SUPERCRITICAL FLUID CARBON DIOXIDE STERILIZATION OF CLINICAL SOLID WASTE

by

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LIST OF ABBREVIATIONS AND SYMBOLS

µg	Microgram
A	Lower asymptote value
A. baumannii	*Acinetobacter baumannii*
A. lwoffii	*Acinetobacter lwoffii*
Acid phosphatase	2-naphthyl phosphate
Alkaline phosphate	2-naphthyl phosphate
API	Analytical profile index
Cal:	Calculated values
CFU	Conoly formimg unit
Cystine arylamidase	L-cystyl-2-naphthylamide
E. faecalis	*Enterococcus faecalis*
E_d	Activation energy
Esterase (C 4)	2-naphthyl butyrate
Esterase Lipase (C 8)	2-naphthyl caprylate
Exp:	Experimental data
HCFs	Healthcare facilities
HCW	Healthcare waste
HLWE	Hospital Lam Wah Ee
ICU	Intensive-care unit
K. pneumoniae	*Klebsiella pneumoniae*

k_{dm}	Inactivation rate
KJ	Kilojoule
L. sphaericus	*Lysinibacillus sphaericus*
Leucine arylamidase	L-leucyl-2-naphthylamide
Lipase (C 14)	2-naphthyl myristate
min	minute
MINITAB	Statistical software package for analyzing data
MOH	Ministry of Health
MPa	Megapascal
N:	Viable cell counts after treatment
N_0:	Initial cell counts
N-acetyl-β glucosaminidase	1-naphthyl-N-acetyl-βDglucosaminide
Naphthol-AS-BI-phosphohydrolase	Napthol-AS-BI-Phosphate
NCBI	National center for Biotechnology Information
P. aeruginosa	*Pseudomonas aeruginosa*
P. mirabilis	*Proteus mirabilis*
psi	Pound per square inch
R	Ideal gas constant
R^2	Regression coefficients
rDNA	Ribosomal deoxyribonucleic acid
RPC	Recycling project committee
RSS	Residual sum of the squares
S. agalactiae	*Streptococcus agalactiae*

S. aureus	*Staphylococccus aureus*
S. epidermidis	*Staphylococcus epidermidis*
S. liquefaciens	*Serratia liquefaciens*
S. marcescens	*Serratia marcescens*
S. marcescens	*Serratia marcescens*
S. mutans	*Streptococcus mutans*
S. pyogenes	*Streptococcus pyogenes*
SCFs	Supercritical fluids
SDS-PAGE	Sodium dodecyl sulphate polyacrylamide gel electrophoresis
SF-CO_2	Supercritical fluids carbon dioxide
T	Absolute temperature
t	Time
Trypsin	N-benzoyl-DL-arginine-2-naphthylamide
t_t	Complete inactivation time
Valine arylamidase	L-valyl-2-naphthylamide
WHO	World Health Organization
α chymotrypsin	N-glutaryl-phenyylalanine-2-naphthylamide
α fucosidase	2-naphthyl-αL- fucopyranoside
α galactosidase	6-Br-2-naphthyl-αD-galactopyranoside
α glucosidase	2-naphthyl-αD-glucopyranoside
α mannosidase	6-Br-2-naphthyl-αD-mannopyranoside
β galactosidase	2-naphthyl-βD- galactopyranoside

β glucosidase	6-Br-2-naphthyl-βD- glucopyranoside
β glucuronidase	Naphthol-AS-BI-βD-glucuronide
λ	Time for Lag phase

PENSTERILAN SISA PEPEJAL KLINIKAL MENGUNAKAN CECAIR KARBON DIOKSIDA LAMPAU GENTING

ABSTRAK

Satu kajian awal mengenai amalan pengurusan sisa klinikal telah dijalankan di Hospital Lam Wah Ee, Pulau Pinang, Malaysia. Amalan pengurusan merangkumi pengasingan, pengumpulan, pengangkutan dan memerlukan pelaburan kewangan yang tinggi. Walaupun amalan ini dipraktikkan, namun risiko jangkitan masih wujud. Program kitar semula didapati tidak mengurangkan jumlah sisa pepejal klinikal, bahaya dan kos pelupusan. Dalam kajian ini, beberapa jenis bakteria patogenik nosocomial dan oportunis telah dikenal pasti dan pensterilan sisa pepejal klinikal adalah perlu untuk mengurangkan risiko jangkitan kepada pekerja. Perbandingan kecekapan sterilisasi autoklaf wap dan karbon dioksida superkritikal (SF-CO_2) pada sisa pepejal klinikal telah dijalankan. Penyahaktifan bakteria melalui kaedah pensterilan wap bergantung kepada suhu (121 ^{0}C), masa rawatan (60 minit) dan jenis spesies bakteria. SF-CO_2 berupaya menyahaktif hampir kesemua spesies bakteria termasuk *E. coli, E. faecalis, S. marcescens* dan *S. aureus, B. sphaericus* pada suhu yang agak rendah iaitu 60^{0}C dan tekanan sederhana pada 20 MPa. Model matematik Gompertz telah digunakan untuk menggambarkan tingkah laku penyahaktifan bakteria dalam sisa klinikal dengan menggunakan keadah SF-CO_2. Pertumbuhan semula bakteria tidak berlaku dalam sisa yang telah dirawat dengan kaedah SF-CO_2. Sisa rawatan sterilisasi autoklaf menunjukkan pertumbuhan semula bakteria selepas 2 hari. Analisa Mikroskop Elektron Pengskanan (SEM), protein selular dan aktiviti enzim yang belum dirawat, dirawat dengan autoklaf dan dirawat dengan SF-CO_2 mendedahkan bahawa autoklaf wap

