. There is also a need of proper disposal of BMW in order to avoid any sort of health related problems outside the hospital i.e. the waste handlers, scavengers and people living in the vicinity of hospital. Proper handling is also needed due to the risk of water, air and soil contamination which may have serious consequences. Proper disposal of BMW is also necessary to avoid reuse of syringes, catheters and surgical blades which may be a source of various viral and bacterial diseases [7]. The principle of good BMW practice based upon 3Rs as Reduce [the generation of BMW], Recycle [the BMW if feasible and risk free] and Reuse [the BMW in not harmful]. The good practice of biomedical waste management [BMWM] intended to be as low generation as possible and proper disposal of biomedical waste in such a way that it does not infect Health worker or the personnel handling the waste, general population and environment [8].

FIGURE 1.1: BMW a potential source of pathogenic bacteria

BMW management can be categorized into;

1. **Waste Segregation:** Proper waste segregation is very important as it helps to reduce the burden of infectious waste. BMW is segregated according to the different color codes defined by WHO as;

(a) Red bag/bin contains infectious waste which is recyclable i.e. catheters, gloves, intravenous tubes, intravenous catheters etc.

(b) Yellow bag/bin contains animal and human soiled waste i.e. dressings, cotton contaminated with blood and body fluids etc.

(c) Black bag/bin contains chemical waste and ash of waste incinerated

(d) White bag/bin contains sharps waste which may cause needle stick or sharps injury i.e. needles, blades, scalpels etc.

(e) Blue bag contains glass ampoules and vials etc.

It is very important to segregate all kind of waste because each class of waste requires separate treatment and disposal. Waste segregation is also important because if hazardous/ infectious waste is not properly segregated from nonhazardous waste it may lead to the contamination of the whole waste which may be difficult to handle [9].

2. **Waste Storage and Transportation:** There must be a proper storage area for BMW provided by the HCE so BMW can be stored until it is transported for further treatment or disposal. Storage area must not be in the reach of general public and selected carefully. Storage area must contain warning signs and away from direct air, water and rodents. BMW should be transported for disposal as soon as possible and must not be stored for longer time [10].

3. **Treatment and Disposal of BMW:** Treatment of BMW means eradicating or minimizing the deleterious effects of the waste. Treatment of BMW also makes the disposal easy and convenient. There are different treatment methods of BMW i.e. Chemical treatment, autoclaving, incineration etc. After making the waste less deleterious by proper treatment waste is disposed off. The most common method of waste disposal is dumping in the landfill. Landfill should be away from water supply so it could not contaminate water. It should also be adequately deep and away from human habitations [11].

Biomedical waste may include a vast variety of microbes ranging from viruses, bacteria and protozoa. These microbes may be pathogenic, opportunistic and nonpathogenic. The most predominant is the genus Bacillus which accounts for 80 – 90%, Staphylococcus and Streptococcus accounts for 5 – 10%. The most common pathogen may be Staphylococcus aureus with a burden of 2 – 10 colonies of this organism per gram of waste. Other pathogens may include Pseudomonas aeruginosa, Escherichia coli and Candida albicans. Other nosocomial pathogens may include Klebsiella, Proteus species, Enterobacter species etc. The prevalence and types of microorganisms present in the biomedical waste depends upon the seasonal and spatial variables of waste collection [12].

According to another study microorganisms present in the BMW were Klebsiella species 9.3%, *E.coli* was as 12.7%, Citrobacter species were 8.5%, Candida albicans species 18.6%, Proteus species were 9.3%, Bacillus species were 9.3%.

Table 1.1: Percent presence of pathogenic bacteria in the BMW

S. No.	Bacteria	Presence [%]
1.	*E.coli sp.*	12.7
2.	*Klebsiella sp.*	9.3
3.	*Proteus sp.*	9.3
4.	*Bacillus sp.*	9.3
5.	*Citrobacter sp.*	8.5

The variety of organisms present in the BMW depends upon the environmental conditions, type of waste and most importantly defense mechanisms of microorganisms to withstand adverse environmental conditions. The three most common methods of defense which microbes use to withstand adverse environmental conditions are; thick cell wall, which helps to maintain cell integrity in adverse environmental conditions. The second and important factor is the secretion of certain chemical substances by the bacterial cells in the environment. These substances help bacteria to oxidize and reduce the toxic substances present in the environment .The third factor is the storage of food inside the cell which helps to maintain survival and growth of bacteria for longer periods in adverse conditions. According to same study microorganisms present in the BMW were Klebsiella species 9.3%, E.coli was as 12.7%, Citrobacter species were 8.5%, Candida albicans species 18.6%, Proteus species were 9.3%, Bacillus species were 9.3% [13].

1.2 Analysis of Efficacy of Routinely used Disinfectants on Pathogenic Bacteria Found in BMW

A disinfectant or disinfecting agent can be described as "a chemical agent [usually] used to kill pathogenic microorganisms except bacterial spores [which are resistant to commonly used disinfectants]. Disinfectants inactivate microorganisms in a variety of ways i.e. by damaging cell wall and cell membrane, interference with electron transport system [interfere with energy generation], or by coagulation and inactivation of bacterial proteins and genetic material.

Disinfectants are commonly chemicals but some nonchemical methods are also used to disinfect surfaces and equipments i.e. Ultraviolet [UV] light is used to inactivate airborne and surface microbes. UV light has very poor penetrability so it is not very effective agent for disinfection. Chemical disinfectants are used to disinfect liquid waste i.e. bacterial cultures, human blood and other blood products. Chemical disinfectants are also used to decontaminate solid infectious waste i.e. by placing disposable culture loops and contaminated swabs in a jar containing disinfectant [14].

1.3 Properties of an Ideal Disinfectant

An ideal disinfectant must possess following properties in addition to be microbicidal;

- Rapid in action
- Non toxic
- Non irritating
- Easy to use
- Do not damage the surfaces
- Show activity in the presence of organic matter
- Show activity in the presence of hard water
- Stable
- Residual activity etc.

While selecting the suitable disinfectant few things should be kept in mind i.e. type of waste [Liquid or solid], types of organisms may be found in the waste [bacteria, viruses, protozoa, fungi etc.] and also the presence of interfering substances.

1.4 Factors Interfering with Disinfectant Efficacy

Different factors [intrinsic or extrinsic] interfere with the action of disinfectant i.e.

- Concentration of disinfectant
- Time of exposure
- Temperature
- Presence of interfering substances i.e. hard water or organic substances
- Number and types of microorganisms to be treated etc[15].

1.5 Classes of Commonly used Disinfectants

Different types of disinfectants are used depends upon the type of material to be disinfected [solid or liquid] and what kind of microorganisms found in the material being disinfected.

- Alcohols (C_3H_8O) 60-90% strength
- Quaternary ammonium compounds ($C_{27}H_{42}C_lNO_2$)
- Phenolics i.e. (C_6H_6O)
- Iodophores ($C_6H_9I_2NO$)
- Hydrogen Peroxide (H_2O_2)
- Glutaraldehyde ($C_5H_8O_2$)
- Hypochlorite (CIO^-) etc.

Hospital acquired infections or nosocomial infections are common worldwide [both in developed and developing countries with different extent]. Approximately 5-10% patients who visit hospitals got some kind of nosocomial infection. Most of these infections are caused by drug resistant microorganisms [multidrug resistant]. In the recent decade resistance of microorganisms to commonly used disinfectants is reported worldwide. So due to this resistance conventional disinfectants used to disinfect medical devices and surfaces becoming less effective to disinfect completely [16].

In healthcare establishments [HCEs] disinfectants are used to disinfect surfaces, to sterilize operation theatres, to disinfect instruments or to treat BMW prior final disposal. There are different kind of disinfectants are available in the market with different chemical composition and different applications. So it is important to use disinfectants properly according to their chemical composition and according to the material to be disinfected. Most of the time disinfectants are used according to the literature provided by the manufacturer.

So keeping in view the above scenario it is important to clean the surfaces and instruments properly to avoid the spread of such nosocomial infections. Most of these infections can be prevented or reduced by cleaning the surfaces with good quality disinfectants, which have the ability to kill most of the pathogenic microorganisms. The choice of disinfectant depends upon the nature of material to be disinfected and type and number of microorganisms [17].

1.6 Problem Statement

BMW is potentially hazardous waste and improper handling and disposal of such infectious waste is associated with many infectious diseases especially bacterial diseases. BMW contains large amounts of antibiotics and disinfectants,this continuous exposure to these antibiotics and disinfectants is leading to the emergence of resistance against these drugs and disinfectants in the bacterial pathogens found in the BMW. This development of resistance is quite alarming.

1.7 Aims and Objectives

The aims and objectives of my research will be:

- To check the pathogenic bacterial strains found in the BMW by isolating and identifying these bacterial pathogens, so to assess the role of BMW to spread infectious bacterial agents.
- To check the efficacy of commonly used disinfectants on pathogenic bacteria found in the BMW and resistance pattern of these bacteria against the commonly used disinfectants. As to assess the role of these disinfectants to prevent BMW borne infections in the hospital settings.

Chapter 2

Literature Review

2.1 Biomedical Waste

Biomedical Waste or BMW is broadly defined as, Any kind of waste which may either be solid, liquid, radioactive or gaseous produced by a healthcare facility i.e. hospitals, diagnostic laboratories, research laboratories, dental clinics etc. during the diagnosis, treatment, vaccination or research process [18].

Hospital waste management is still a major issue in most of the developing countries i.e. Pakistan, where healthcare personals and general population is exposed directly or indirectly to the hazardous effects of BMW. BMW may include a large array of toxic waste include used syringes, blood bags, bandages, scalpels, surgical blades, human tissues, organs, blood and body fluids and radioactive waste [19].

Other hazardous materials i.e. antineoplastic chemicals, anesthetic gases, photographic chemicals, mercury, chemicals used in histopathology i.e. formaldehyde, picric acid etc. and other corrosive, irritants and miscellaneous chemicals are also considered a part of BMW. Healthcare sewage may also contain a variety of chemical and biological substances which may directly or indirectly harm the human health [20]. Improper dumping of BMW may be hazardous it may either directly cause harm to the human health or indirectly by attracting rodents and other disease carrying vectors. It may also contaminate soil and water and as a result water borne or soil borne infections. Burning of BMW may also contribute to global warming by producing methane gas [21]. According to a study done in the department of community medicine, Army Medical College Rawalpindi, Pakistan regarding the awareness of health hazards associated with mishandling of health-care waste. Surprisingly it was found that none of them were available about the risks associated with the mishandling of BMW. Even they had never received any training regarding the safe handling of BMW [22]. This study shows the seriousness of condition in the country. Healthcare waste also called as Biomedical waste [BMW] is the kind of waste generated by healthcare establishments, clinics and research facilities during the diagnosis, treatment, vaccination and research process. Solid healthcare waste is the solid waste being generated from healthcare facilities. According to the level of infectiousness the major fraction of the waste [75-90%] is noninfectious waste also called as domestic waste and consists of paper, plastic bags, disposable tea cups, cards etc. The other 10-25% of waste is infectious or hazardous waste which poses a serious health problem if not handled with care [23]. Various terms are used interchangeably for BMW i.e. biohazardous waste, Infectious waste, hospital waste, Healthcare waste; Health facility generated waste, infective waste, pathological waste, microbiological waste, red bag waste and medical waste. All of these terms are used less or more synonymously to define the infectious nature of this waste. This waste has the potential to cause harm to the humans and other livings when exposed to it [24].

There are different classes of medical waste. According to the Environmental Protection Agency [EPA] medical waste is classified into the following types;

1. Blood and blood products
2. Bacterial cultures and stock cultures
3. Pathological waste
4. Used sharps i.e. needles, scalpels
5. Contaminated waste generated by laboratory

6. Animal carcasses and their body parts
7. Contaminated waste generated as a result of patient care
8. Discarded biological items
9. Contaminated instruments and equipment
10. Miscellaneous waste which is potentially infectious [25].

The legislation regarding the management and handling of healthcare waste in Pakistan is covered under Pakistan environmental protection act [PEPA] 1997. This act covers the disposal and handling of healthcare waste. This act is about the contamination of air, water and land due to the healthcare waste. It states that the pollution level of air, water and land should not exceed the prescribed limits. This act prohibits handling of hazardous which are to be dealt after acquisition of license. In response to the increased concerns regarding the handling and management of BMW the government of Pakistan developed rules for management of BMW in 2005. These rules define the different categories of hospital waste. These rules provide guidelines about the development and management of hospital waste system. These rules also describe the safe handling i.e. collection, segregation, storage, transportation and treatment of hospital waste. According to an estimate approximately 250,000 tons of BMW is generated annually from all kind of health facilities i.e. government and private hospitals, clinics, laboratories and small centers [26].

2.2 Types of BMW

BMW generated in the HCEs falls under two major categories as Nonhazardous and Biohazardous waste with few subcategories. Categories of BMW;

1. Nonhazardous or Non-risk waste
2. Biohazardous or risk medical waste

Biohazardous waste also has two subcategories as;

1. Infectious waste
2. Noninfectious waste.

2.2.1 Nonhazardous or Non-risk Waste

The constituents of this waste are noninfected cardboards, food remnants, plastic packaging material, fruit peels, paper and envelops etc. This waste contributes major portion [75-90%] of waste generated by the HCEs. Nonhazardous material is noninfectious and not hazardous for the hospital staff and environment. So this kind of waste is treated along with other domestic waste. Noninfectious material is stored and transported in the white dustbin. Nonhazardous waste should be segregated with care so it should not get contaminated from hazardous/infectious waste. So nonhazardous waste should be stored, transported and treated separately [27].

2.2.2 Biohazardous or Risk Medical Waste

Hazardous waste or biohazardous waste is the kind of waste which has the potential to cause hazard or damage to the healthcare workers, personnel handling it or the environment. This waste contributes 10-25% of total waste generated in the HCE. Hazardous waste may directly or indirectly cause injury or damage. Hazardous waste is again divided into the infectious waste and noninfectious waste according to the level of infectiousness [28].

2.2.2.1 Infectious Waste

Infectious waste consist of used sharps i.e. syringes, needles, surgical blades, scalpels, prickers etc. Other items are bacterial cultures, blood and blood products, body fluids, infected dressings, cotton, catheters, body organs etc. This kind of waste is highly dangerous so should be handled carefully. It has the potential to cause infection and disease in case of poor handling. So care should be taken to handle this class of waste.

2.2.2.2 Hazardous Noninfectious Waste

This class of waste is though noninfectious but has the potential to cause damage if not managed carefully. This class of waste includes Chemical waste i.e. chemicals used in histopathology

- Radioactive waste i.e. waste in the radiology department
- Cytotoxic waste i.e. acids and bases used in the laboratory
- Carcinogenic waste i.e. Ethidium bromide used in gel electrophoresis [29].

Table 2.1: Categories of Health Care Waste [30].

Category of HCW	Recommended category	Class of regulated medical waste
Isolation waste	Yes	Yes
Bacterial cultures and stock cultures	Yes	Yes
Blood and blood products	Yes	Yes
Pathological waste	Yes	Yes
Used sharps i.e. blades and scalpels	Yes	Yes
Animal carcasses and body parts	Yes	Yes
Surgical wastes of humans	Optional	May be
Laboratory waste	Optional	May be
Waste from Dialysis units	Optional	May be
Contaminated equipment	Optional	No
Unused sharps	No	Yes

Hospital waste may contain biological or non-biological items include: infectious waste, pathological waste, hazardous chemical waste, radioactive waste, sharp items i.e. disposable syringes, needles scalpels etc. Other items include bandages, dressings, wrappers and food items. Other type of waste may include drugs and chemicals used in X-rays processing etc[31]. It should be kept in mind that if both these types of wastes are mixed together i.e. infectious and noninfectious waste the whole waste becomes harmful. So care must be taken so that infectious waste should not be mixed with noninfectious waste otherwise handling such big amount of waste becomes extremely difficult. Infectious waste can be categorized into several categories i.e. human organs, tissues and body parts, carcasses of animals used for research purpose, vomits, cultures, urine, sharps i.e. syringes, blades, needles and prickers, saws, drugs and chemicals etc[32]. Due to the environmental conditions of health facilities it is safe to mark all the waste as infectious waste and special treatment and disposal system should be considered [33].

2.3 BMW Management Strategies

Proper management of BMW is of great importance because improper handling of it may adversely affect the health of people who come into contact with it. Proper collection, segregation, storage, transportation and safe disposal of waste are the key steps in proper management of BMW [34]. According to the "Occupational health and safety administration USA May 30, 1989" during the handling of biological waste the major concern is the risk of exposure to potentially infectious agents. BMW disposal and treatment facility means any specific place or setting where final treatment and disposal is done [35]. There are different steps involved in the management and handling of BMW starting from generation to the final disposal of waste. The steps involved in the management of BMW are;

- Segregation
- Storage

- Transportation
- Disposal [or]Final treatment

2.3.1 Segregation of Waste

BMW segregation plays an important role in the proper management of waste. It is important to properly segregate the infectious or contaminated waste from noninfectious waste, otherwise the whole waste becomes infected and to hold such a big quantity of waste is very difficult [36]. BMW is segregated into different colored bags and dustbins according to the coloring codes system as given below:

2.3.1.1 Red Colored Dustbin/Container

Red colored dustbin is used to collect recyclable contaminated waste i.e. intravenous tubes, intravenous and urinary catheters, urine bags, syringes, gloves etc.

2.3.1.2 Yellow Colored Dustbin/Container:

Yellow colored dustbin is used to collect anatomical waste, other items contaminated with blood, blood products and other body fluids i.e. dressings, cotton swabs, expired medicine, linen, bed sheets etc.

2.3.1.3 Black Colored Dustbin/Container

Black colored dustbin is used to collect incineration ash.

2.3.1.4 White Colored Dustbin/Container

White colored dustbin is used to collect contaminated sharps i.e. needles, scalpels, surgical blades, syringes etc. that may cause needle stick injuries and transmission of infectious agents.

2.3.1.5 Blue Colored Dustbin/Container

Blue colored dustbin is used to collect glass i.e. ampoules, medicine vials. Metallic body implants are also collected in blue colored dustbins [37].

According to a study conducted by Fazil Hakim Khattak in 2009 about 19% of the hospitals did not segregate the hospital waste, while in 27% of the hospitals there was a practice of segregation of sharps. In 48% of healthcare facilities there was a practice of segregating noninfectious waste from infectious waste. 32% of hospitals were using waste containers properly according to their color codes [38].