menyahaktifkan bakteria secara fizikal dan mengubah sifat enzim selular. Dalam rawatan SF-CO_2 tekanan menjadi faktor yang menyebabkan kerosakan pada dinding sel, perpecahan sel dan anjakan pada bahagian luar membran. Ketiadaan protein semasa analisis SDS-PAGE mencadangkan bahawa protein selular dan enzim telah terlarut dalam SF-CO_2. Keputusan keseluruhan kajian ini menunjukkan bahawa teknik pensterilan sisa pepejal klinikal SF-CO_2 adalah lebih berkesan untuk digunakan dalam pengurusan sisa klinikal, terbukti berupaya mengurangkan risiko pendedahan kepada jangkitan dan keupayaan untuk memusnahkan sel-sel bakteria secara kimia dan fizikal. Dengan pengurangan risko, pihak hospital secara tidak langsung dapat menyediakan persekitaran yang selamat bagi pesakit, penjagaan kesihatan dan kakitangan klinikal.

SUPERCRITICAL FLUID CARBON DIOXIDE STERILIZATION OF CLINICAL SOLID WASTE

ABSTRACT

There is growing awareness on safe handling and management of clinical solid waste. The aim of the present study was to determine an effective sterilization method for safe handling and recycle-reuse of clinical solid waste materials. A preliminary study on the clinical waste management practice was conducted at Hospital Lam Wah Ee, Penang, Malaysia. The management practices encompasses segregation, collection, transportation and require high financial investments. Despite these practices, the infectious risk is still at hand. The existing recycling programs of general solid waste materials remains unchanged of the amount of clinical solid waste generation, its hazard and the disposal cost. In this study, several types of nosocomial and opportunistic pathogenic bacteria have been identified and sterilization of clinical solid waste is requisite to minimize infectious risks to the workers. Comparison on the sterilization efficiency of steam autoclave and supercritical carbon dioxide (SF-CO_2) on clinical solid waste was conducted. Steam sterilization inactivation of bacteria depended on temperature and treatment time and types of bacterial species. The most effective experimental condition for the autoclave treatment was found to be temperature 121 ^{0}C and 131 ^{0}C for the exposure time 60 min and 30 min, respectively. SF-CO_2 inactivates the bacteria in clinical solid waste including *E. coli, E. faecalis*, *S. marcescens* and *S. aureus*, *B. sphaericus* at a relatively lower temperature at 60 ^{0}C and moderate pressure of 20 MPa. Gompertz mathematical model was used to describe the inactivation

behavior of bacteria in clinical solid waste using SF-CO_2. No re-growth of bacteria was detected in SF-CO_2 treated wastes, unlike bacterial re-growth in autoclave treated waste in 2 days. Analysis of Scanning Electron Microscope (SEM), cellular protein and enzymatic activity of bacterial cells revealed that steam autoclave physically inactivates the bacteria and denatures cellular enzymes. Meanwhile, SF-CO_2 inactivates the bacteria both physically and chemically. Both Pressure and temperature were the factors that cause cell wall damage and extracted out the cytoplasmic materials of bacterial cell. The absence of proteins and enzymes in the SDS-PAGE and APIZYM analysis, respectively, suggests that the cellular protein and enzymes have been dissolved in the SF-CO_2. The overall results of this study suggest that SF-CO_2 sterilization of clinical solid waste is a more effective technique to be employed in the clinical waste management. SF-CO_2 was proven to have reduced the risk of exposure to infection based on its capability to destroy the bacteria cells. With the reduced risk, the hospital could provide a safer environment for patients, healthcare and clinical staffs.

Chapter One: Introduction

1.1 Clinical solid waste management in Malaysia

In the last few decades, human activities and changes associated with lifestyles and consumption patterns have resulted in the generation of huge volumes of different types of wastes. The wastes have threatened the survival of humans and other living things, as well as the natural resources, those are necessary for human existence. Consequently, in little more than two decades public concern over the waste management and the pollution problems associated with waste generation have attracted significant attention and a great deal of researches have been conducted to evaluate appropriate waste management options in order to minimize environmental pollution and maximize resource recovery (Williams, 2005). In recent years, concern over the solid waste from healthcare facilities (HCFs) has increased throughout the world (DenBos and Izadpanah, 2002). Clinical solid waste, arising principally from hospitals and clinics, is potentially dangerous since it can spread infectious diseases due to the inadequate management of clinical solid waste (Abd El-Salam, 2010; Al-Khatib and Sato, 2009).

There is growing awareness on effective control and safe handling of clinical solid waste in worldwide due to the common concern for hospital hygiene (Alagoz and Kocasoy, 2008; Bdour et al., 2007). Clinical solid waste is prescribed by many as

infectious, requires pertain approach during handling and disposal of clinical solid waste (Abd El-Salam, 2010). The amount of clinical solid waste generation increases significantly in Malaysia with increasing public healthcare facility and advance technology (Tabasi and Marthandan, 2013). The existing clinical waste management practice in Malaysia is not able to adequately preserve human health and environmental contamination. The Ministry of Health (MOH, 2009) reported that the most common issue for the inadequate clinical solid waste management practice in Malaysia is the improper waste segregation at source. General waste is mixing with clinical solid waste and vice versa due to improper segregation practices in hospitals (DOE, 2009).

The increasing treatment and disposal cost of clinical solid waste and its hazards to human health and environment are relating to the miss classification, improper segregation of the waste (Blenkharn, 2005; Diaz et al., 2008; Lee et al., 2004). The technologies used at present to dispose the clinical solid waste is not environmentally friendly and do not cope with clinical solid waste in a safe manner. For example, the most used technology to dispose clinical solid is incineration. The incineration is considered as an inappropriate technology for treating clinical solid waste due to release a wide variety of pollutants including dioxins, furans, heavy metals, acid gases, carbon monoxide, and nitrogen oxide (Coker et al., 2009; Lee et al., 2004; Singh et al., 2011). Moreover, the incineration technology requires high financial start-up cost and occupational capital to implement the facilities (Alagöz & Kocasoy, 2008; Lee et al., 2004).

Recycling-reuse of clinical solid waste materials is the most desirable way to reduce the waste generation and to prevent materials from entering the waste stream (Lee et al., 2004; Tsakona et al., 2007). Clinical solid waste contains enormous volumes of recyclable materials (Lee et al., 2004; Marinkovic et al., 2008). Therefore, the development of recycling clinical solid waste can serve as a means of reducing rising quantities of waste generation and its treatment cost (Blekharn, 2005; Jang et al., 2006; Lee et al., 2004; Ozbek and Sanin, 2004; Park and Jeong, 2001; Patil and Shekdar, 2001; Tsakona et al., 2007; Tudor, 2007). Clinical solid waste must be free from infectious agents prior to recycling the waste materials. On this basis, the clinical solid waste must be sterilized at the point of generation in order to avoid possible infectious threat of clinical solid waste (Marinkovic et al., 2008; Tsakona et al., 2007).

The definition of the term 'sterilization' is the complete destruction or removal of all living microorganisms on or within a substance, including bacteria or spores, viruses, and fungi (Maurer, 1978; Williams, 2005; Zhang et al., 2006a). Sterilization of clinical solid waste presents a challenge to current sterilization technology due to the major portions of clinical solid waste are heat sensitive plastic or polymer materials. In medical practice, the most common sterilization techniques used are stream autoclaving, ethylene oxide, and gamma-radiation (Dempsey and Thirucote, 1989; Zhang et al., 2006a). Though, all these methods assure a satisfactory microbial inactivation, but still exists a number of limitations (Nik Norulaini et al., 2008; Spilimbergo et al., 2003). Steam autoclave, despite inactivate the microorganisms, can destroy the temperature sensitive materials (White et al., 2006). Additionally, the steam sterilization technique is

expensive and difficult to control because of the extremely high temperature required (Spilimbergo et al., 2002, 2003; White et al., 2006). Gama–radiation sterilization may change tensile strength and transparency of reusable waste material (Dillow et al., 1999). Ethylene oxide, on the other hand, is a toxic and flammable gas. It is a known carcinogen and can cause hemolysis (Dillow et al., 1999). Ethylene oxide sterilization can also chemically destroy the polymer materials. Hence, the available sterilization technologies in medical care are not suitable for the sterilization of clinical solid waste, since the heat sensitive recyclable and reusable clinical solid waste materials may destroy either thermally or chemically. Because of the limitation of the current sterilization technology, a low temperature sterilization technology must be evaluated to deal with clinical solid waste in order to propose cost effective and safer clinical solid waste management practice (Marinkovic et al., 2008).