Figure 2.1: Segregation of BMW in different colored containers [Properly labeled with coloring codes]

2.3.2 Storage of Waste

It is important that hospital must provide a place for storage of BMW, where BMW is stored until it is collected for final treatment/disposal. The selection of storage area is very important as storage of waste in an unsafe and improper place may lead to the serious problems. Storage area should be well protected and not in the reach of general public. There must be warning signs and symbols exhibited at the storage area. Storage area should be well protected from rodents, insects, water and wind. Care should be taken while storing expired chemicals or chemically contaminated items as mixing of different chemicals may leads to the explosion. It also important that waste should not be stored longer than 3 months [39]. According to the Dr. J.S. Badshah it is safe and good practice to incinerate potentially infectious waste as soon as possible. It is good to incinerate waste within 24 hours of generation of waste, otherwise store the waste in a refrigerated storage in a well-protected place. Proper ventilation and temperature should be maintained. According to OSHA to store BMW, the following recommendations should be followed;

- Containers having infectious waste should be closed or sealed well.
- Food items should not be stored in the refrigerator where BMW is stored. Because storing food items in such place may lead to the transmission of infectious agents.
- There should be limited access to the storage area so that authorized persons can only enter the area.
- There should be warning signs displaying signs of biohazard around the storage area [40].

2.3.3 Transportation of Waste

Transportation of waste is a critical step in proper waste handling. It should be kept in mind that BMW is potentially hazardous so care should be taken while loading or unloading the waste. BMW should be transported in special trucks which are leak proof and thick walled.

2.3.4 Disposal [or] Final Treatment of Waste

Disposal or treatment of BMW refers to the process by which deleterious effects of BMW are completely eradicated or reduced to a safe level. There are several treatment strategies or methods are available depending upon the nature of the waste. Disposal of BMW minimizes hazardous effects on environment. Most commonly used methods for disposal of BMW are autoclaving, chemical treatment, incineration and irradiation etc.

2.3.4.1 Incineration

Incineration is the process by which BMW is converted into noninfectious ash and flue gases. The temperature range of an incinerator is 800-1400℃. Incineration is the ideal process to treat many types of infectious waste. Incineration reduces the waste mass by 90-95% and also reduces the hazardous effects of waste [41].

FIGURE 2.2: Incinerator for BMW final treatment (Disposal of BMW)

2.3.4.2 Autoclaving

Autoclaving is used as an alternate of incineration. For autoclaving a temperature of 121℃ is applied at a pressure of 15 [psi] for 20-30 minutes. Autoclaving kills or inactivate the infectious agents.

Autoclaving is also commonly used to sterilize the medical equipment. Autoclaving is less expensive and carries very little risk. Autoclaving is the method of choice for some types of infectious waste [42].

2.3.4.3 Chemical Method

Chemical treatment is used to treat liquid waste. Several chemical reactions are involved i.e. precipitation, oxidation, reduction, pH change to convert hazardous substances into less hazardous substances. Different chemicals are used according to the nature of waste i.e. calcium oxide, chlorine, sodium hydroxide etc. [43].

2.3.4.4 Irradiation

Less frequently irradiation is also used to treat BMW. Different rays are used i.e. UV rays, X-rays, gamma rays etc. Waste is treated in an enclosed chamber by exposing the waste to gamma rays. Gamma rays are lethal for microorganisms. As compared to other methods irradiation is very expensive method. Care should be taken, as unprotected exposure to rays may lead to cancer and DNA damage [44].

2.3.5 BMW Management System in Pakistan

In Pakistan BMW is handled under the "Pakistan Environmental Protection Act [PEPA] 1997". This act covers the safe handling and disposal strategies for BMW according to the "National Environmental Quality Standards [NEQS]. Rules regarding the handling of hospital waste in Pakistan were developed in 2005. These rules define the roles and responsibilities of healthcare workers to safely handle i.e. to collect, segregate, store, transport and dispose of BMW properly. It is estimated that around 250,000 tons of healthcare waste or BMW is generated annually [45]. According to a study done by "Junaid Habib-Ullah" in the Lahore city only six hospitals were following the practice of weighing healthcare waste.

These six hospitals were generating an average of 0.292 kg of BMW/bed each day. According to this study "Fatima Memorial hospital" was generating the highest weight of BMW per bed as 0.322 kg. A private hospital named "Ali hospital" was generating the least quantity of 0.21 kg per bed/day. It was estimated that a quantity of 1366 kg of infectious waste was being generated by these hospitals per month. Overall large amounts of potentially infectious biomedical waste is generated on the daily basis in the hospitals of Pakistan. But the management of such big amount of waste is very poor. Staff handling the waste is also not properly trained and there is also lack of resources.

TABLE 2.2: Generation rate of BMW in the big hospitals of Lahore city

HCF	No. of Beds	Occupancy rate	Kg/ Bed	Kg/ Day	Kg/ Month
DHQ Sheikhupura	300	200	0.305	61	1586
DHQ Kasur	350	161	0.312	50.2	1305
DHQ Gujranwala	350	185	0.302	55.8	1450
Fatima Memorial Hospital	510	281	0.322	90.4	2350
Shalamar Hospital	350	151	0.301	45.4	1180
Ali Hospital	150	60	0.210	12.6	327
Average	355	156	0.292	52.82	1366

2.4 Status of Awareness and Training of Healthcare Staff Regarding The Handling of BMW

As healthcare waste contains potentially infectious agents so it should be handled with great care. Handling and management of BMW requires special expertise, so healthcare staff should be trained to safely handle the BMW. According to a study conducted by "Junaid Habib-Ullah" in the Lahore city regarding the handling of BMW only 33% of healthcare workers were aware about the hazards associated with BMW and safe handling of BMW.

Other 67% were either totally unaware or were not following the standard rules regarding the management of BMW. Only one hospital trained its staff regarding the hazards associated with BMW handling and how to manage an exposed or injured healthcare worker. Only one hospital maintained the record of such incidents [46].

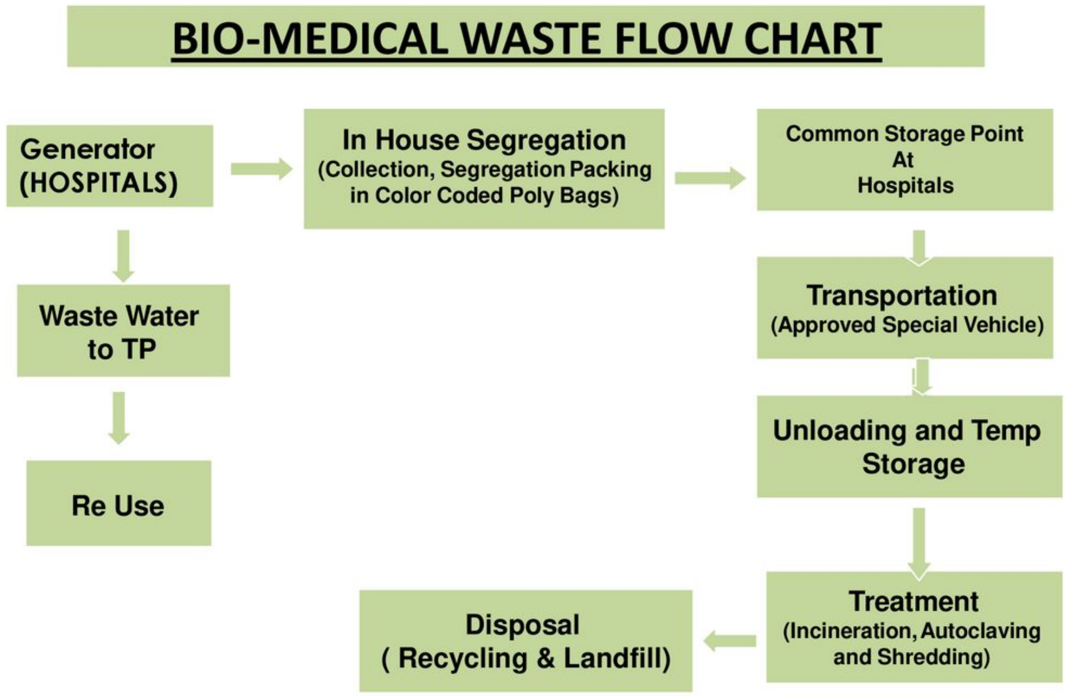

FIGURE 2.3: Flow chart of BMW Management in Pakistan

2.5 Necessity of Proper BMW Management

As BMW is a potential source of many biological and chemical hazards so it is very important to treat BMW properly so to protect the healthcare workers, personnel's dealing with the BME management and the environment. It is also important to dispose of this potentially infectious waste properly so to protect the waste handlers, scavengers and the general public living in the vicinity. It is also important to keep in mind that improper incineration and smoke may also contaminate the environment. Expired drugs and other medicines should also be disposed of properly so to avoid the repacking and selling of such drugs. Recycling and reuse of improperly treated syringes, needles, prickers, IV catheters etc. is also a major problem. Proper collection, segregation, storage, transportation and disposal are the key steps in the effective management of BMW [47].

Medical waste or BMW generated in the hospitals as a result of diagnosis, treatment, management or vaccination of patients has raised serious concerns owing to the fact that there is no appropriate management of it. With a tremendous rise in the population, advancement of diagnostic and treatment strategies and specially introduction of disposable items i.e. needles, syringes, IV catheters etc. as a result there has been an increase in the production of BMW [48].

In the past few years there has been an increase concern over the handling of BMW because of potentially infectious nature of this kind of waste. The amount of BMW generated in the HCEs is relatively small as compared to other kind of waste generated in the community but to handle this kind of waste [BMW] is very difficult and risky. Management of BMW is considered as a critical issue throughout the world so special techniques and equipment's are required to handle this waste [49].

The amount of BMW generated by HCEs depends upon multiple factors including type of HCE i.e. primary care hospital, tertiary care hospital or a small clinic, number of patients attended, health status of the community, number of disposable items used and number of reusable items in the establishment. BMW is considered as special type of waste because of its infectious nature. Improper handling of such waste i.e. uncontrolled burning or open dumping may create a health risk for healthcare workers, patients, waste handlers and for the environment too. Although health services are to reduce the incidence of diseases in a community but such type of waste if not handled carefully may become a substantial source of diseases for the community [50].

Over the last few years there has been an increased concern over the generation and management of solid waste, this is because of the fact that this type of waste is potentially dangerous for the patients, healthcare workers and environment if not handled with care. Following qualities make BMW hazardous i.e. Infectious, Toxic (Cytotoxic and irritating), Injurious etc. BMW is hazardous since it has the ability to disseminate various infections i.e. both within the hospital settings Known as hospital acquired infections [nosocomial infections] and also outside the hospital setting to the waste handlers and general public. It is reported that almost 60% of all hospital staff get injuries and infections from sharps during performing different procedures and handling such waste. Hospital waste handlers are at a great risk of getting infections from such injuries because most of them are illiterate and not aware of risks associated with such injuries [51].

According to a study done in the 8 teaching hospitals [generally >200 bedded hospitals] of Karachi city it was found that only few [12%] hospitals arranged training sessions for their staff to train them to properly manage the BMW. No any record was maintained to handle the BMW [52].

According to a study done in the Lahore highlighted that majority [85%]of sweepers were aware of the fact that their job is associated with risk to their health but they have to continue their jobs for economical reasons.

2.6 Problems Associated with Improper Handling of BMW

As we know well that BMW is a potential source of many infectious agents. It is therefore necessary to handle it with extreme care in order to protect the personnel handling it and also the environment. General public can be infected directly i.e.

by needle stick injury, wound, inhalation or ingestion or indirectly via contaminated water or food. Dumping the BMW in the open can have adverse effects on the environment.

The dumping of BMW along with the routine municipal waste is a bad practice which could be a source of contamination of water, soil and air. Similarly if BMW is not incinerated properly it may contaminate the air band may have bad health impact on the surrounding inhabitants. The most dangerous and the most common hazard is sharps, which are a major source of transmission of infectious agents [53].

Figure 2.4: Openly dumped syringes and needles. Continuous risk for humans and environment

In the Germany approximately 500,000 needle stick injuries occur each year among the healthcare workers in the hospitals. Most of these injuries occur due to syringes, needles and other sharp objects [54].

Chemicals present in the BMW i.e. heavy metals can also contaminate the soil and water. Poor landfill may also cause water pollution in the form of leachates. In the case of excess nutrient leachate from the BMW such as phosphates and nitrates can cause eutrophication [development of algal body on the surface of water].

This kind of leachate of chemicals from landfill can alter the pH and chemical composition of water. This chemically altered water in not only dangerous to humans but also for the other aquatic animals and plants. Consumption of such contaminated animals and vegetation may be harmful for human health [55].

Although the chemical and physical characteristics are important but the potential of BMW to spread infectious diseases is a matter of concern. Literature points out problems associated with improper handling of BMW such as accidental exposure of healthcare workers and environmental contamination. The literature from various studies also highlights the role of waste in the spread of infectious diseases among the general public via direct or indirect exposure [56].

One of the greatest concerns associated with healthcare waste is the presence of microbial pathogens in the waste. The selective pressure of chemicals, antibiotics and compounds found in the BMW may lead to the increase burden of these potentially pathogenic organisms. Main pathogenic organisms found in the hospital waste are bacteria that may be resistant to antibiotics and contaminate the hospital equipment, surfaces and other materials. As these organisms are discharged with solid waste as well as in the liquid waste so they may contaminate the sewerage system as well as the disposal site of waste i.e. landfill [57].

2.7 Role of BMW in Disease Transmission

According to the "Guide on infectious waste management" the exposure of a person to the infectious agent could have serious consequences. This exposure may lead to the direct disease transmission via an open wound, skin lesion, cut or scratches or via splashes and evaporation to the mucus membranes, nose, eye and mouth. Infectious agents may also spread by inhalation or ingestion. Disease transmission may also be indirect via consumption of contaminated water or food [58]. Transmission of infectious diseases in the hospital settings mostly occur by needle stick injuries while recapping and reusing needles and syringes. Major viral infectious agents spread through needle stick injuries are Hepatitis B Virus [HBV], Hepatitis C Virus [HCV] and Human Immunodeficiency Virus [HIV]. According to WHO the chance of seroconversion from single needle stick injury is 0.3 – 0.5% in case of HIV and 2 – 5% in case of HCV [59].

FIGURE 2.5: Improperly dumped BMW

FIGURE 2.6: A garbage picker is collecting a blood bag for re-use

According to the WHO, in the health sector alone unsafe use of injections and syringes leads to the 30,000 new cases of HIV transmission each year, approximately

1.2 million new cases of HCV and 8 million new cases of HBV transmission worldwide each year. Immunization against HBV by vaccination is recommended for healthcare workers who are at constant risk of interaction with the HBV while performing their duties [60].

Scavengers and hospital workers who are involved in recycling/ reuse of infectious waste constantly at risk of developing infections and also a source of transmission of such infections to the community. In recent years with the introduction of disposable syringes, needles and other similar items the situation has become worse as it is very difficult to handle such a big amount of waste. In the recent years tons of the medical waste is generated daily. Improper handling of such waste is associated with the contamination of soil, water and air [61].

A large number of bacterial diseases can also be transmitted through contact with BMW i.e. Respiratory tract infections, urinary tract infections, wound infections, septicemia, bacteremia and skin infections [62]. Hospital infectious waste is a potential source of infectious microorganisms that can infect healthcare workers, patients and the general public if not handled with care. Infections may be transmitted directly, indirectly through air, soil or water or through the vectors i.e. rodents [63]. There are multiple routes of exposure through injury, inhalation, ingestion, or through contact with mucous membranes. Moreover hospital waste is a potential source of potentially dangerous microorganisms that can infect people and if not disposed of properly can also contaminate the environment. Healthcare waste contains a large variety of organisms their number and type depends upon the source of generation. Commonly identified organisms among the healthcare waste are Pseudomonas spp; Escherichia coli, Staphylococcus spp; and Corynaebacterium diphtheria. Therefore hospital waste should be carefully monitored and handled to avoid the transmission of hospital acquired infections [64]. Despite the significant improvement in patient healthcare, anesthesia, surgical techniques and sterilization it is estimated that 2.5% of total 6 million patients who receive some surgical procedures in the hospitals of U.S each year got some sort of hospital acquired or nosocomial infection. This leads to the increase health and economic burden on the patient and the government, also a source of transmission of infections to the community [65]. Nowadays proper assessment of hospital environment i.e. Air, water and surfaces has become a routine part of good healthcare facilities which has lead to the improved patient care. Operation theatres are also checked time to time for any sort of infectious agent as a part of good surgical protocols and improved healthcare quality [66].

2.8 What Kind of Bacterial Pathogens Found in BMW

Different types of pathogenic organisms may be found in the hospital waste. Most common organisms found in the BMW are bacteria, fungi and viruses. The most predominant bacterial organism is genus Bacillus [80-90%], Staphylococcus and Streptococcus vary between 5-10%. Staphylococcus aureus is the most common pathogen [colony count 2-10 colonies of organism per gram of waste]. *Pseudomonas aeruginosa, Escherichia coli* are also found in varying numbers. Other nosocomial pathogens may also found in little numbers i.e. *Klebsiella, Enterobacter* and *Proteus* species. Few numbers of fungal organisms are also found in the BMW. The most common fungal pathogen is *Candida albicans.* Viruses are also found in very low numbers. It is observed that BMW contains such material that is able to carry viruses. However viral titer decreases with the passage of time. Hepatitis B virus is detected from BMW but its potential to cause infection is not established yet. The pathogens can leach out from medical waste and contaminate the environment. Care must be taken while dumping the medical waste. BMW should not be dumped near the water supply or in open access to rodents[67]. Plate count of aerobic heterotrophic organisms cultivated from soil hospital waste dumpsite showed an insignificant number [$P > 0.05$]. The reason behind this insignificant difference is that hospital waste does not provide favourable environment for pathogens as it contains antiseptics, presence of predators, extreme pH, moisture content and high temperature [68]. The count of fungi in the hospital dumpsite soil was high due to the presence of organic material in the BMW. BMW is a rich source of organic material so as fungi are heterotrophic organisms this provides a rich source of growth for fungi [69].