Supercritical fluid carbon dioxide (SF-CO_2) is an effective sterilization method that has notable benefits over the existing sterilization method. The fluid carbon dioxide at the supercritical state (31.1 °C, 7.4 MPa) is non-toxic and nonflammable. Carbon dioxide is easily available as an industrial byproduct and thus is inexpensive. SF-CO_2 is proven to be effective against any sort of microorganisms, as it impacts target microorganisms both physically and chemically (Jimenez et al., 2008; Kim et al., 2009; Spilimbergo et al., 2002). SF-CO_2 has been potentially used to sterilize biomedical device for being effective against bacteria (Dillow et al., 1999; Spilimbergo et al., 2002), viruses (Fages et al., 1998), and spores (Zhang et al., 2006b). This technology sterilizes the heat sensitive biomedical device without any damage and lowering its quality

(Dillow et al., 1999; Zhang et al., 2006a). Although, SF-CO_2 has been proven as an effective sterilization technology, limited researches have been conducted to sterilize the clinical solid waste using SF-CO_2. Thus, the adoption of SF-CO_2 sterilization technology in clinical solid waste management is receiving potential interest with regards to determine a safer and resource recovery clinical solid waste management practice.

1.2 Problem statement

Many studies have documented to determine a safer clinical waste management practice within an affordable cost by the healthcare facilities. Patwary et al. (2009a) reported that segregation of general waste could dramatically impact on lowering the clinical waste generation. Studies conducted by Lee et al. (2004) and Tudor et al. (2007) reported that the recycling of healthcare waste is a good solution as a means of reducing rising quantities of clinical solid waste and its treatment cost. Lee et al. (2004) further reported that it must ensure that the recyclable healthcare waste must be free from infectious agent prior to conducted recycling program. Although, segregation practice would protect the mixing of general solid waste with the infectious waste, how it could affect the clinical solid waste generation rate and the treatment cost is not well described in literature. Most of the developing country's hospitals are facing financial constrain, lack of regulatory guideline in country level, inadequate segregation materials and trained clinical staffs, those are crucial to conduct effective segregation, resource recovery and recycling program of healthcare solid waste (Ozbek and Sanin, 2004; Sabour et al., 2007; Shinee et al., 2008). Therefore, effective source segregation and

recycling practice of healthcare solid waste in a safe manner is impossible for most of the HCFs of developing countries.

One of the major reasons of improper clinical solid waste management practice in a healthcare facility is that the healthcare worker are not aware of possible infectious risk of clinical solid waste (Alagoz and Kocasoy, 2008; Coker et al., 2009; Saini et al., 2004). There is limited scientific information available in literature on the role clinical waste as a reservoir of infectious diseases. It is obligatory to characterize the types of microorganisms present in clinical solid waste in order to achieve a reliable infectious risk of the clinical solid waste.

Available technologies (i.e., incineration, Autoclave, microwave) used to treat clinical solid waste are not environmentally friendly and not able to preserve human health and the environment (Alagoz and Kocasoy, 2008, Marinkovic et al., 2008). Marinkovic et al., (2008) declared that sterilization using a mobile device at its source is the most acceptable solution to infectious medical waste (infectious waste and sharp objects). Sawalem et al., (2009) suggested adopting a low operating cost, easily implementable, and low maintenance sterilization method in clinical waste management to prevent contamination. Sterilization of the clinical solid waste with the view of conducting resource recovery is challenging due to major portions of clinical solid waste materials are made of heat sensitive plastics or bio-polymers. However, numerous studies reported that SF-CO_2 is a gentle terminal sterilization technology, which could

sterilize the heat sensitive high density plastics and polymers without damage and lowering the quality (Dillow et al., 1999; Ellis et al., 2010; White et al., 2006, Zhang et al., 2006a). No study has been conducted yet to determine the acceptable sterilization technology to sterilization clinical waste at its generation source. It is therefore, bearing considerable concern to determine a reliable sterilization technology to handle the clinical solid waste in a safe manner.

Many studies have been carried out to inactivate the bacteria in environmental waste using various sterilization technologies. Little attention has been paid on the re-growth bacteria from the sterilized waste. Bacteria are cellular microorganisms, able to re-grow and multiply under a favorable nutrient requirement (Chong et al., 2010; Rusin et al., 1997). Therefore, it must be ensured the complete inactivation of the bacteria in the cellular level in order to avoid unexpected re-growth of bacteria prior to decide any sterilization technology. Studies reported that pressure, temperature and medium are substantial during inactivation of bacteria in the SF-CO_2 treatment (Dillow et al., 1999; Kim et al., 2009; Spilimbergo et al., 2003), but there is not yet clear understanding of this effect. Several hypotheses have been proposed as an inactivation mechanism including cell rupture, lipid modification, changes of protein, loss of enzymatic activities, acidification, etc., (Dillow et al., 1999; Lin et al., 2008; Spilimbergo and Bertucco, 2003). However, there is limited evidence available in literature to acquire clear understating and confirm the proposed mechanisms.