A. israelli has about 3.17% prevalence in the soil of dumpsite and about 5% prevalence in the soil adjacent to the dumpsite soil. This organism resides the buccal cavity of humans but it also causes suppurative disease of foot called actinomycosis. *C. equi* has the 1.59% appearance in the soil associated with dumpsite and 5% appearance in the soil adjacent with dumpsite. This organism is associated with human infections. According to a study different fungal species were identified in a sample of soil obtained from dumpsite. These species were included *Aspergillus spp, Penicillium rubrum, Penicillium viricedum, Aspergillus niger, Aspergillus nidulans* and *Trichothecium roseum.*

Aspergillus spp. is capable of utilizing multiple substrates for food because they have multiple enzymes to degrade these substances. *Aspergillus spp.* is capable of producing disease in animals and humans called as *aspergillosis*. *R. nigricans* was the second most common non pathogenic fungi in the dumpsite soil [70].

According to a study various opportunistic pathogens were found in the various medical wastes. These organisms were included *Pseudomonas spp; Kocuria spp; Microbacterium oxydans, Bevibacillus spp* etc. In addition to bacterial organisms various viruses were also identified in the BMW i.e. Norvoviruses and Hepatitis B virus were isolated from human tissues. Other pathogenic organisms commonly found in the hospital waste are *Corynaebacterium diphtheriae, Pseudomonas spp; Escherichia coli, Staphylococcus spp; Salmonella spp;* and Respiratory synctial virus [RSV]. Medical waste should be handled and monitored carefully to prevent hospital acquired infections [71].

TABLE 2.3: Occurrence of bacterial isolates and frequency of isolation from BMW

Bacterial Isolates	Number of Isolates	Frequency of Isolates [%]
E.coli	30	39
S. aureus	25	32

S. pyogenes	12	15
B. subtilis	10	10
K. pneumoniae	3	4
Total	78	100

A study conducted on hospital waste has shown that dressings and bed sheets contaminated with body fluids of patients had the highest microbial load. This was due to the long periods of contact of these items with patients. According to this study *E.coli* was the predominant organism with the highest rate of prevalence followed by *S. aureus*. Other bacterial organisms isolated from these samples were *S. pyogenes, B. subtilis* and *K. pneumoniae* etc. with lower frequency.

Results of this study has also shown that there was a high microbial load in dressings 25 and bedding 13, 32% and 17% respectively, while microbial load of used lancets and expired cytotoxic drugs was 1 [1.3%] and 2 [3%] respectively [72].

Hospital waste may contain a vast variety of pathogenic bacteria that come from patients i.e. blood and body fluids. The number and type of bacterial spp; depends upon antibacterial agents found in the BMW i.e. antibiotics, detergents, antiseptic agents and the environmental conditions i.e. temperature, humidity, pH and nutritional state. The mixing of healthcare waste water with municipal sewage system creates hazards for the general public and imbalance of microbial flora in the sewage system. High number of faecal coli forms, total coliforms, *Streptococci, Staphylococci, Pseudomonas* and *Klebsiella* were reported in the sewage system in the neighborhood of a hospital from Baghdad city [73].

Causative agents [Type]	Diseases
Bacteria	Wound infections Tetanus, Cholera, Shigellosis, Diarrhea, Toxemia etc.
Viral	Hepatitis B and C, HIV/AIDS, Poliomyelitis, Rabies etc.

Parasitic	Amoebiasis, Giardiasis, Ascariasis, Leishmaniasis
Fungal	Candidiasis, Cryptococcoses, Coccidiodomycosis

2.9 Role of BMW in The Emergence of Antibiotic Resistant Strains in The Environment

In the previous few years antibiotic resistance has become a serious issue due to the excessive and uncontrolled use of antibiotics especially in the developing countries. Excessive use of antibiotics leads to the prevalence of antibiotic resistant genes in the environment. BMW plays an important role in the emergence of antibiotic resistance in the environment. According to a study on "deleterious effects of BMW" it is estimated that approximately 5.2 million people [especially children] die each year due to the hazardous effects of BMW. Bacteriophages found in the BMW play an important role in the transfer [horizontal] of antibiotic resistance genes [75].

Due to the increased and uncontrollable use of antibiotics in the human medicine as in case of self medication and incomplete courses of antibiotics there is an increasing trend of antimicrobials resistance in the bacteria. Antibiotics are also used in the field of veterinary i.e. in veterinary feeds emergence of antibiotic resistant bacteria and evolution of multidrug resistant strains has become a great challenge. Drug resistant Salmonella is a big problem in all the countries of the world. Multidrug resistant Salmonella is becoming a big challenge in the poor countries of the world [76].

A large variety of antimicrobial, radionuclide's and other substances are used to diagnose, treat or vaccinate patients or to disinfect surfaces and equipment and for research purpose. Many non-metabolized drugs are released in the environment that may lead to change in the microbial ecology and also emergence of antibiotic resistance in environment. Bacterial pathogens may also acquire antibiotic resistance by cellular mutation or by acquiring plasmids or transposons which contain resistance genes [R-genes]. Specific environments such as hospitals contain high number of resistant bacteria. Such complex environments also promote the growth of resistant bacteria which leads to the increased number of such bacteria with enhanced resistance to antibiotics.

A vast number and species of bacteria are found in such complex environments so they exchange their genetic material and gain more complex resistance patterns. Hospital waste especially wastewater may contain drug resistant bacteria which may lead to the emergence of drug resistant pathogenic bacteria in the environment. These dug resistant bacteria may cause complicated infections to humans and animals that cannot be easily treated by conventional antibiotics. So hospital wastewater is a serious matter of concern for those who receive such water which contains high number of drug resistant pathogens [77].

2.10 Analysis of Efficacy of Routinely used Disinfectants Bacterial Pathogens Found in BMW

2.10.1 Disinfectants

Hospital acquired, nosocomial or Healthcare associated infections are caused by transmission of infectious agents from hospital surfaces and medical devices. These infections are responsible for a significant number of morbidity and mortality.

Recently it is observed that most of these infections are caused by methicillin resistant *Staphylococcus aureus* [MRSA], *Clostridium perfringens, Vancomycin resistant Enterococcus* and *Norvoviruses.* Most of these infectious agents are shed by patients and they contaminate hospital equipment and hospital surfaces in a sufficient number to cause infection in patients [78].

Contamination prevention to achieve aseptic conditions requires choosing best chemical to eliminate all the microorganisms from a surface. There are three main categories of chemical agents used to attain asepsis, which include Sanitizers, sporicidals and disinfectants.

Sanitizers decrease the number of vegetative organisms below the level agreed by public health ordinance. While disinfectants remove or destroy vegetative organisms. Sporicidals kills both the vegetative as well as spore forms of microbes [79].

Microorganisms are also present in the hospital waste water which is one of the major public health and environmental concern. According to a research conducted by Tsai et al. reported that *coliforms, pseudomonas aeruginosa* and *Salmonella spp;* were found in the hospital waste water sludge.

Salmonella spp; were found in the high frequency of 37% [in 10 samples out of total 27 samples]. This high frequency of *Salmonella* may cause serious public health problems by consumption of such contaminated water [80].

TABLE 2.5: Properties of commonly used disinfectants

Disinfectant	Active ingredient	Advantages of use	Disadvantages of use
Alcohol	Ethyl alcohol Isopropyl alcohol	Rapid bactericidal action No bacteriostatic action Cheap	No sporicidal action Swell and harden plastic and rubber Inflammable
Chlorine and it's compounds	Hypochlorite Chlorine peroxide	Bactericidal Mycobacteridida Inexpensive Fast action	Corrosive for metals Irritant for skin Damage plastic

Peroxigens	Hydrogen peroxide Per acetic acid	Bactericidal Sporicidal Biodegradable environmental friendly	Irritant Corrosive to copper and steel Unstable
Quaternary ammonium compounds	Alkyl/ Benzyl/ Didecyl Dimethyl ammonium Chloride	Most commonly usedBactericidal Virucidal Bacteriostatic Deodorizer	Less efficient against Gram negative bacteria and non enveloped viruses

2.10.2 Role of Disinfectants to Prevent Hospital Acquired Infections

Proper cleaning and disinfection plays an important role to prevent nosocomial infections. Different methods of disinfection are used i.e. chemical disinfectants, UV radiation and heat etc. Chemical disinfectants are commonly used in the healthcare establishments and food industry because it is easy to use and have broad spectrum of microbicidal activity. For high touch surfaces pre-impregnated wipes or ready to use wipes are commonly used because of their reliable and easy application. Although these pre-impregnated wipes are commonly used but their effectiveness is to be understand yet [81].

Before using any disinfectant literature should be viewed thoroughly to understand the cleaning procedure i.e. ho to apply, contact time required to kill microbes, temperature required for maximum activity and its interaction with other chemicals used in combination with other chemicals i.e. deodorants, surfactants and enzymes. Qualification of proper disinfecting agents is a crucial process that requires better planning, careful execution of planning and proper scrutiny.

Vegetative bacteria, bacterial spores and viruses often shed from infected patients on the surface of hospital and also on the instruments. These bacterial and viral pathogens may cause severe infections. So it is important to disinfect hospital surfaces properly to kill these infectious agents. The role of disinfectants to control nosocomial infections is shown by multiple studies. A study from London showed a significant decrease in the number of *Clostridium difficile* infections after the introduction of sporicidal chemicals in the chemicals used for disinfection [82].

2.10.3 Bacterial Resistance Against Commonly used Disinfectants

In the recent years currently used procedures to control hospital acquired infections are becoming less effective. Resistance against commonly used disinfectants is becoming widespread. So emergence of multidrug resistant pathogens which are also resistant to commonly used disinfectants is becoming a serious issue for the clinicians and healthcare establishments. So this situation requires strict adherence to follow protocols of disinfection and sterilization. As commonly used disinfectants are becoming less effective so it is the dire need of time to test and develop newer and more effective disinfectants [83].

Large quantities of different disinfectants are used to disinfect surfaces and equipment in the hospitals. Disinfectants are also used to disinfect skin to reduce the number of microorganisms found on the skin. Large quantities of residual disinfectants reach the waste water. So these residual disinfectants kill the sensitive microbes and make room for resistant bacteria. Bacteria found in the hospital waste water may show enhanced resistance against a wide variety of disinfectants. This resistance may develop due to genetic mutations or by acquisition of mobile genetic elements [plasmids or transposons] [84].

Bacterial resistance against antibiotics is a long term phenomenon but recently bacterial resistance against disinfectants is a matter of discussion. It is observed that *Mycobacterium chelonae* isolated from endoscope washer showed an enhanced resistance against glutaraldehyde as compared to other strains of *Mycobacterium*. It was observed that the mechanism behind this resistance was the presence of arabinogalatan/arabinomannan in the cell wall of *Mycobacterium chelonae* [85].

2.10.4 Disinfectant Wipes

In the hospitals ready to use disinfectant impregnated wipes [RTUDIW] are commonly used to disinfect instruments and surfaces, because they are easy to use and more reliable. Wipes provide both mechanical cleaning to remove large organic debris as well as chemical cleaning by disinfectant.

The wipes are mostly made of textile [cellulosic fiber] and sometimes of thermoplastic fibers [polyethylene terephthalate]. These are made of inexpensive materials i.e. cellulosic fibers. A good quality wipe should have high water retention and storage capacity. Polyfine fibers of wipe provide high tensile strength and also provide resistance against solvents and abrasion. Most of the wipes used for the surface disinfection are made up of combination of viscous fibers and polyester [86].

Ready to use disinfectant impregnated wipes or RTUDIW consist of towels impregnated with diluted disinfectant and other chemicals i.e. deodorants, surface cleaners and preservatives etc. Chemical interactions between disinfectants and other chemicals used should be kept in mind. Different factors influence the efficiency of i.e. type of wipe, type of disinfectant used, wiping strategy, interaction between different chemicals used for disinfection, storage time and most importantly contact time of disinfectant with the contaminated material or infectious agents.

Chapter 3

Material and Methods

The research work was carried out in wet lab of department of Bioinformatics and Biosciences, Faculty of Health and Life Sciences, Capital University of Science and Technology, Islamabad. Growth on selective media was done in the microbiology section of "Cure laboratories" Islamabad. Biochemical tests for identification and disinfectant efficacy tests were also performed in the microbiology section of "Cure Laboratories" Islamabad.

Table 3.1: List of culture media that were used:

Name of culture media	Used to isolate	Manufacturer
Nutrient agar	Total bacterial count (TBC)	Conda
XLD (Xylose Lysine Deoxycholate agar)	*Salmonella typhi*	Oxid
MSA (Mannitol Salt agar)	*Staphylococcus aureus*	Conda
VRB (Violet Red Bile agar)	*E.coli*	Conda
Lactose agar	*E.coli*	Conda
Citramide agar	*Pseudomonas aeruginosa*	Merck
Tryptic Soy agar	For disinfectant efficacy test	Conda

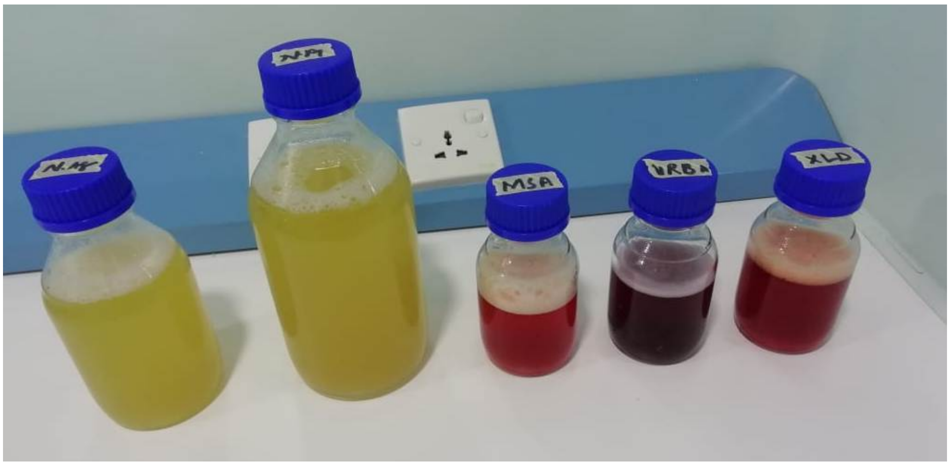

FIGURE 3.1: Preparation of MSA, VRB, XLD, Citramide and Nutrient agar

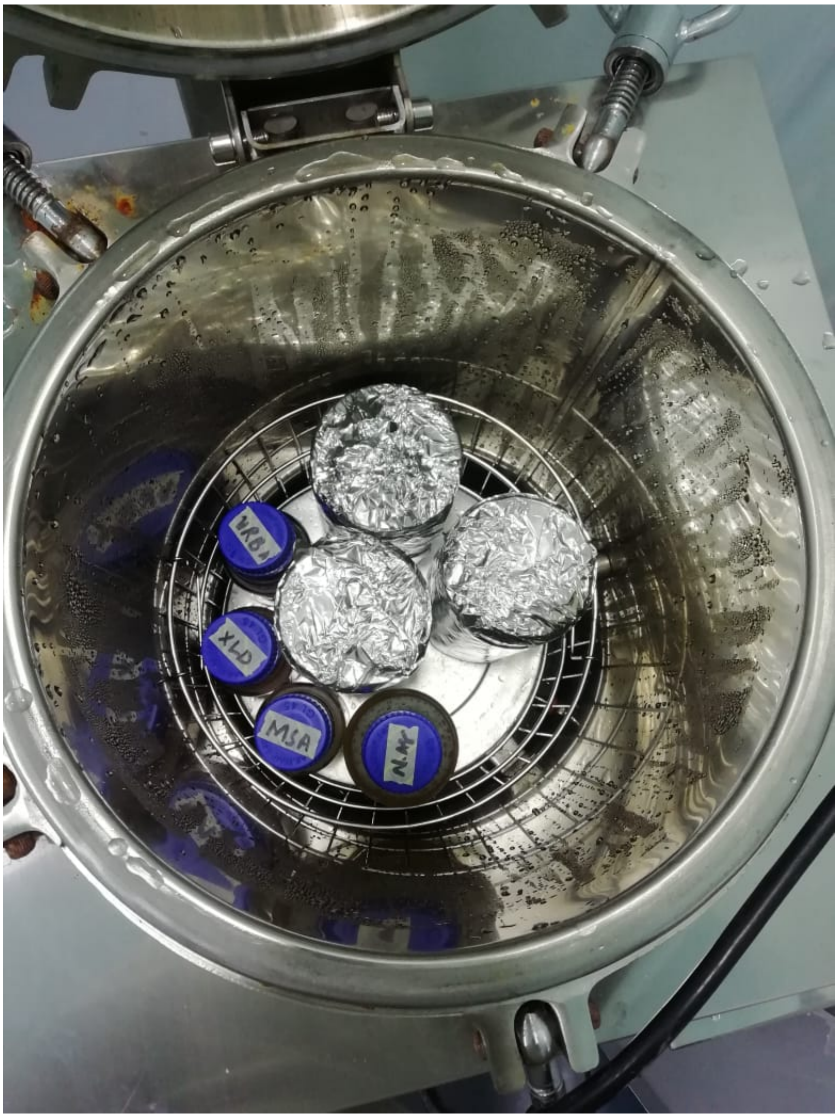

FIGURE 3.2: Sterilization of media by autoclaving

TABLE 3.2: List of disinfectants tested against the isolates

Disinfectant	Concentration tested
Dettol	20%
IPA (Isopropyl Alcohol)	70%
Phenol	5%

FIGURE 3.3: Disinfectants used (Dettol, Phenol and IPA)

3.0.1 List of biochemical tests used

Following biochemical tests were used for the identification of isolates

- Oxidase
- Catalase
- Urease
- Citrate utilization test
- Indole
- H_2S production

3.1 Methodology

Following methodology was adopted for the collection and processing of samples;

3.2 Sample Collection

Samples were collected from different Health Care Establishments [HCEs] of District Jhelum i.e. different wards of District headquarters hospital Jhelum, Tehsil headquarters hospital Sohawa, Tehsil headquarters hospital Sara-eAlamgir, Ahmed hospital Jhelum, Islamabad diagnostic centre and PIMS hospital Islamabad. Samples of solid waste were collected in sterile zipper plastic bags having enough capacity to hold sufficient amount of sample. Total 5 grams of sample was collected from each site. Samples were collected from dustbins of wards, central storage area and incinerator area of hospitals. After collection plastic zippers were labeled with sample number, collection area, date and time of collection. After collection and proper labeling, samples were transported to the microbiology laboratory for further processing. There were 20 samples collected from different healthcare establishments and after sample collection samples were processed according to SOPs [87].