1.3 Objectives

The objectives are:

1. To determine the current status of clinical waste management practice in a hospital of Penang, Malaysia.
2. To identify the bacteria in clinical solid waste, sharp waste and general solid waste.
3. To determine the effectiveness of the SF-CO_2 sterilization on the inactivation of microorganisms in clinical solid waste.
4. To study the inactivation mechanisms of bacteria in clinical solid waste and the re-growth of bacteria in sterilized clinical solid waste.

Chapter Two: Literature Review

2.1 Clinical Solid Waste Management

Safe Clinical solid waste management is crucial due to avoid the potential hazards to human health and environmental. Clinical solid waste is perceived by many as hazardous or infectious (Blenkharn, 1995; Miyazaki and Une, 2005; Phillips, 1999; Salkin, 2003). Although surveys refer that about 10-25% of waste contains the infectious agent (Bendjoudi et al., 2009; Mohee, 2005; Shinee et al., 2008), but Saini et al. (2004) reported that general waste may contain pathogenic bacteria and the microbial flora present in clinical waste and general waste might similar. Besides, there is a possibility of the contamination of non-clinical waste (general waste) with infectious agents during poor segregation, collection, storage and transportation (Blenkharn, 1995; Shinee et al., 2008). Hence, effective attention must be placed during treating clinical solid waste so that clinical waste cannot mix with non-clinical waste. Accordingly, clinical solid waste should be handled, stored, transported and disposed of in a controlled manner to safeguard public health and to prevent environmental pollution. Infectious pathogenic microorganisms may infect the human body during unsafe handling via direct contact (puncture, abrasion or cut in the skin) or indirect conduct (mucous membranes, inhalation or ingestion) (Pruss et al., 1999). A particular concern on the handling of sharps clinical solid waste, it represents the most acute potential hazards to health (Alagoz and Kocasoy, 2008). The management of clinical solid waste, particularly in developing countries is often poor and fraught with difficulties.

Unless clinical waste is properly handled and disposed, it can present risks to healthcare staffs, the public and the environment (Al-Khatib and Sato, 2009; Shinee et al., 2008). There is not yet clear understanding of the infectious risk of the inadequate clinical solid waste management, which is often implemented. A Number of studies have been conducted in many countries to define the best appropriate clinical waste management plan in order to minimize the health hazards and associate environmental pollution (Alagoz and Kocasoy, 2008; Bdour et al., 2009; Bendjoudi et al., 2009; Cheng et al., 2009; Da Silva et al., 2005; Hassan et al., 2008; Sawalem et al., 2009; Shinee et al., 2008). All such studies have indicated that the planning and implementation of waste management practices would reduce waste generation, minimize health hazard and disposal cost.

The management of clinical solid waste is considered as problematic due to its enormous volume of generation, serious threat to the human health as well as disposal cost (Bendjoudi et al., 2009; Da Silva et al., 2005; Diaz et al., 2008; Jang et al., 2006; Saini et al., 2004). Many developed countries have devised codes of practices and guidelines for handling and disposal such waste (Bdour et al., 2007; Da Silva et al., 2005; Lee et al., 2004). Although significant progress has been found, yet it still requires further modification in all aspects of clinical waste management practices. In most developing countries, clinical waste has not received adequate attention despite the fact that clinical waste labeled as hazardous or infectious (Alagoz and Kocasoy, 2008; Coad, 1992; Da Silva et al., 2005; Jang et al., 2006; Tsakona et al., 2007). In developing countries, clinical solid waste has been handled and disposed together with the non-

clinical waste, which is creating inevitable risks to the health care workers, publics and the environment (Alagoz and Kocasoy, 2008; Bendjoudi et al., 2009; Da Silva et al., 2005; Marinkovic et al., 2008; Shinee et al., 2008). WHO in 2002 conducted an investigation survey on the clinical waste management in 22 developed countries. The survey reported that the proportion of healthcare facilities that do not use proper waste disposal methods ranges from 18-64% (WHO, 2004). Healthcare workers are not educated and most of them have not had any special training on the clinical waste management (Coker et al., 2009; Diaz et al., 2008; Shinee et al., 2008). Generally, they use two hands during collection and sorting the waste (Shinee et al., 2008). Most of the healthcare institutions do not have appropriate color coded bags or containers for sorting the waste (Alagoz and Kocasoy, 2008). Some of the healthcare facilities have used plastic bags, paper bags or cardboard boxed to collect the clinical solid waste (Coker et al., 2009; Shinee et al., 2008). Besides, healthcare waste is not sorted because of the high fee of their disposal cost, therefore both clinical and non-clinical waste are mixed together and dump illegally (Alagoz and Kocasoy, 2008; Coker et al., 2009; Shinee et al., 2008). Even most of the hospitals have not any special place for the storage the clinical waste prior to disposal. Clinical waste is placed in an unsecured area until collected and it is fully accessible to the animals (Alagoz and Kocasoy, 2008; Da Silva et al., 2005).