FIGURE 3.4: Sampling site for BMW at DHQ hospital Jhelum

FIGURE 3.5: Sampling site at THQ Sohawa

FIGURE 3.6: Zipper bag for sample collection of BMW

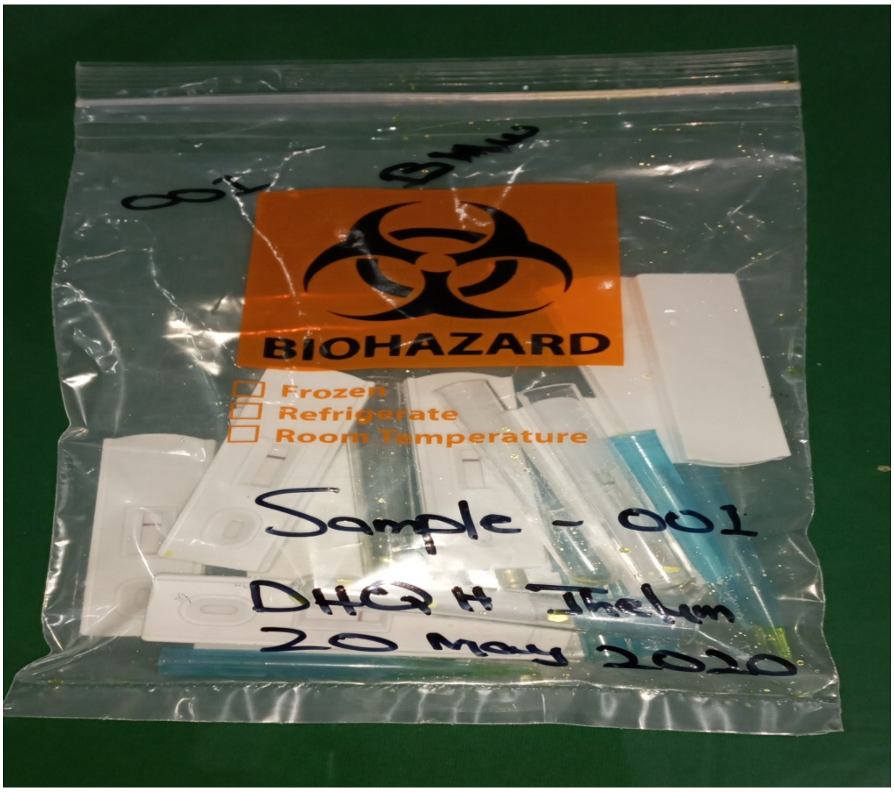

FIGURE 3.7: Sample of BMW collected in a zipper bag

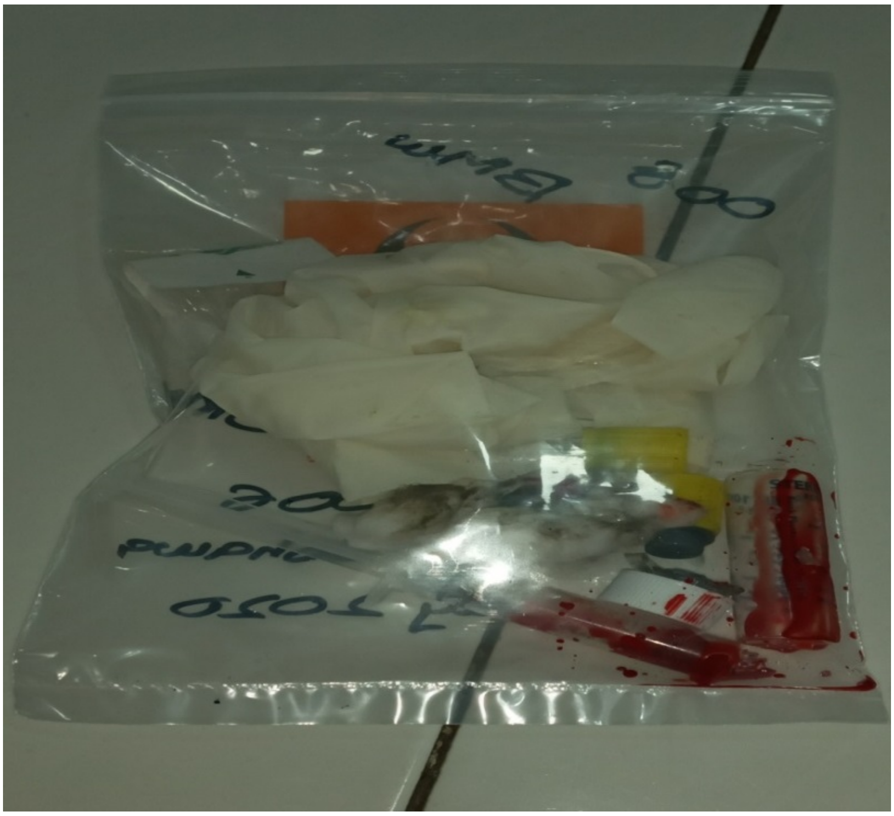

FIGURE 3.8: Sample of BMW collected from PIMS Hospital

3.2.1 Sample Preparation

Following methodology was used for sample preparation for further processing;

1. 5 grams of sample was collected from each site.
2. Out of 5 grams 1 gram sample weighed and processed further.
3. In a screw capped bottle 1 gram of sample was added in 9 ml of sterile distilled water (1:10 dilution) and mixed well for 2-3 minutes.
4. This was called parental dilution and was proceeds further.

Figure 3.9: Distilled water used for sample dilution

3.2.2 Sample Processing

TBC (Total Bacterial Count)

1. Nutrient agar was prepared (as manufacturer's recommendation) in a sterile glass flask and mixed well.
2. Then nutrient agar was autoclaved at 121℃ for 15 minutes at 15 lb psi.
3. After autoclaving media was allowed to cool at 42℃.

4. 1 ml sample (parental dilution) was added in a petri dish of 90 mm.
5. Then 25 ml of already sterile media was added in this plate (pour plate method).
6. Few of the samples were streaked on the nutrient agar with the help of a sterile swab (streak plate method).
7. Then inoculated plates were incubated at 30-35℃ for 48 hours.
8. After incubation plates were observed for CFU (TBC) and colonies were counted with the help of a colony counter and calculated as; CFU/ml= No. of colonies counted* 10.
9. On some plates colonies were TNC (Too numerous to count) then 100 μl sample was added/streaked on the media and colonies were counted.
10. If 100 μl sample was added then colonies were counted as No. of colonies * 10*10. CFU/ml= No. of colonies counted* 10* 10. (ISO certified method)

3.2.3 Bacterial Identification

By culturing on the selective media

1. For the identification of isolated colonies culturing on selective media and biochemical tests were employed.
2. For the isolation and identification of *Salmonella spp.*, 1 ml of parental dilution (1:10) was inoculated on XLD agar.
3. MSA was used for the isolation and identification of *S.aureu*s.
4. For the isolation and identification of *E.coli* VRB and Lactose agar were used.
5. For *P. aeruginosa* Citramide agar was used.

6. To obtain further purified colonies restreaking on the respective selective media was done.

7. For the confirmation of microbes biochemical tests (Oxidase, Catalase, Urease, Citrate utilization test, Indole, H_2S production) were also performed.

3.3 Biochemical Tests for Identification of Isolates

Following biochemical tests were performed.

3.3.1 Oxidase test

FIGURE 3.10: Positive and Negative Oxidase Test

3.3.1.1 Principe

This test is done to check the presence of cytochrome oxidase enzyme in the bacterial cell. This enzyme oxidizes the reduced colorless reagent into an oxidized colored product.

3.3.1.2 Procedure

1. A small piece of filter paper was soaked in Kovacs oxidase reagent and then allowed to dry.

2. An 18 – 24 hour old colony of test organism was rubbed on the filter paper containing Kovacs reagent.

3. Then filter paper was observed for color change (deep purple) within 5-10 seconds.

3.3.1.3 Results

Results of this test were interpreted as;

Oxidase positive: A change in color was observed within 5-10 seconds.
Delayed oxidase positive: A change in color was observed within 60-90 seconds.
Oxidase negative: No color change was observed within 2 minutes [88].

Result should be interpreted cautiously especially between rapid positive and delayed positive organisms.

3.3.1.4 Oxidase Positive Organisms

P.aeruginosa

3.3.2 Catalase Test

3.3.2.1 Principle

This test is used to detect the presence of catalase enzyme in the bacteria. Catalase enzyme breaks down the hydrogen peroxide into oxygen and water. This reaction is evident by formation of bubble.

3.3.2.2 Procedure

1. A microscope slide was placed in a petri dish.

2. A bacterial colony (18-24 hours old) was taken with the help of an inoculating wire loop and placed on the slide.

3. With the help of dropper few drops of 3% H_2O_2 were placed on the colony and observed for any gas bubbles.

3.3.2.3 Result

A positive test is indicated by the formation of clear gas bubbles [89]. A positive result is indicated by formation of clear gas bubbles. Formation of bubbles is means catalse negative.

3.3.2.4 Catalase Positive Organisms

Staphylococcus aureus

3.3.3 Citrate Utilization Test

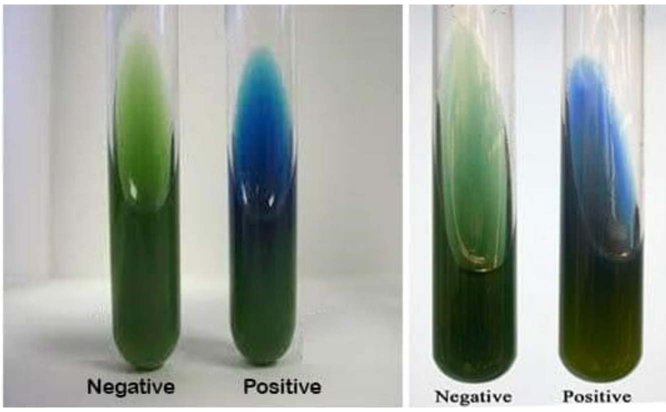

FIGURE 3.11: Citrate Utilization Test

3.3.3.1 Principle

Citrate utilization test is used to identify citrase producing organisms i.e. *Salmonella*. Citrase producing organisms can use citrate as a source of carbon for metabolism. This test is performed on Simmons Citrate agar.

3.3.4 Procedure

1. An 18-24 hour old colony of testing organism was taken and inoculated on the slant of Simmons citrate agar.

2. Incubated media was inoculated for up to 7 days at 35℃ – 37℃.

3. After 7 days observed for any color change from green to blue.

3.3.4.1 Result

Positive result is indicated by change in the color from green to blue [90]. Positive result indicated by the change in the color from Green to blue. No change of color means organism is negative for Citrate Utilization.

3.3.4.2 Citrate Positive Organisms

Salmonella spp.

3.3.5 Urease Test

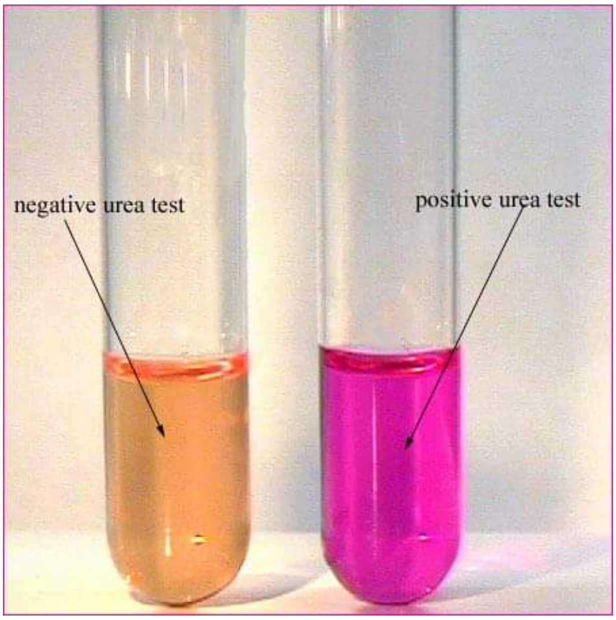

FIGURE 3.12: Positive and Negative Urease Test

3.3.5.1 Principle

Urease enzyme is produced by some bacteria which converts urea into ammonia and carbon dioxide. So this test is used to identify those bacteria which can produce this enzyme.

3.3.5.2 Procedure

1. Stuart's Urea broth was inoculated with a heavy inoculum of 18-24 hours old pure culture of test organism.
2. Tube was shaken gently to suspend the bacteria.
3. Tube was incubated at 35℃ with loosened cap and observed for any color change at 8, 12, 24 and 48 hours.

3.3.5.3 Result

A positive result is indicated by the change of broth color in to the bright pink color [91]. A positive result is indicated by the change of broth color in to the bright pink color. No change of color of broth means organism is utease negative.

3.3.5.4 Urease Positive Organism

Proteus spp.

3.3.6 Indole Test

3.3.6.1 Principle

This test is used to identify bacteria which can convert amino acid tryptophan into indole. This test is used to differentiate between members of Enterobactericiae family.

3.3.6.2 Procedure

1. A colony of test organism was inoculated in the tryptone broth.
2. Then it was incubated at 35℃ for 24-48 hours.
3. After incubation 5 drops of Kovacs reagent were added to the tube.
4. Then tube was observed for any color change.

3.3.6.3 Result

A positive reaction is indicated by formation of pink to red color [92]. Positive result is indicated by formation of pink to red color. While no color change means indole negative organism.

3.3.6.4 Indole Positive Organisms

Escherichia coli

3.3.7 H_2S (Hydrogen Sulfide) Production Test

3.3.7.1 Principle

This test is used to check the microorganism's ability to reduce sulfur (sulfur containing compounds) to hydrogen sulfide. This test is used as a tool for the identification of enterobactericiae.

3.3.7.2 Procedure

1. Organism to be tested was inoculated on the media by stab inoculation.
2. Inoculated tubes were incubated for 24-48 hours at 37℃.
3. Then tubes were observed for the formation of black precipitates.

3.3.7.3 Result

Positive result is indicated by the formation of black color precipitate [93]. Positive result is indicated by formation of black color precipitate. While no formation of black color precipitate means negative result.

3.3.7.4 H_2S Positive Organisms

E.coli spp. and Salmonella spp.

3.4 Reference Strains for Further Confirmation

For further confirmation of isolates reference strains were used along with growth characteristics on selective media, biochemical characteristics

3.5 Disinfectant Efficacy Test

1. This test was performed to check the efficacy of routinely used disinfectants on the bacteria isolated from BMW.

2. Disinfectants that are commonly used in hospital were tested i.e. Dettol, IPA and Phenol were tested.

3. A tile was used as a surface. First tile was autoclaved at 121℃ for 15 minutes at 15 lb PSI.

4. Then surface of the tile was artificially contaminated with organism to be tested i.e. *Salmonella, E.coli, P.aeruginosa* and *S.aureus* in a biosafety cabinet.

5. Then disinfectant to be tested was applied and sampling was done from the surface of tile at 0, 5 and 30 minutes with the help of a sterile swab. A control tile (without treatment) was also used.

6. Swabs were inoculated on selective media and incubated at 35℃ for 48 hours.

7. Colonies were counted and log reduction was determined for treated tile and control tile [94].

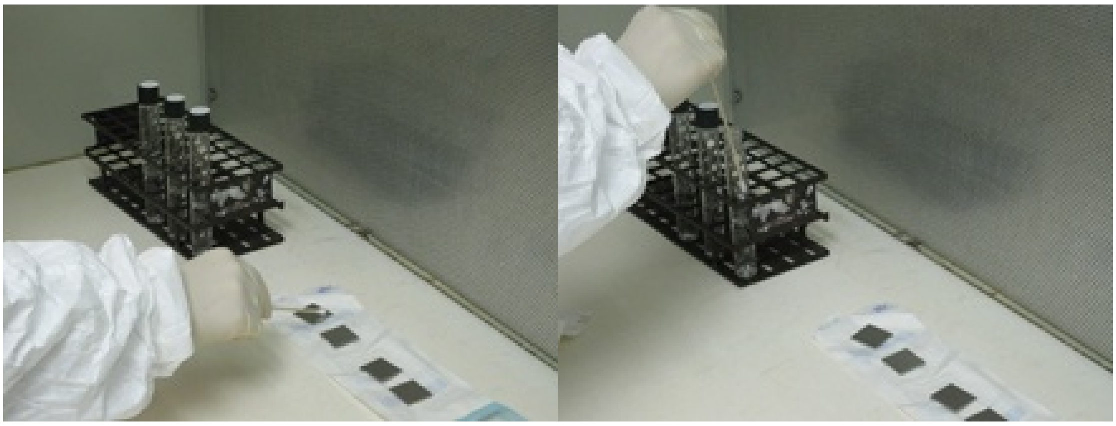

FIGURE 3.13: Surface test for disinfectant efficacy

Chapter 4

Result and Analysis

This study showed that different types of microorganisms were found in the samples of BMW in very high numbers. Among these microorganisms 4 of the major pathogens found in the BMW (Hospital waste) were analyzed. Total 20 samples were collected and processed from different government and private hospitals and different HCEs. 05 samples of solid BMW were collected from different areas of District headquarters hospital Jhelum. 03 samples were collected from Tehsil headquarters hospital Sohawa. 03 samples were collected from different areas of THQ hospital Sara-e-Alamgir. 02 samples were taken from Ahmed hospital Jhelum. 02 samples were collected from Islamabad Diagnostic Centre.05 samples were collected from different areas of PIMS hospital Islamabad. Samples were collected and transported for microbial analysis as soon as possible.

Samples of solid waste were collected and analyzed to identify commonly found pathogens in the BMW so to assess what kind of pathogens are found in the hospital based waste. Efficacy of commonly used disinfectants in the hospital settings against these isolates was also analyzed to assess the role of these disinfectants to prevent BMW borne infections.

All of the 20 samples were analyzed for the presence of Escherichia coli, *S.typhi*, *Staphylococcus aureus, Pseudomonas aeruginosa* and *Proteus spp.* These bacterial pathogens were selected for isolation from BMW on the basis of literature review.

FIGURE 4.1: Sample of BMW collected from THQ hospital Sohawa

Bacteria were isolated from all of the 20 samples with different numbers and types of pathogens.

4.1 Analysis of TBC

All the 20 samples were positive for bacteria. Samples were analyzed for TBC or Total viable count and they showed little variation among the number of TBC. The mean bacterial count that was determined from samples of BMW collected from DHQ hospital Jhelum was 1.8 x 10^5CFU per gram of sample (1.8 x 10^4CFU per ml of sample). Highest TBC or viable bacterial count was shown by samples collected from THQ hospital Sara-e-Alamgir that was 5.5 x 10^5CFU/ gram of sample (5.5 x 10^4CFU/ ml of sample dilution). The lowest TBC or Viable bacterial count was obtained from samples of BMW that were collected from Islamabad Diagnostic

Centre that was 0.9 x 10^5 CFU/ gram of sample (0.9 x 10^4 CFU/ ml of sample dilution).

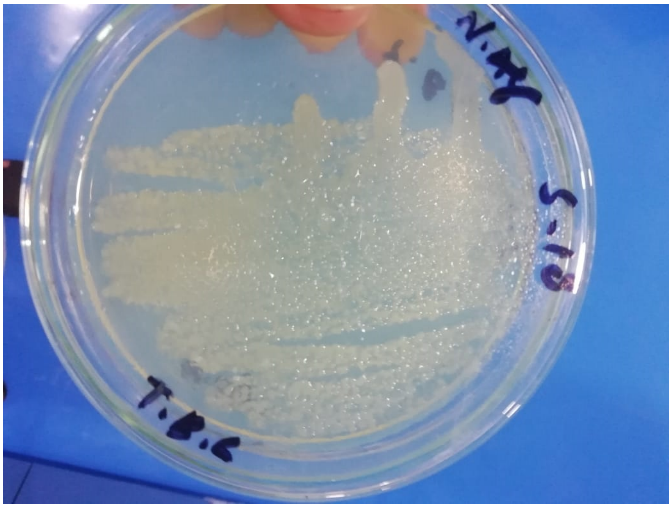

FIGURE 4.2: Growth on nutrient agar for TBC.

The average TBC or mean viable count from all of these 20 samples was 2.43 x 10^5 CFU /gm of sample. Table 4.1 is showing the TBC of samples collected from different hospitals.

TABLE 4.1: Hospital wise total bacterial count (TBC)

HCE	TBC/100 μ/l	TBC/ml	TBC/gram
DHQ Hospital Jhelum	1800 CFU	1.8 x 10^4 CFU	1.8 x 10^5CFU
THQ Hospital Sohawa	2300 CFU	2.4 x 10^4 CFU	2.4 x 10^5CFU
THQ hospital Sara-e-Alamgir	5500 CFU	5.5 x 10^4 CFU	5.5 x 10^5CFU
Ahmed hospital Jhelum	1300 CFU	1.3 x 10^4 CFU	1.3 x 10^5CFU
Islamabad Diagnostic Centre	900 CFU	0.9 x 10^4 CFU	0.9 x 10^5CFU
PIMS Hospital Islamabad	2700 CFU	2.7 x 10^4 CFU	2.7 x 10^5CFU

Samples of BMW from THQ hospital Sara-e-Alamgir showed the highest number of total viable count (5.5 x 10^5CFU/gram of sample). This number was very high than the TBC of samples collected from Islamabad Diagnostic Centre (0.9 x 10^5CFU / gram of sample. PIMS hospital Islamabad was second to THQ hospital Sara-e-Alamgir in terms of TBC which was (2.7 x 10^5CFU / gram of sample). Samples from other hospitals showed variable number of TBC. Samples from THQ Sara-e-Alamgir showed the highest number of TBC. Figure 4.3 is showing the TBC (x 10^5 CFU/gram of sample) from different healthcare facilities.

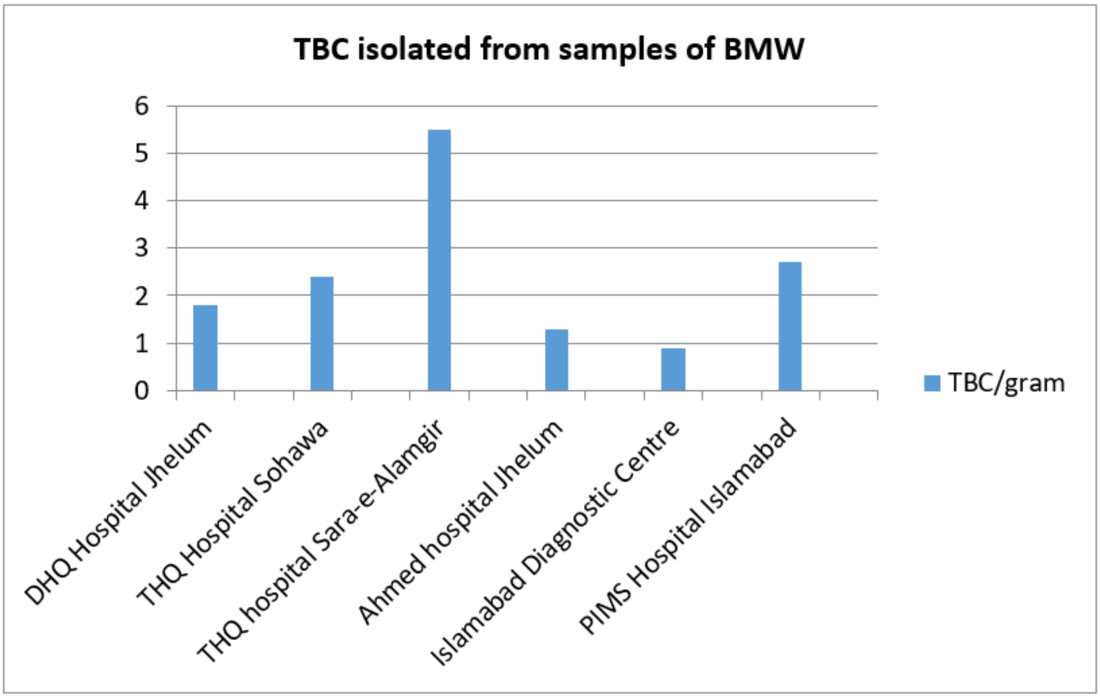

FIGURE 4.3: TBC isolated from different HCEs (x 10^5) CFU/gram of sample

4.2 Prevalence of Isolates in the Samples of BMW

All of the 20 samples were analyzed for the presence of *E.coli, S.typhi*, *P.aeruginosa* and *E.coli*. Out of these 20 samples 13 samples were positive for *E.coli* (65%), 10 samples were positive for *S.aureus* (50%), 8 samples were positive for *P.aeruginosa* (30%) and 5 samples were positive for *S.typhi* (25%). Table 4.2 is showing the occurrence and frequency of isolates

TABLE 4.2: Prevalence of isolates in the samples of BMW

Isolate	Prevalence of isolates	Frequency of prevalence
E.coli	13	65 %
S.aureus	10	50 %
P.aeruginosa	8	30 %
S.typhi	5	25 %

When the samples were analyzed for the presence of *E.coli*, samples from Ahmed hospital Jhelum and THQ Hospital Sohawa showed the highest frequency (100 %) of prevalence of this organism from these sites. While all the samples from Islamabad Diagnostic centre were negative for the presence of *E.coli* (0%). Over all 13 samples out of 20 were positive for the presence of *E.coli*. So the mean prevalence rate of this organism was 13/20 or 65%. 10 samples of BMW out of

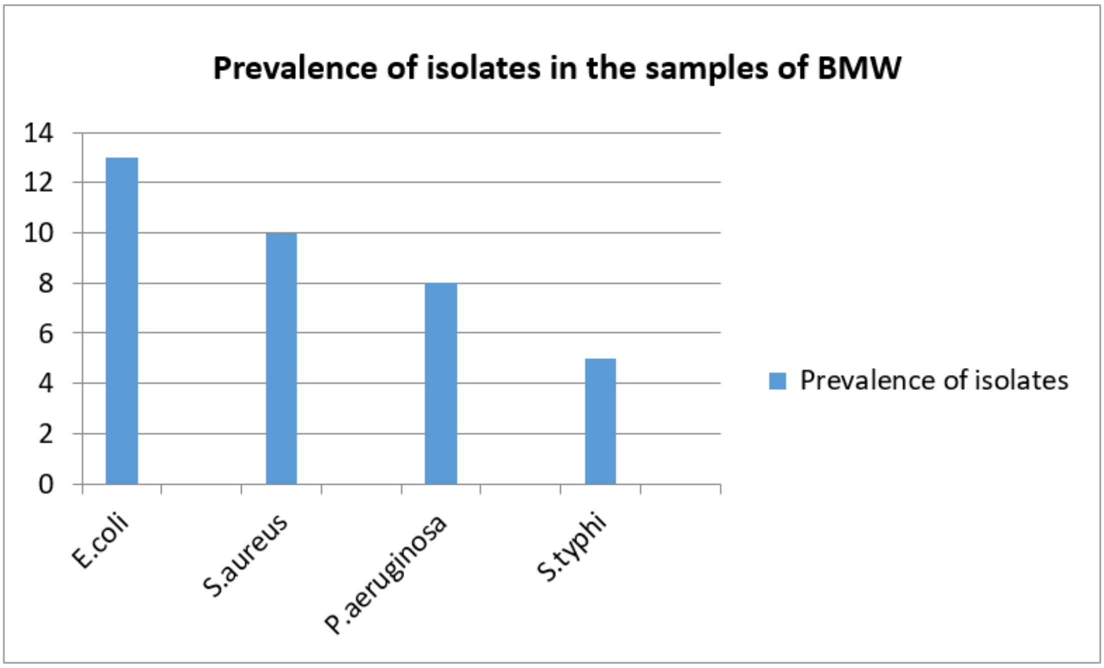

FIGURE 4.4: Prevalence of isolates in the samples of BMW

20 were positive for the presence of *S.aureus* with a mean prevalence rate of 50%. Samples from DHQ hospital Jhelum showed the highest isolation rate (80%) of *S.aureus*, while both the samples from Ahmed hospital Jhelum were negative for

the presence of *S.aureus*. The third most prevalent organism was the *P.aeruginosa* with the average prevalence rate of 8/20 or 30% in the samples of BMW. Both the samples from Ahmed hospital Jhelum were positive for this organism with a prevalence rate of 100%. *S.typhi* was also isolated from the samples in least numbers. 5 samples out of 20 were positive for this organism with a mean prevalence rate of 25%. Samples from three sites (DHQ Hospital Jhelum, Ahmed hospital Jhelum and Islamabad Diagnostic Centre) were negative for this organism. THQ Hospital Sohawa showed the highest prevalence of this organism with 66.66% prevalence.

Table 4.3 below is showing the prevalence rate of different organisms among the samples collected from different HCEs.

TABLE 4.3: Prevalence of isolates in the samples of BMW

Isolate	Prevalence of isolates	Frequency of prevalence
E.coli	13	65 %
S.aureus	10	50 %
P.aeruginosa	8	30 %
S.typhi	5	25 %

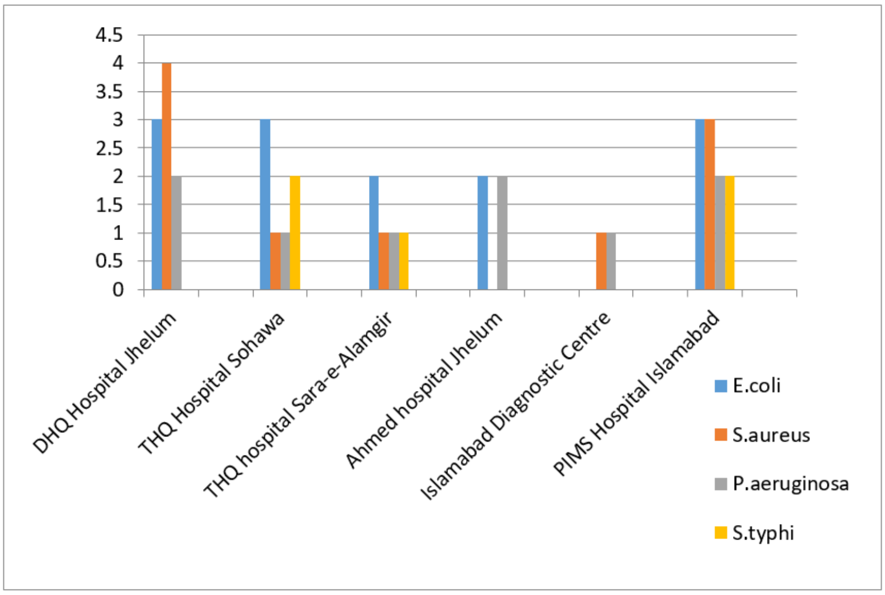

FIGURE 4.5: Prevalence rate of pathogens in the samples of BMW among different HCEs

4.3 Number and Types of Pathogens Isolated from BMW

Samples of BMW were analyzed for the presence of commonly found pathogens in the hospital environment. Samples from different hospital settings showed different number and types of isolates. On average samples from PIMS hospital Islamabad showed the highest number of isolates (22.88 x 10^3) per gram of samples on the other hand samples from Islamabad Diagnostic Centre showed the least number (0.30 x 10^3) of isolates per gram of samples. Samples from PIMS hospital showed the highest number of *E.coli* (16 x 10^3) while samples from 2 samples from Islamabad Diagnostic Centre showed no isolation of *E.coli*. The average number of *E.coli* isolation from all these hospitals per gram of BMW was 4.78 x 10^3 organisms.

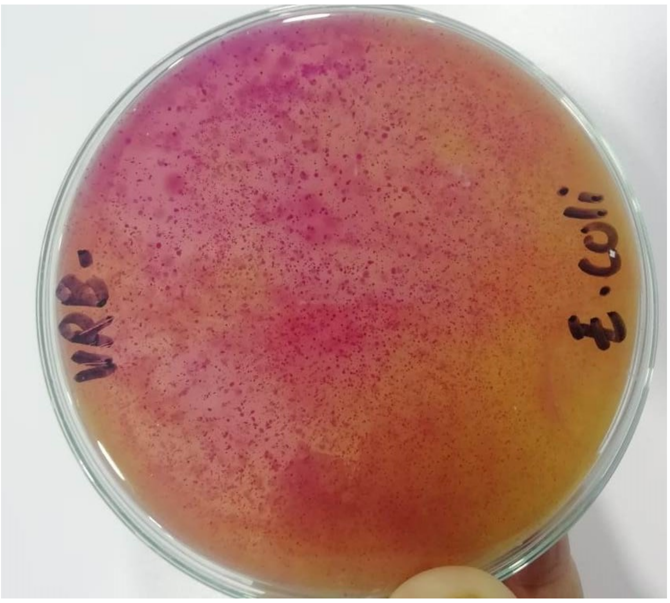

FIGURE 4.6: Colonies of *E.coli* on VRB agar

The highest number of *S.aureus* / grams of sample were isolated from samples collected from DHQ hospital Jhelum (8.7x 10^3). While no *S.aureus* was isolated from the samples of BMW collected from Ahmed hospital Jhelum. The average number of isolation of *S.aureus* per gram of BMW samples was 2.7 x 10^3 organisms. This number was well below than the isolation rate of *E.coli* from these samples.

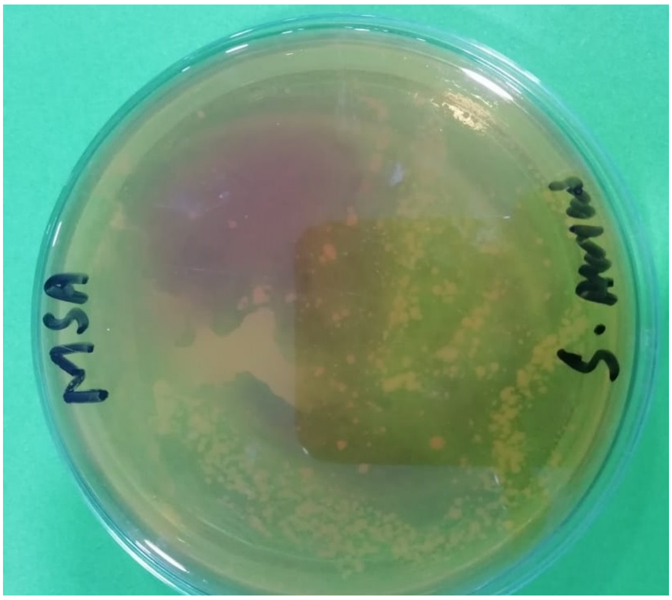

FIGURE 4.7: Colonies of *S.aureus* on the MSA agar

Samples were also analyzed for the presence and number of *P.aeruginosa* . The number of isolates was analyzed per gram of samples. The highest load of *P. aeruginosa* per gram of samples was isolated from THQ hospital Sohawa (0.9 x 10^3). While the least number of this organism isolated from Islamabad Diagnostic Centre (0.14 x 10^3). The average isolation rate of this organism per gram of sample was 0.14 x 10^3. This number was well below than the average number of *E.coli* and *S.aureus* isolated from the same samples.

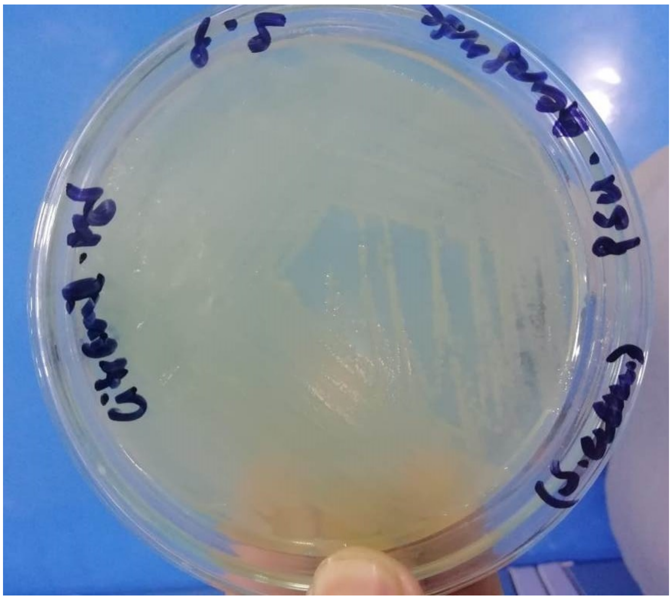

FIGURE 4.8: Colonies of *P.aeruginosa* on Citramide agar

The fourth organism which was isolated and characterized from BMW was *S.typhi* but the frequency of this organism was very low than the other isolates. *S.typhi* was not isolated from the samples of BMW collected from DHQ hospital Jhelum, Ahmed Hospital Jhelum and Islamabad Diagnostic Centre.