World Health Organization defined an effective clinical solid waste management in a clinical facility depends on dedicated waste management plan, good administration, adequate financing and participation by trained clinical staff (WHO 2005),. In addition,

clear definition and classification of the waste (Askarian et al., 2004; Shinee et al., 2008), source segregation of the waste (Moreira and Gunther, 2013), the estimation of the amount and type of waste generated (Tsakona et al., 2007), and the use of appropriate disposal technology (Lee et al., 2004; Diaz et al., 2008) are crucial in order to decide an effective clinical solid waste management.

2.2 Definition and classification of Clinical solid waste

The waste generated in Healthcare facilities (HCFs) has not clearly been defined in the literature. There are currently several terms used to describe the waste that is generated in healthcare facilities, as presented in Table 2.1. It can lead to problems as it is important to have a specific definition of those wastes derived from healthcare premises. This is because, there are practical considerations to differentiate between the waste and the waste from HCFs, and in relation to choosing a right waste disposal method, which depends on the clear understanding (Bendjoudi et al., 2009; Moritz, 1995; Nemathaga et al., 2008). In literature, the terms ‘clinical waste’, ‘health care waste’, ‘infectious waste’ and ‘medical/hospital waste’ are typically encountered, they may have similar meanings or be subsets of one another, which substantially inhibits using and comparing data from different countries (Bendjoudi et al., 2009; Diaz et al., 2008; Jang et al., 2006; Lee et al. 2002; Mato and Kaseva, 1999; Moritz, 1995; Nemathaga et al., 2008).

Table 2.1 Definitions and general classification of waste arising from healthcare facilities.

Definition	Classification	Reference
Health care waste	General waste and medical Waste	Shinee et al., 2008
Hospital waste	General waste, medical waste and sharp	Nemathaga et al., 2008
Medical waste	Infectious waste and general medical waste	Cheng et al., 2009
Medical waste	General waste and special waste	Lee et at., 2004
-	Infectious waste and non-infectious waste	Miyazaki and Une, 2005
Hospital waste	General waste and Hazardous waste	Sawalem et al., 2009
Healthcare waste	Hazardous and non-hazardous waste	Mohamed et al., 2009
Medical waste	Domestic waste and hazardous waste	Abd El-Salam, 2010
Hospital waste	Hazardous and non-hazardous waste	Kaisar Alam Sarkar, et al., 2006
Healthcare waste	Medical waste and general waste	Ruoyan et al., 2010
Medical/Hospital waste	Infectious and municipal waste	Tsakona et al., 2007
Medical waste	Tissues and other	Jang et al., 2006
Medical waste	Hazardous and non-hazardous waste	Patwary et al., 2009a

Lee et al., (2002) used the term medical waste to deal with all types of wastes produced by HCFs. It includes all types of waste generated by HCFs, such as hospitals, clinics, physician office and other medical laboratory and research facilities (Hall, 1989; Jang et al., 2006). Medical waste is a subcategory of healthcare waste, which potentially indicates the infectious waste except sharps (Lee et al., 2002). Nemathaga et al. (2008)

delineated the definition of hospital waste is any type of waste generated from healthcare facilities. This includes both non-clinical and clinical waste constituents. The World Health Organization refers to the waste generate from HCFs as healthcare waste (HCW). According to Bendjoudi et al. (2009), HCW results from the treatment, diagnosis, or immunization of humans and/or animals in hospitals, veterinary and health-related research facilities, and medical laboratories. This type of waste contains infectious waste, toxic chemicals and heavy metals, and may contain substances that are genotoxic or radioactive. Generally, a small portion of the total healthcare waste bears the infectious agent. Clearly, 10-25% of total healthcare wastes are infectious (Bendjoudi et al., 2009; Mohee, 2005; Pruss et al., 1999), therefore waste arising from HCFs cannot be defined as infectious waste. Besides, all waste cannot be addressed as clinical waste. There are some categories of waste, those are not falling within the definition of clinical waste (Moritz, 1995).

Healthcare waste can be classified as non-clinical waste (non regulated HCW, also can define as general waste), and clinical waste (special waste, regulated HCW) (Lee et al., 2002, 2004; Mato and Kassenga, 1997). Non-clinical waste is such type of waste, which is not posing any infectious risk to human health and environment. Examples of non-clinical waste include packaging materials such as cardboard, office paper, leftover food, cans etc. (Lee et al., 2002, 2004; Diaz et al., 2008; Pruss et al., 1999). Conversely, clinical solid waste is the type of solid waste materials, which generates in clinical facilities during diagnosis, treatment, immunization, in research pertaining thereto and biological testing (WHO, 2000, 2004). Examples of clinical solid

waste are discarded surgical gloves, glassware, instruments, needles, lancets, culture, stocks and swabs and remove body organs (Nemathaga et al., 2008; Jang et al., 2006; Oweis et al., 2005; WHO, 2000). Clinical waste can be categorized as infectious waste, radioactive waste, chemical waste, pathological waste, pharmaceutical waste and sharps (Pruss et al., 1999). Examples of different types of clinical solid waste are given in Table 2.2 (Lee et al., 2002; Nemathaga et al., 2008; Shinee et al., 2008).