This organism was isolated from three other hospitals in very low frequencies. The highest frequency of this organism was isolated from samples of BMW obtained from PIMS hospital Islamabad (0.27 x 10^3). The average number of this organism from all the settings was 0.10 x 10^3.

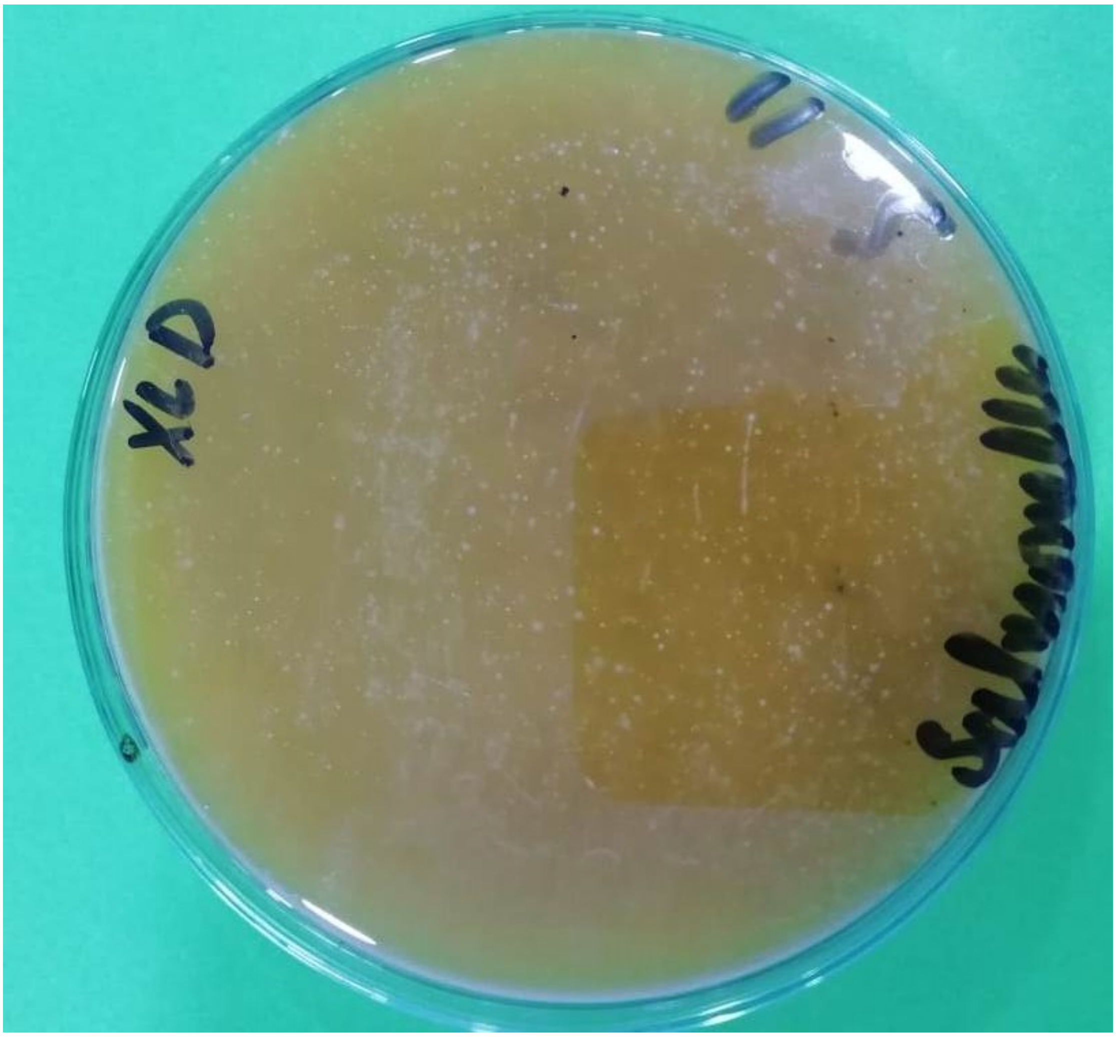

Figure 4.9: Colonies of Salmonella on XLD agar

Table 4.4 is showing the types and number of different organisms isolated from BMW per gram of samples.

Table 4.4: Number and types of isolates / gram of BMW

HCE	*E.coli*	*S.aureus*	*P.aeruginosa*	*S.typhi*	Total
DHQ Hospital Jhelum	2.3x 10^3	8.7 x 10^3	0.67 x 10^3	0.0 x 10^3	11.04 x 10^3
THQ Hospital Sohawa	3.7x 10^3	0.94 x 10^3	0.9 x 10^3	0.21 x 10^3	5.95 x 10^3
THQ hospital Sara-e-Alamgir	6.4x 10^3	0.31 x 10^3	0.35 x 10^3	0.16 x 10^3	3.02 x 10^3
Ahmed hospital Jhelum	0.31x 10^3	0.0 x 10^3	0.62 x 10^3	0.0 x 10^3	0.93 x 10^3
Islamabad Diagnostic Centre	0.0x 10^3	0.16 x 10^3	0.14 x 10^3	0.0 x 10^3	0.30 x 10^3
PIMS Hospital Islamabad	16.0 x 10^3	6.5 x 10^3	0.31 x 10^3	0.27 x 10^3	22.88 x 10^3
Total	28.71 x 10^3	16.61x 10^3	2.99 x 10^3	0.64 x 10^3	48.95 x 10^3

The results of this study showed that *E.coli* was the predominant pathogen in the BMW. The average number of this organism per grams of sample was 4.78 x 10^3. This number was much higher than the other organisms. While the isolation rate of *S.typhi* was very low i.e. average number per grams was 0.10 x 10^3. The other 2 organisms were found in variable numbers. On the other hand samples from PIMS hospital Islamabad showed the highest load of pathogens under study. The total number of pathogens was much higher than the other hospitals. On the other hand least number of these pathogens was found in the samples from Islamabad Diagnostic centre.

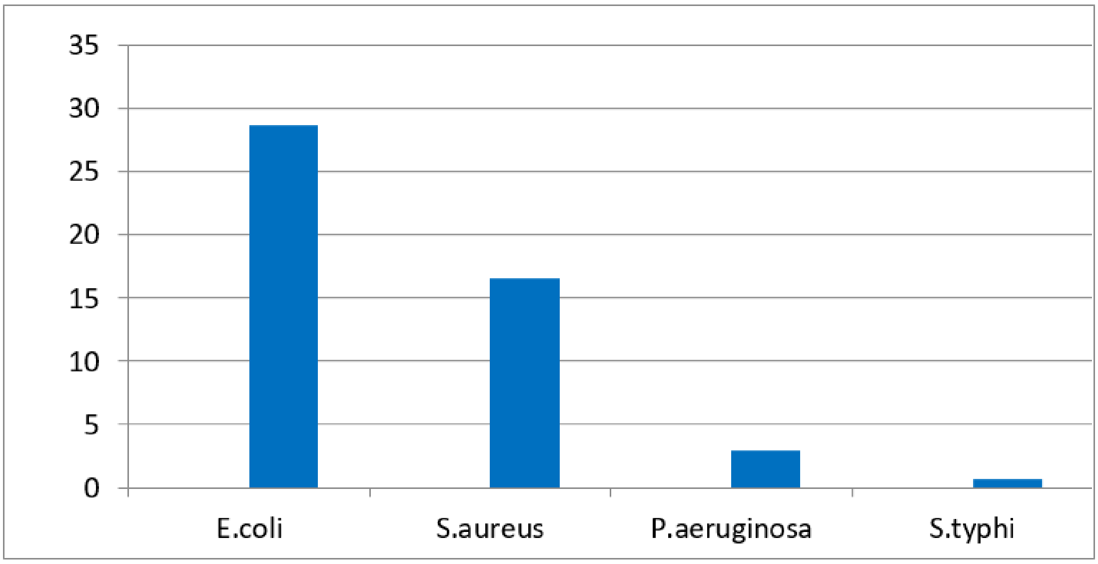

FIGURE 4.10: Number and types of isolates (x 10^3)/ Gram of sample From all HCEs

4.4 Morphological and Biochemical Characteristics of Isolates

Biochemical tests that were performed for the characterization and identification of isolates revealed that both Gram positive and Gram negative organisms were present in the BMW. Most of the isolates were rod shaped (*E.coli*, *S.typhi*, *P.aeruginosa*) while few of them were cocci (*E.coli*). On the basis of oxygen requirement some isolates were obligate aerobe (*P.aeruginosa*) while others were Facultative anaerobe.

The results of biochemical tests were also varying organism to organism. Colony morphology on selective media was also used for differentiation and identification. Colony morphology as size, shape, color and consistency was different from organism to organism i.e. colony of *S.aureus* was yellow where as colony of *E.coli* was pink on lactose agar. Table 4.5 is showing the complete description of biochemical and growth characteristics of isolates.

Table 4.5: Biochemical characteristics of isolated organisms

Test	*S.aureus*	*E.coli*
Shape	Cocci	Rod
Spores	No	No
Motility	Non-Motile	Motile with peritrichous flagella
Oxygen requirement	F. anaerobe	F. anaerobe
Selective media	Mannitol Salt agar	Violet Red Bile agar Lactose agar
Colony	Yellow colonies on Mannitol Salt agar	Red/Purple fluorescent colonies on VRB agar
Gram stain	Positive	Negative
Oxidase	Negative	Negative
Catalase	Positive	Positive
Urease	Positive	Negative
Citrate utilization test	Positive	Negative
Indole	Negative	Positive
H2S production test	Negative	Negative
Any other special feature	Beta hemolysis on blood agar Coagulase positive	Being lactose fermenter so produce pink colonies on MacConkey agar
Test	***P.aeruginosa***	***S.typhi***
Shape	Rod	Rod
Spores	No	No
Motility	Motile with polar flagella	Motile with peritrichous flagella
Oxygen requirement	Obligate aerobe	Aerobe

Selective media	Citramide agar	Xylose Lysine Deoxycholate agar
Colony	Bright green colonies on Citramide agar	Red colonies on XLD agar
Gram stain	Negative	Negative
Oxidase	Positive	Negative
Catalase	Positive	Positive
Urease	Negative	Negative
Citrate utilization test	Negative	Negative
Indole	Negative	Negative
H2S production test	Negative	Positive
Any other special feature	Produce different water soluble pigments i.e. Pyocyanin	

4.5 Disinfectants Efficacy Test Against Isolates of BMW

Three of the most commonly used disinfectants (Dettol, IPA and Phenol) were tested against the isolates to check their efficacy against these isolates. Isolates were exposed to recommended concentrations of disinfectants for specific time and samples were swabbed after specific time intervals as recommended by ISO. 0.01 ml of microbial culture was placed on the surface of coupons and then disinfectant was applied. Immediately after treatment time elapsed test surfaces were placed in a solution to neutralize the action of disinfectant. Log reduction in CFU was determined for treated coupons. At least 3 log CFU reduction was considered for bactericidal activity. Suspension of test organism was prepared in tryptic soy broth with a cell density of almost 1 x 10^6 CFU / ml.

4.5.1 Efficacy of Disinfectants Tested Against *E.coli*

Efficacy of same disinfectants was also checked against *E.coli* isolated from BMW. There was 2 logs reduction after 0 minutes of exposure against Dettol. There was no growth of *E.coli* after 15 minutes of exposure. There was 2 logs reduction in CFU after 0 minutes of exposure against IPA and 4 logs reduction after 5 minutes of exposure. There were only <10 organisms left after 15 minutes of exposure and no growth was observed in the samples taken after 30 minutes of exposure. Effectiveness of Phenol (5%) was also checked against the isolated *E.coli*. There was 1 logs reduction in CFU after 0 minutes of exposure and 3 logs reduction after 5 minutes of exposure to phenol. There was no *E.coli* after 30 minutes of exposure to phenol.

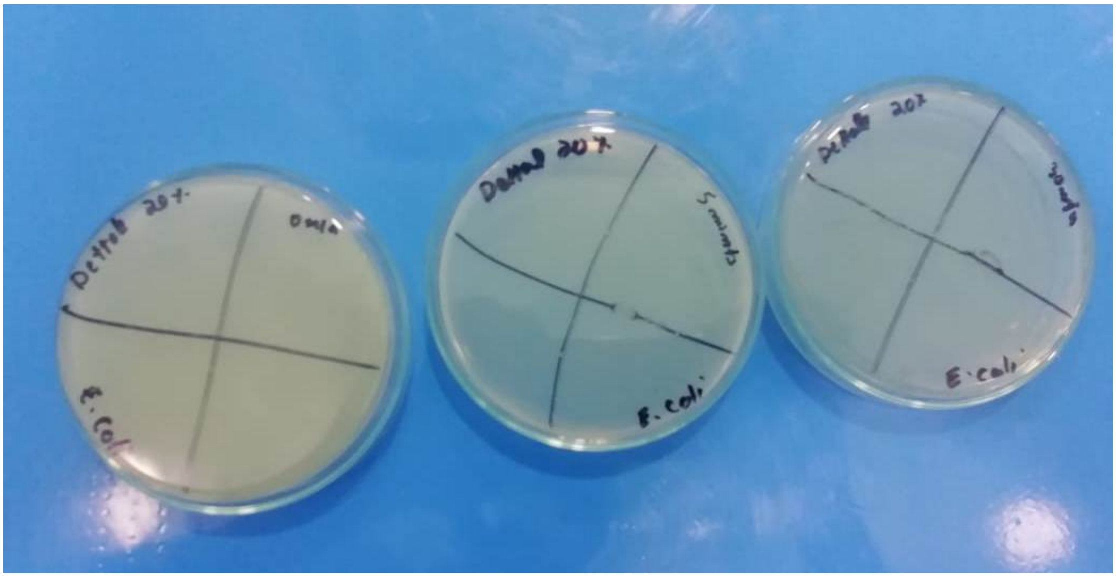

FIGURE 4.11: Culture media inoculated with *E.coli* after 0, 5 and 30 minutes of contact with Dettol

Dettol was the most effective disinfectant against *E.coli*. There was no CFU of *E.coli* after 15 minutes of exposure to Dettol. Table 4.6 is showing the results of efficacy of disinfectants against *E.coli*.

TABLE 4.6: Efficacy of disinfectants against *E.coli*

S.no	Disinfectant	0 min	5 min	15 min	30 min
1	Dettol (20%)				
	Recovery (CFU)	3.2 x 10^4	2.4x 10^2	0	0
2	IPA (70%)				
	Recovery (CFU)	5.5 x 10^4	6.9x 10^2	10	0
3	Phenol (5%)				
	Recovery (CFU)	2.4 x 105	1.8 x 10^3	0.83 x 10^2	0

4.5.2 Efficacy of Disinfectants Tested Against *S.aureus*

Dettol (20%) was tested against *S.aureus* isolated from BMW for 0, 5, 10 and 30 minutes. At 0 minute there was a 2 logs reduction in the number of *S.aureus*, at 5 minutes exposure there was 4 logs reduction and after 15 minutes all the organisms were killed completely. The second disinfectant was IPA (70%) which showed 1 log reduction after 0 minute exposure and 3 logs reduction at 5 minutes and there were very few organisms were left after 15 minutes exposure and no growth after 30 minutes.

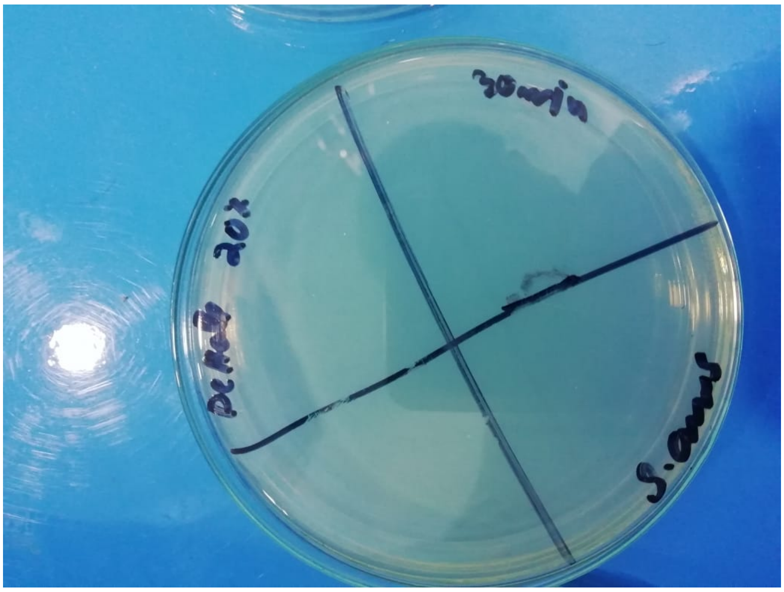

FIGURE 4.12: Culture media inoculated with *S.aureus* after 30 minutes of contact with Dettol

Phenol (5%) was also tested there was 1 log. reduction at 0 minute and 3 log. reduction at 5 minutes. There was no growth after 30 minutes of exposure. Table 4.7 below is showing the results of Dettol, IPA and Phenol tested against *E.coli*.

TABLE 4.7: Efficacy of disinfectants against *S.aureus*

S.no	Disinfectant	0 min	5 min	15 min	30 min
1	Dettol (20%)				
	Recovery (CFU)	2.8×10^4	4.7×10^2	0	0
2	IPA (70%)				
	Recovery (CFU)	1.6×10^5	8.1×10^3	34	0
3	Phenol (5%)				
	Recovery (CFU)	6.5×10^5	2.5×10^3	0.9×10^2	0

4.5.3 Efficacy of Disinfectants Tested Against *P.aeruginosa*

Third isolate from BMW was *P.aeruginosa* . Efficacy of these disinfectants against *P.aeruginosa*was also assessed. Dettol showed more effectiveness against these isolates as there was only <7 CFU after 15 minutes of contact with this disinfectant.

IPA showed almost same effectiveness as Dettol but there was 61 CFU after 15 minutes of contact and no growth at all after 30 minutes of contacts.

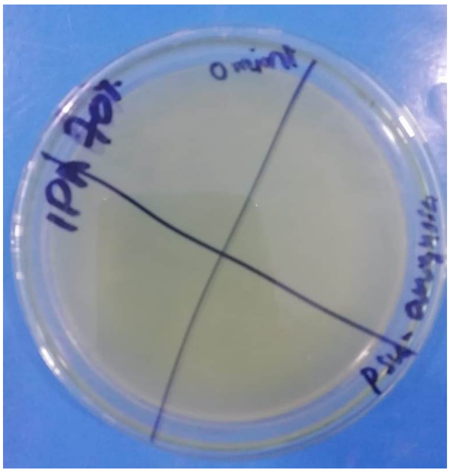

FIGURE 4.13: Media inoculated with *P.aeruginosa* after 0 minutes of contact with IPA

Phenol was the least effective as there was 2 logs decrease in CFU after 0 minutes of contact and 3 logs decrease after 5 minutes of contact. There were <12 CFU after 30 minutes of contact. So some organisms resisted phenol even after 30 minutes of contact.

Table 4.8 is showing the results of efficacy of Dettol, IPA and Phenol tested against *P.aeruginosa* .

TABLE 4.8: Efficacy of disinfectants against *P.aeruginosa*

S.no	Disinfectant	0 min	5 min	15 min	30 min
1	Dettol (20%)				
	Recovery (CFU)	3.4 x 10^4	2.6x 10^2	7	0
2	IPA (70%)				
	Recovery (CFU)	2.8x 105	6.9x 10^3	61	0
3	Phenol (5%)				
	Recovery (CFU)	7.1 x 105	4.7 x 10^3	2.2 x 10^2	12

4.5.4 Efficacy of Disinfectants Tested Against *S.typhi*

S.typhi showed least resistance against all of three disinfectants. There was 3 logs reduction in CFU after 0 minutes of exposure to Dettol and there was 4 logs reduction after 5 minutes of exposure and no CFU after 15 minutes of exposure.

IPA was also tested against *S.typhi*. There was 2 logs reduction in CFU after 0 minutes of exposure and 4 logs reduction after 5 minutes of exposure and no CFU after 15 minutes of exposure. In case of phenol there was 2 logs reduction in CFU after 0 minutes contact and 4 logs reduction after 15 minutes of exposure. There were only 8 CFU after 15 minutes contact with phenol and no CFU after 30 minutes of contact.

Table 4.9 is showing results of efficacy of Dettol, IPA and phenol against *S.typhi*

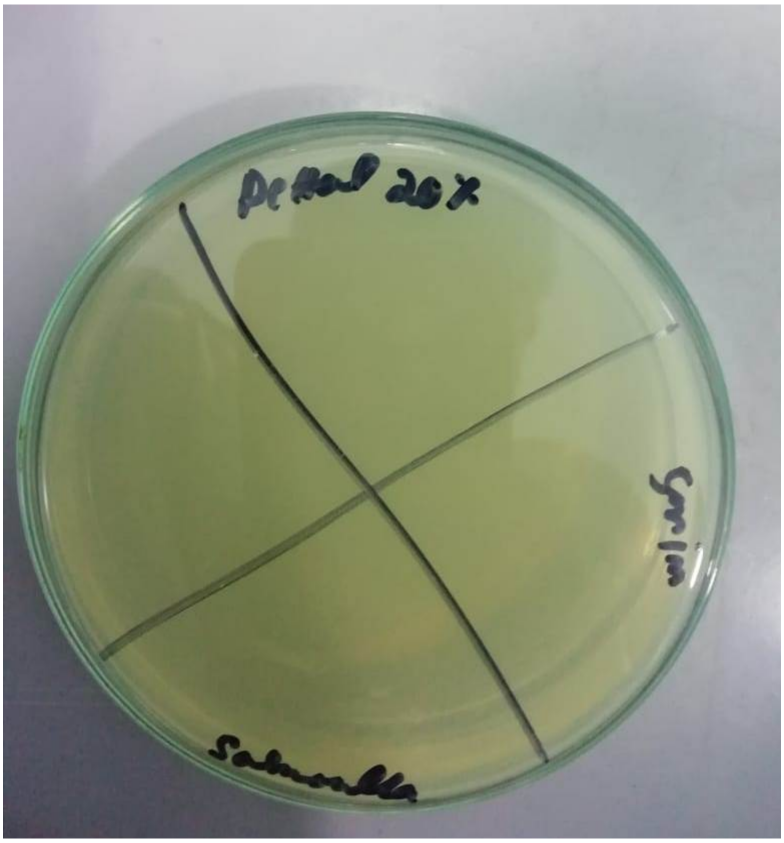

FIGURE 4.14: Culture media inoculated with *S.typhi* after 5 minutes of contact with Dettol

TABLE 4.9: Efficacy of disinfectants against *S.typhi*

S.no	Disinfectant	0 min	5 min	15 min	30 min
1	Dettol (20%)				
	Recovery (CFU)	6.2 x 10^3	0.9 x 10^2	0.0	0
2	IPA (70%)				
	Recovery (CFU)	4.8x 10^4	2.8 x 10^2	0.0	0
3	Phenol (5%)				
	Recovery (CFU)	6.7 x 10^4	6.9 x 10.	8	12

4.6 Discussion

BMW (Biomedical waste) is generated in big quantities in different healthcare facilities of Pakistan but the way it is handled is not very safe.

Very small proportion of this waste is hazardous (infectious) <15% but improper segregation of infectious and non-infectious waste leads to the contamination of all the waste. Proper segregation and handling of BMW is important to protect healthcare staff, persons handling the waste and also the environment.

This study concluded that different kinds of pathogens were found in the BMW collected from different healthcare facilities. Samples of BMW were analyzed for the presence of 4 commonly found pathogens (*S.aureus*, *E.coli*, *P.aeruginosa* and *S.typhi*). All the samples were positive for bacteria. As Cheremisinoff and Shah found that medical waste contains number of infectious agents so it is important to dispose of this kind of waste properly [95].

4.6.1 Total Bacterial Count (CFU)

Samples from THQ Sara-e-Alamgir showed the highest burden of bacteria i.e. 5.5 x 10^5 CFU/gram of sample and samples from Islamabad Diagnostic Centre showed the lowest burden of bacteria i.e. 0.9x 10^5 CFU/gram of sample. There was a difference between the number of viable count or TBC among different HCEs due to the different reasons i.e.

1. Staff of Islamabad Diagnostic Centre was well aware and trained about the handling and management of BMW while on the other hand staff from THQ Sara-e-Alamgir and other government hospitals was unaware about the handling of BMW.

   Segregation of waste was a big problem as in these hospitals there was no proper segregation of infectious and non-infectious waste so non-infectious waste was also contaminated from infectious waste making it difficult to handle such big amount of infectious waste to handle. This finding was same as a study done in the Karachi, Pakistan by Sultana Habibullah and Salahuddin Afsar showed that sanitary workers from most of the hospitals were unaware about the proper disposal of healthcare waste and 71.4% healthcare facilities which were included in the study were disposing off their waste in the public dustbins [96].

2. Lack of resources was also a big problem in the government hospitals.

3. Dustbins were emptied after every shift in the private settings, while after a day or two in the government hospitals (THQ Sara-e-Alamgir) so bacteria multiply and their number was increased in the BMW in the government HCEs. So the number of bacteria (CFU) was more in these settings.

4. Infectious waste was decontaminated with disinfectant before disposing of in the Islamabad Diagnostic Centre so the number of CFU was less in the samples of BMW. While BMW was not decontaminated properly before disposing of in most of the government hospital settings so this was also a reason behind increase number of CFU in the samples from government hospitals.

4.6.2 Prevalence of Pathogens in the BMW

This study revealed that *E.coli* was the most common pathogen in the BMW. Out of total 20 samples 13 samples (65%) were positive for this pathogen. *S.aureus* was found in 10 samples out of 20 samples (50%) making it second most common pathogen found in the BMW samples. *P.aeruginosa* was found in 8 samples out of 20 (30%). *S.typhi* was found in just 5 samples out of 20 samples (25%) of

BMW. *E.coli* was the most common pathogen isolated from the samples of BMW followed by *S.aureus* which was same as found by Anitha et al, who reported that *E.coli* was the most prevalent organism isolated from samples of BMW [97]. This finding was in variance with the study of Giroletti who reported that Bacillus subtilis was more prevalent in the BMW than other organisms and *E.coli* was found in low numbers [98].

These findings were might be observed because these 4 isolates have the high ability to resist bad environmental conditions. These bacteria protect themselves from bad environmental conditions by any one of these three methods as; Thick cell wall, release of chemicals which oxidize and reduce toxic substances which may harm the cell and ability of cells to keep food inside. These three methods help the bacteria to survive bad environmental conditions and resist chemicals i.e. disinfectants which may harm the cell.

E.coli was most prevalent organism found in the samples of BMW from different healthcare facilities this was also in agreement with the study conducted in India that found a high prevalence of *E.coli* (22.9%) in the samples of BMW followed by Klebsiella sp., Proteus vulgaris and Citrobacter sp[99].

S.aureus was the second most prevalent bacteria in the BMW after *E.coli*. 10/20 (50%) samples were positive for this organism. These findings were in agreement with the research findings of Rachael Ngozi Osagie et al. who reported that *E.coli* was the most prevalent organism in the BMW (39%) followed by *S.aureus* (32%) and S.pyogenes was at third with a prevalence rate less than *E.coli* and *S.aureus* (15%) [100].

4.6.3 Pathogenesis of Isolates

Both Gram positive and Gram negative organisms were isolated from BMW, some were non motile while others were motile. The organisms which were isolated from BMW are responsible for the causation of many diseases including nosocomial infections. *E.coli* is a Gram negative rod commonly found in the gut as normal flora. Most of the time they are harmless but sometimes causes serious food poisoning, UTI and meningitis.

S.aureus is Gram positive cocci that normally resides upper respiratory tract and skin of human hosts. Sometimes it causes bacteremia, infective endocarditis, soft tissue abscesses and pneumonia. *P.aeruginosa* is a Gram negative aerobic rod. It is associated with most of the hospital acquired infections and sepsis. *S.typhi* is a Gram negative rod which causes systemic infections i.e. typhoid fever [101].

4.6.4 Disinfectant Efficacy Against Isolated Bacteria

3 commonly used disinfectants (Dettol, IPA and Phenol) were tested against isolated pathogens. Dettol was the most effective disinfectant against *E.coli.* There was 6 logs reduction in the CFU of *E.coli* in <15 minutes of contact with Dettol. Two other disinfectants IPA and Phenol also showed bactericidal effect but less effective than Dettol. Phenol was least effective against *E.coli.* But all three disinfectants reduce 6 logs CFU of *E.coli* after 30 minutes of contact. Dettol was most effective against *S.aureus* there was 6 logs reduction in the number of *S.aureus* in <15 minutes of contact with this disinfectant. The two other disinfectants also showed 6 logs reduction in CFU after 15 minutes but <30 minutes of contact time. When same disinfectants were tested against *P.aeruginosa* Dettol showed 4 logs reduction within 5 minutes of contact, while only 7 organisms were left after 15 minutes of contact and no CFU was isolated after 30 minutes contact time. IPA showed 4 logs reduction in CFU after 5 minutes of contact and there was no growth after 30 minutes of contact with IPA.Some *P.aeruginosa* (12) were left even after 30 minutes of exposure to phenol.

All the three disinfectants showed almost similar effectiveness against *S.typhi* after 5 minutes of contact (4 logs reduction in CFU) and 6 logs reduction after 15 minutes of contact. Only few organisms were left after 15 minutes of contact with phenol. Over all Dettol was most effective against all the bacteria isolated from BMW.

Dettol showed 6 logs reduction in CFU of *E.coli*, *S.aureus*, *P.aeruginosa* and *S.typhi* in <15 minutes of contact. While on the other hand phenol was least effective against these isolates. IPA showed intermediate effectiveness between Dettol and Phenol. Phenol was least effective against P.aeruginosa. There were 12 organisms left even after 30 minutes of contact with phenol. *S.typhi* was most sensitive to all three disinfectants among all the isolates from BMW.

Dettol was the most effective disinfectant against *E.coli*, *S.aureus*, *P.aeruginosa* and *S.typhi* as compared to IPA and phenol this was in contrast to the findings of

Mannur Sharada et al. who found that Dettol was 90%,Savlon was 80.95% effective against *E.coli*, *S.aureus* and *P.aeruginosa* as compared to phenol. So according to their findings phenol was more effective as disinfectant than Dettol. In the same study they also concluded that zone of inhibition (ZOI) of Savlon against *S.aureus* and *E.coli* was 33 and 34 mm respectively and against Dettol was 38 mm and 37 mm respectively. The zone of inhibition of these disinfectants against *P.aeruginosa* was 24 mm and 30 mm against Savlon and Dettol respectively. So *P.aeruginosa* was less sensitive against these disinfectants [102].

In another study Chioma C. Okore et al, found that the zone of inhibition (ZOI) of Izal and Z-germicide against *E.coli* was 17 mm and 19 mm respectively while zone of inhibition of Dettol against *E.coli* was 21 mm. So Dettol was more effective against *E.coli* and phenol coefficient of Dettol against *E.coli* was 6.25 (Which means Dettol was 6.25 times more effective than phenol). Dettol was also more effective against *S.aureus* and phenol coefficient of Dettol against this organism was 6.25 [103].

In another study conducted by Raut Gargi et al, stated that when Dettol was tested against *E.coli*, *S.aureus* and *S.typhi* it showed a phenol coefficient of 9, 8 and 10 respectively and when efficacy of hydrogen peroxide was tested against these organisms it showed a phenol coefficient of 5, 5 and 2 respectively. So Dettol was more efficient against these organisms than phenol and hydrogen peroxide [104].

Disinfectant is a chemical agent which has the ability to kill microorganisms which can cause disease except bacterial spores. Disinfectants kill microorganism by many ways i.e. by damaging proteins, damaging nucleic acid and interfering with metabolism. A good quality disinfectant must be cheap, rapid in action, has residual activity, non toxic and active in the presence of organic matter Dettol has both bactericidal and bacteriostatic action depends upon its concentration. It is effective against both Gram positive and Gram negative organisms. Dettol kills microorganisms by disrupting the cell membrane. IPA is both bactericidal and bacteriostatic depending upon its concentration. It is non sporicidal. Phenol is the most commonly used disinfectant especially in the developing countries of the world because it is inexpensive. Phenol is bactericidal, tuberculocidal and fungicidal [105].

Chapter 5

Conclusions and Recommendations

BMW is generated in large quantities in the HCEs as a result of diagnosis, prognosis, treatment and vaccination of patients. Proper management of BMW is very critical to reduce the risks associated with it. Segregation of waste is the first and most important step in the management of BMW. A wide number and types of bacteria found in the BMW which may cause disease and spread of antibiotic resistance in the environment. *E.coli* was the most predominant bacteria found in the BMW while other organisms i.e. S.aureus, *P.aeruginosa* and *S.typhi* were at 2nd, 3rd and 4th respectively. These bacterial species are associated with hospital acquired infections. Efficacy of routinely used disinfectants was also checked against these isolates of BMW. Dettol was found to be most effective against all of these isolates. Contact time of at least 15 minutes was required to kill all the bacteria. Phenol was found to be least effective against all of the isolates. Phenol was least effective against *P.aeruginosa* therewere few pathogens left even after 40 minutes of contact with phenol. *S.typhi* wasfound to be least resistant against all of these disinfectants.

BMW should be handled carefully to reduce the risks associated with it. There should be proper segregation of infectious waste from non-infectious waste for the proper management of BMW. There should be proper training of the healthcare staff for the safe handling and management of BMW.

BMW should be stored, transported and disposed of properly in a close environment. Disinfectants should be used for long enough time to eradicate all the bacteria. Short contact time of disinfectants with bacterial organisms is responsible for the development of resistance against these disinfectants. Efficacy of disinfectants used in the hospital settings should be checked time to time to evaluate development of resistance against them.

Bibliography

[1]. Caltivelli, E., In medical waste treatment Ispracourses. Waste Treatment Manage, Journal of Medical Society, 1990. 1973: 10(16): p. 1-12.

[2]. Need of Biomedical Waste Management System in Hospitals - An Emerging issue - A Review. Current World Environment Journal of Virology, 19(20): p. 117-124.

[3]. Aggarwal, H. and P. Kumar, Need for biomedical waste management. Journal of Medical Society, 2015. 29(1): p. 58-58.

[4]. Abdulla, F., H. Abu Qdais, and A. Rabi, Site investigation on medical waste management practices in northern Jordan. Waste Manag, The Scientific World Journal, 2008. 28(2): p. 450-8.

[5]. Mishra, K., A. Sharma, and A.S. Sarita, A study: biomedical waste management in India. IOSR Journal of Environment Science, Technology and Food Technology , 2016. 10(5): p. 64-67.

[6]. Sarsour, A., et al., Assessment of medical waste management within selected hospitals in Gaza strip Palestine: A pilot study. International Journal of Scientific Research in Environmental Sciences, 2014. 2(5): p. 164.

[7]. Ghasemi, M.K. and R.B. Yusuff, Advantages and Disadvantages of Healthcare Waste Treatment and Disposal Alternatives: Malaysian Scenario. Polish Journal of Environmental Studies, 2016. 25(1): Pp. 10-44.

[8]. MISHRA, S., et al., Chapter eleven solid waste management in mumbai metropolitan region (mmr): current practices and future opportunities. The urban environmental crisis in india: new initiatives in safe water and waste management, Health and Human Rights Journal, 2017. 3(6) p. 163-165.

[9]. Al-Fatimi, M., et al., Biological activities of selected basidiomycetes from Yemen. Pharmazie, Journal of health affairs, 2013. 68(3): p. 221-6.

[10]. Gardner, J.F. and M.M. Peel, Introduction to sterilization and disinfection. 1986: Churchill Livingstone. 3(2): pp. 14-39.

[11]. Hoeltge, G.A., Managing hazardous waste in the clinical laboratory. Clinics in laboratory medicine, International Journal of Medicine, 1989. 9(3): p. 573-586.