Table 2.2 Examples of types of Clinical solid waste

Category	Examples
Infectious waste	Lab cultures and stocks of infectious agents, wastes from isolation wards, tissues, materials or equipment contact with infected patients
Pharmaceutical waste	Expired or unnecessary pharmaceuticals and drugs.
Pathological waste	Body parts, human fetuses, blood, other body fluids.
Chemical waste	Solid chemicals from diagnostic and experimental work, cleaning materials,
Radioactive waste	Radioactive substances from radiotherapy or lab work
Sharps	Needles, syringes, blades, broken glass, scalpels etc.

The ministry of Health of Malaysia categorises the healthcare waste in the guideline as general waste and special regulated waste (MOH, 2009). The clinical waste is a one of the sub categories of the regulated waste. The clinical waste has been defined

as scheduled waste under the Environmental Quality Regulations, 1989 and further classified as infectious, pathological and sharp waste (MOH, 2009). According the MOH, (2009), the classification of healthcare waste is presented in Figure 2.1. Clinical waste is defined by MOH, (2009) as:

a. Any waste which consists entirely or partly of human or animal tissue, blood or other body fluids, excretions, drugs or other pharmaceutical products, swabs or dressings or syringes, needles or other sharp instruments, being waste which unless rendered safe may prove hazardous to persons coming into contact with it; and

b. Any other waste arising from medical, nursing, dental, veterinary, pharmaceutical or similar practice, investigation, treatment, care, teaching or research or the collection of blood from transfusion, being waste which may cause infection to any person coming into contact with it.

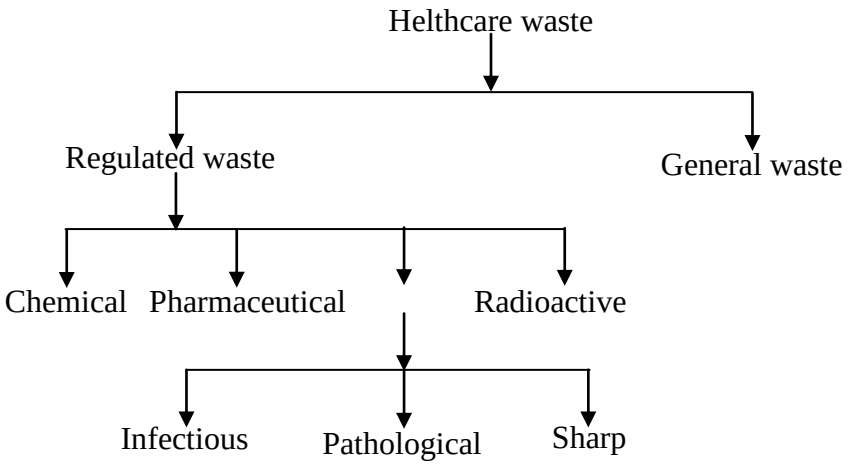

Figure 2.1 The classification of healthcare waste (Source: MOH, 2009).

2.3 Source of clinical solid waste

The principal sources of clinical solid waste are hospitals and clinics, particularly those providing acute services, i.e, offering Operating theatres, Maternity ward, Accident & Emergency, Mortuary, Intensive Care, Isolation Wards, Pharmacy, Pathology Laboratories and other research facilities (Bendjoudi et al., 2009; Blenkharn, 1995; Da Silva et al., 2005; Marinkovic et al., 2008). Other sources of clinical waste are ambulance services, public health laboratories, blood donation centers and blood banks, practice center of doctors, dentists, veterinary surgeons, immunization/vaccination clinics and hospitals, clinics and nursing homes providing community care, care of the elderly and services related to mental health and learning disabilities (Hagen et al., 2001; Marinkovic et al., 2008; Pruss et al., 1999).

There has been an increase in the amount of clinical waste coming from households. This is due in part to changes in health care policies. The establishment of home health and medical care services has, in recent years, become a basic requirement for the population (Blenkharn, 2008; Slack et al., 2004). Both medical devices and instruments are used while treating patients at home, thereby producing a variety of waste materials. Self-injecting diabetics and people changing colostomy bags at home can also generate significant quantities of clinical waste (Blenkharn, 2008; Harsh et al., 2010). The wastes generated from the treatment of patients suffering from infectious diseases may spread infection either through direct contact or indirectly through the environment. Waste materials originating from home health and medical care services are still included in general household waste materials, even when the wastes are infectious (Blenkharn, 2008; Miyazaki et al., 2007). However, the management of household infectious waste material has not received any attention yet, even in a developed country like Japan (Miyazaki et al., 2007).