[12]. Bridier, A., et al., Comparative biocidal activity of peracetic acid, benzalkonium chloride and ortho-phthalaldehyde on 77 bacterial strains. Journal of Hospital Infection, 2011. 78(3): p. 208-213.

[13]. Aslim, B., N. SAĞLAM, and Y. Beyatli, Determination of some properties of Bacillus isolated from soil. Turkish Journal of Biology, 2002. 26(1): p. 41-48.

[14]. Singh, Z., et al., An introduction to essentials of bio-medical waste management. Medical journal, Armed Forces India, 2001. 57(2): p. 144-147.

[15]. Hossain, M.S., et al., Clinical solid waste management practices and its impact on human health and environment–A review. Waste management, Journal of Hospital infections, 2011. 31(4): p. 754-766.

[16]. Eguvbe, A., An assessment of healthcare workers awareness of hazards of healthcare waste and its management in Oshimili South Local Government Area of Delta State, Nigeria. Eguvbe AO, Akpede N, Journal of Medicine 2010. 28(3): p. 50-51

[17]. Jeswani, H.K., W. Wehrmeyer, and Y. Mulugetta, How warm is the corporate response to climate change? Evidence from Pakistan and the UK. Business

Strategy and the Environment, International Journal of biology, 2008. 17(1): p. 46-60.

[18]. Chaudhry, M.A., et al., Health hazards of hospital waste to sanitary workers at combined military hospital Rawalpindi, Pak. Armed Forces Med. J, 2004. 54(2): p. 253-258.

[19]. Habibullah, S. and S. Afsar, Waste disposal of government health-care facilities in urban area of Karachi: A KAP survey. Pakistan Journal of Medical Research, 2007. 46(1): p. 1-4.

[20]. Rutala, W.A. and C.G. Mayhall, Medical waste. Infection Control & Hospital Epidemiology, International Journal of Medicine, 1992. 13(1): p. 38-48.

[21]. Rutala, W.A., R.L. Odette, and G.P. Samsa, Management of infectious waste by US hospitals. Jama, 1989. 262(12): p. 1635-1640.

[22]. Khan, M.R., F. Fareedi, and B. Rashid, Techno-economic disposal of hospital wastes in Pakistan. Pak J Med Res, 2006. 45(2): p. 41-5.

[23]. Datta, P., G.K. Mohi, and J. Chander, Biomedical waste management in India: Critical appraisal. Journal of laboratory physicians, 2018. 10(1): p. 6-14.

[24]. Visvanathan, C., Hazardous waste disposal. Resources, conservation and recycling, International Journal of Biology1996. 16(1-4): p. 201-212.

[25]. Organization, W.H., Management of solid health-care waste at primary health-care centres: A decision-making guide. Pak J Med 2005. 15(5): P:21-26.

[26]. Oyeleke, S.B., The microbiological effects of hospital wastes on the environment. African journal of biotechnology, 2009. (8): pp. 12-30.

[27]. Organization, W.H., et al., Global status report on alcohol 2004. 2(5): pp. 13-32.

[28]. Control, C.f.D., Update: acquired immunodeficiency syndrome and human immunodeficiency virus infection among health-care workers. MMWR. Morbidity and mortality weekly report, 1988. 37(15): p. 229.

[29]. Sales, J.W. and R. Workman, EPA guide for infectious waste management, in EPA guide for infectious waste management. 1986, 12(16): pp 8-12.

[30]. Organization, W.H., W.H.O. Staff, and Ś.O. Zdrowia, World report on knowledge for better health: strengthening health systems. 2004: World Health Organization. 5(8): p.18.

[31]. Kumar, R., et al., A descriptive study on evaluation of bio-medical waste management in a tertiary care public hospital of North India. Journal of environmental health science & engineering, 2014. 12: p. 69-69.

[32]. Chartier, Y., Safe management of wastes from health-care activities. 2014: World Health Organization, 2014. 12(17).pp. 18-26.

[33]. Wafula, S.T., J. Musiime, and F. Oporia, Health care waste management among health workers and associated factors in primary health care facilities in Kampala City, Uganda: a cross-sectional study. BMC public health, 2019. 19(1): p. 203-203.

[34]. Khattak, F.H., Hospital waste management in Pakistan. Pak J Med Res, 2009. 48(1): p. 19-23.

[35]. Sarsour, A., et al., Assessment of Medical Waste Management within Selected Hospitals in Gaza Strip Palestine: A Pilot Study. International Journal of Scientific Research in Environmental Sciences, 2014. ijsres: 6(8) p. 164-173.

[36]. Safety, O. and H. Administration, Occupational exposure to bloodborne pathogens: proposed rule and notice of hearing. Federal Register, 1989. 54(May 30): p. 23042-23139.

[37]. Ferdowsi, A., M. Ferdosi, and M.J. Mehrani, Incineration or autoclave? a comparative study in Isfahan hospitals waste management system (2010). Materia socio-medica, 2013. 25(1): p. 48.

[38]. Chudasama, R.K., et al., Biomedical Waste Management: A study of knowledge, attitude and practice among health care personnel at tertiary care hospital in Rajkot. Journal of Research in Medical and Dental Science, 2013. 1(1): p. 17-22.

[39]. Hirani, D.P., K.R. Villaitramani, and S.J. Kumbhar, Biomedical waste: an introduction to its management. International Journal of Innovative Research in Advanced Engineering (IJIRAE), 2014. 1(8): p. 82-87.

[40]. Zimmermann, K., Microwave as an emerging technology for the treatment of biohazardous waste: A mini-review. Waste Management & Research, Journal of Molecular Biology, 2017. 35(5): pp. 471-479.

[41]. Ullah, J.H., Managing the health care solid waste in punjab, pakistan. 2010, national college of business administration & economics, Pakistan Journal of Med. Res, 2010. 20(3): pp. 29-46.

[42]. Organization, W.H., Teacher's guide: management of wastes from health-care activities. Amman: WHO Regional Centre for Environmental Health Activities, 2003. 12(19): pp. 9-16.

[43]. More, S., et al., Awareness Among Medical Students, Hospital Medical and Paramedical Staff Regarding Biomedical Waste Management: A Study and Review. International Journal of Medical Toxicology & Legal Medicine, 2015. 18(1): p. 9-14.

[44]. Oweis, R., M. Al-Widyan, and O. Al-Limoon, Medical waste management in Jordan: A study at the King Hussein Medical Center. Waste management, Annual Review of Microbiology, 2005. 25(6): p. 622-625.

[45]. Cheng, Y., et al., Medical waste production at hospitals and associated factors. Waste Management, Indian Journal of Medicine, 2009. 29(1): p. 440-444.

[46]. Alhumoud, J.M. and H.M. Alhumoud, An analysis of trends related to hospital solid wastes management in Kuwait. Management of Environmental Quality: An International Journal, 2007. 12(15): pp. 21-25.

[47]. Acharya, D. and M. Singh, The hook of biomedical waste management. Ist ed. 2000, 5(8): pp. 2-18. Minerva Press. New Delhi.

[48]. Morgan, D., Needlestick and infection control: policies and education. AIDS Letter, 1999. 68(77): p. 1-4.

[49]. Hofmann, F., N. Kralj, and M. Beie, Needle stick injuries in health care-frequency, causes und preventive strategies. Gesundheitswesen (Bundesverband der Arzte des Offentlichen Gesundheitsdienstes (Germany)), 2002. 64(5): p. 259-266.

[50]. Rengarajan, N., Municipal solid waste management in India: Finding sustainable pathways for the city of Bangalore. Indian Jor 2013: Arizona State University, 2013. 16(12): pp. 37-48.

[51]. Diaz, L., G. Savage, and L. Eggerth, Alternatives for the treatment and disposal of healthcare wastes in developing countries. Waste Management, International Journal of Medicine, 2005. 25(6): p. 626-637.

[52]. Marino, C.G.G., et al., Cut and puncture accidents involving health care workers exposed to biological materials. Brazilian Journal of Infectious Diseases, 2001. 5(5): p. 235-242.

[53]. Eitel, D.F., Managing medical waste technology: how US hospitals adapted to change. International Journal of Technology Management, 1998. 15(3-5): p. 402-420.

[54]. Brunekreef, B. and S.T. Holgate, Air pollution and health. The lancet, 2002. 360(9341): p. 1233-1242.

[55]. Macher, J., et al., Effect of ultraviolet germicidal lamps on airborne microorganisms in an outpatient waiting room. Applied Occupational and Environmental Hygiene, EPA 1992. 7(8): p. 505-513.

[56]. Matthews, S., et al., Trends in Alcohol-Related Violence in Australia, Journal of Open Life Sciences, 1991/92-1999/00. 2002. 22(18): pp. 35-39.

[57]. Zia, A., et al., Influence of income level and seasons on quantity and composition of municipal solid waste: A case study of the capital city of Pakistan. Sustainability, Gomal Journal of Medical Sciences, 2017. 9(9): p. 1568.

[58]. Soliman, S.M. and A.I. Ahmed, Overview of biomedical waste management in selected Governorates in Egypt: A pilot study. Waste management, International Journal of Medicine, 2007. 27(12): p. 1920-1923.

[59]. Nascimento, T.C., et al., Occurrence of clinically relevant bacteria in health service waste in a Brazilian sanitary landfill and antimicrobial susceptibility profile. Revista da Sociedade Brasileira de Medicina Tropical, 2009. 42(4): pp. 6-14.

[60]. Horan, T.C., et al., Nosocomial infections in surgical patients in the United States, January 1986-June 1992. Infection Control & Hospital Epidemiology, 1993. 14(2): p. 73-80.

[61]. Verdeil, X., et al., Epidemiologie clinique et microbiologique des infections nosocomiales en chirurgie: Etude prospective portant sur 3422 malades hospitalises au chr de Toulouse. Médecine et maladies infectieuses, 1990. 20(5): p. 222-228.

[62]. Fauteux, P., National affairs. Environmental policy and law, 2016. 42(46): p. 2.

[63]. Ayliffe, G.A., Control of hospital infection. A practical handbook, 1992. 3(6):pp. 18-26.

[64]. Oyeleke, S. and N. Istifanus, The microbiological effects of hospital wastes on the environment. African Journal of Biotechnology, 2009. 8(7) pp. 25-36.

[65]. Neu, J., Ninety-Fourth Critical Bibliography of the History of Science and Its Cultural Influences (to January 1969). Isis, 1969. 60(5): p. 5-219.

[66]. Nascimento, T.C., et al., Ocorrência de bactérias clinicamente relevantes nos resíduos de serviços de saúde em um aterro sanitário brasileiro e perfil de susceptibilidade a antimicrobianos. Revista da Sociedade Brasileira de Medicina Tropical, 2009. 42(4): p. 415-419.

[67]. Ethica, S.N. and A. Sabdono. Bio-remediation potential of hydrolytic bacteria isolated from hospital liquid biomedical waste in Central Java. in Proceedings of the 3rd World Congress on New Technologies. PLOS Biology, 2017 4(2): pp 18-19. [68]. Al-Shahwani, M., et al., Bacterial densities of hospital sewage in Baghdad City. Environmental Pollution Series A, Ecological and Biological, 1986. 41(1): p. 1-10.

[69]. Akter, N. and J. Tränkler, An analysis of possible scenarios of medical waste management in Bangladesh. Management of Environmental Quality: An International Journal, 2003. 2(5): pp. 18-22.

[70]. Colomer-Lluch, M., et al., Bacteriophages carrying antibiotic resistance genes in fecal waste from cattle, pigs, and poultry. Antimicrobial agents and Chemotherapy, International Journal of Medicine, 2011. 55(10): p. 4908-4911.

[71]. Khachatourians, G.G., Agricultural use of antibiotics and the evolution and transfer of antibiotic-resistant bacteria. Cmaj, 1998. 159(9): p. 1129-1136.

[72]. Goud, S., et al., The detection and antimicrobial susceptibility profile of Shigella isolates in and around Hyderabad, Telangana. J. Pharm. Innov., Indian Journal of Pharmaceutical Sciences 2018. 5(7): pp. 24-39.

[73]. Romano, R., D. Lu, and P. Holtom, Outbreak of community-acquired methicillin-resistant Staphylococcus aureus skin infections among a collegiate football team. Journal of athletic training, 2006. 41(2): p. 141.

[74]. Bergeson, L.L. and C.N. Hutton, FIFRA-Chemical Testing Issues. Envtl. L. Rep. News & Analysis, 2005.18(35): p. 10141.

[75]. Tsai, C., J. Lai, and S. Lin, Quantification of pathogenic micro-organisms in the sludge from treated hospital wastewater. Journal of applied microbiology, 1998. 85(1): p. 171-176.

[76]. Carter, Y. and D. Barry, Tackling C difficile with environmental cleaning. Nursing times, Journal of applied microbiology, 2011. 107(36): p. 22-25.

[77]. Chakraborty, B., et al., Action of newer disinfectants on multidrug resistant bacteria. Journal of Evolution of Medical and Dental Sciences, 2014. 3(11): p. 2797-2814.

[78]. Russell, A., Biocide use and antibiotic resistance: the relevance of laboratory findings to clinical and environmental situations. The Lancet infectious diseases, 2003. 3(12): p. 794-803.

[79]. Van Klingeren, B. and W. Pullen, Glutaraldehyde resistant mycobacteria from endoscope washers. Journal of Hospital Infection, 1993. 25(2): p. 147-149.

[80]. Rengasamy, R., Composite nonwovens in wipes, in Composite Non-Woven Materials. 2014, Elsevier. 2(5): p. 89-119.

[81]. Cheesbrough, M., District laboratory practice in tropical countries, part 2. 2006: Cambridge university press. 2006. 18(22): pp. 24-32.

[82]. Alexander, S.K. and D. Strete, Microbiology: a photographic atlas for the laboratory. 2001: Benjamin-Cummings Publishing Company.

[83]. Clarke, P.H. and S. Cowan, Biochemical methods for bacteriology. Microbiology, International Journal of Microbiology, 1952. 6(1-2): p. 187-197.

[84]. Boyce, J.M., Modern technologies for improving cleaning and disinfection of environmental surfaces in hospitals. Antimicrobial Resistance & Infection Control, International Journal of Medicine, 2016. 5(1): p. 1-10.

[85]. Christensen, W.B., Urea decomposition as a means of differentiating Proteus and paracolon cultures from each other and from Salmonella and Shigella types. Journal of bacteriology, 1946. 52(4): p. 461.

[86]. Baron, E.J., L.R. Peterson, and S.M. Finegold, Bailey and Scott's diagnostic microbiology. Vol. 693. 1994: Mosby St. Louis.

[87]. Aljumaily, M.J., N.B. Mehdi, and S.K. Abbas, Diagnosis of some Bacteria Causing Gingivitis in People with Type 2 Diabetes and Investigating some of Their Biochemical and Immunological Parameters. Rafidain Journal of Science, 2019. 28(3): p. 48-54.

[88]. Van Klingeren, B., et al., Assessment of the efficacy of disinfectants on surfaces. International biodeterioration & biodegradation, 1998. 41(3-4): p. 289-296.

[89]. Shaner, H. and G. McRae, Eleven recommendations for improving medical waste management. Burlington (VA): The Nightingale Institute for Health and the Environment, 2006. 10(13): pp. 29-43.

[90]. Karamat, K., et al., Strategic framework for bio-safety and bio-security in public sector hospitals Pakistan. Karachi, Pakistan. PMRC Ministry of Health. 2005. 5(8): pp. 18-22.

[91]. Anitha, J. and I.A. Jayraaj, Isolation and identification of bacteria in biomedical wastes (BMW). International journal of pharmacy and pharmaceutical sciences, 2012. 4(5): p. 386-388.

[92]. Anitha, J., Isolation and identification of bacteria from biomedical waste (BMW). International Journal of Pharmacy and Pharmaceutical Sciences, 2012. 2(4): p. 386-388.

[93]. Biswas, A., A. Amanullah, and S. Santra, Medical waste management in the tertiary hospitals of Bangladesh: an empirical enquiry. ASA university review, 2011. 5(2): p. 149-158.

[94]. Osagie, R.N., A.A. Eyaufe, and F. Ireye, Microbial Analysis of Biomedical Wastes from Selected Health Facilities in Parts of Edo South and its Public Health Implication. International Journal of Public Health Science, 2016. 5(1): p. 51-54.

[95]. Panda, R., P. Panda, and C. Mishra, The effect of hospital solid wastes on the environment. Ecoscan, International Journal of Biology, 2012. 6(1&2): p. 37-40.

[96]. Sharada, M., et al., Comparative effectiveness of disinfectants with phenol on multidrug resistant bacteria and fungi isolated from the clinical sample–an in vitro preliminary study. Journal of Evolution of Medical and Dental Sciences, 2013. 2(18): p. 3055-3062.

[97]. Sumathy, V.J.H., Antimicrobial Activi. Pharm, Res, Indian Journal of Medicine 2016. 4(6): p. 355-361.

[98]. Gargi, R., et al. Efficacy study of some antiseptics and disinfectants. Indian Journal of Medicine, 2017. 3(5): pp. 61-68.

[99]. Latimer, J.M. and J.M. Matsen, Microwave oven irradiation as a method for bacterial decontamination in a clinical microbiology laboratory. Journal of Clinical Microbiology, 1977. 6(4): p. 340-342.

[100]. Singhal, L., A.K. Tuli, and V. Gautam, Biomedical waste management guidelines 2016: What's done and what needs to be done. Indian Journal of Medical Microbiology, 2017. 35(2): p. 194.

[101]. Mahmoudkhani, R., A. Mokhtari Azar, and M.R. Khani, A survey of tehran hospitals wastewater. Int. J. Environ, 2012. 12(41): p. 531-538.

[102]. DARON, R., Clinical use of virtual study models versus traditional plaster study models for orthodontic treatment diagnosis. 2004, Thesis submitted to the Faculty of the Graduate Studies and Research, International Journal of Medicine 2004.20: pp. 16-33.

[103]. Okore, C.C., et al., Antimicrobial efficacy of selected disinfectants. American Journal of Biology and Life Sciences, 2014. 2(2): p. 53.

[104]. Gargi, R., et al., Efficacy study of some antiseptics and disinfectants, International Journal of Microbiology,2017. 6(3): pp. 15-19.

[105]. Sanborn, M., S. Wan, and R. Bulard, Microwave sterilization of plastic tissue culture vessels for reuse. Applied and environmental microbiology, 1982. 44(4): p. 960-964.