2.4 Clinical solid waste generation

Generally, healthcare waste generation rate depends on the type of healthcare establishment, availability of instrumentations, general condition of HCFs area, ratio of disposable item in use and number of patient care (Alagoz and Kocasoy, 2008; Bdour et al., 2007; Cheng et al., 2009; Mohee, 2005). Also, the economic, social and cultural status of the patients might change the amount of waste generation (Askarian et al., 2004; Hassan et al., 2008). Among the factors, the number of day-care patients has a

significant effect on waste generation rate (Bdour et al., 2007; Patwary et al., 2009a). For example, Bdour et al. (2007) and Patwary et al. (2009a) reported that, due to the higher number of day-care patients, public healthcare facilities produce larger amount of healthcare waste than private healthcare facilities.

The proportion of clinical waste per bed is similar in both public and private hospitals because of the mismanagement of HCW and a lack of segregation of waste for sorting the clinical waste in surveying hospitals (Patwary et al., 2009a). Marinkovic et al., (2008) reported that the healthcare waste generation rate depends on the size and the type of the medical institution, which might differ from country to country based on the level of the economic development (Nemathaga et al, 2008). The developed countries generate higher amounts of healthcare waste than that of the developing countries (Marinkovic et al., 2008, Nemathaga et al, 2008, Pruss et al., 1999). Data from World Health Organization reveals that North America produces 7-10 kg of healthcare waste per bed/day, whereas South America produces 3 kg of waste per bed/day. This difference was also found in Europe and Asia. Western Europe produces 3-6 kg, whereas Eastern Europe 1.4-2 kg of waste per bed/day. In Asia, richer countries produce 2.5 kg per bed/day, and poorer countries 1.8-2 kg per bed/day (Pruss et al., 1999). From the data, it was evident that amount of healthcare waste generation rate depends on the level of economic development of the region. It was also noticed that, due to a higher level of economic development, the North America produces the largest amount of waste. This is might be due to the developed nation's lifestyle demands consumption of a high amount of goods and services, which tends to generate a higher amount of waste

(Marinkovic et al., 2008). Furthermore, the use of disposable instruments and packaging materials rather than the use of reusable items in healthcare centers in developed countries might increase the amount waste generation.

The clinical waste generation rate depends on waste management plan and segregation activities (Alagoz and Kocasoy, 2008). Cheng et al., (2009) reported that the total amount of healthcare waste generation is much higher at medical centers and private hospitals, but the proportion of clinical waste is much higher at local hospitals. This is due to poor segregation practice followed during sorting the clinical waste in the local hospital, which contaminated the non-clinical waste, hence the amount of clinical waste generation increased. The contribution of clinical wastes to the total waste stream varied from about 12.5–69.3% (Abd El-Salam, 2010; Da Silva et al., 2005; Hassan et al., 2008; Nemathaga et al., 2008; Sawalem et al., 2009; Shinee et al., 2008). The healthcare waste generation rate in different countries is given in Table 2.3. It is evident from the Table 2.3, developing countries in Africa (South Africa, Algeria, Egypt, Libya) and Asia (Bangladesh, Mongolia) continent generate the lower amount of HCW, but the proportion of clinical waste among total waste higher than that of middle develop countries in Europe continent (Croatia, Greece). This is because, the developed nations are following advanced legislation and guidelines during waste collection, and state of various possible ways during waste handling, storage and transportation to minimize the clinical waste generated (Alagoz and Kocasoy, 2008; Almuneef and Memish, 2003; Tudor, 2007). Clinical waste has not yet fully appreciated in the developing countries, still handled and disposed together with non-clinical waste (Alagoz and Kocasoy, 2008).

Though, in the beginning, minor proportion of the total waste may be considered as clinical waste. Later, cross-contamination might occur due to mixing with the non-clinical waste, which is rendering the entire load of clinical waste (Blenkharn, 1995; Patwary et al., 2009a, b).

Quantity and quality of clinical waste generated at its source are the key issues to decide an effective clinical waste management practice (Coker et al., 2009; Shinee et al., 2008). Therefore, it is important to minimize clinical waste generation rate at generation source. Appropriate segregation and sorting of clinical waste at source can minimize the clinical solid waste generation rate. One of the critical obstacles to conduct source segregation of clinical solid waste is lack of knowledge on risk exposure of clinical solid waste.

Table 2.3 Average health care waste generation rate in different countries hospitals

Country/City	Waste generation rate	Non-clinical waste, %	Clinical waste, %	Generation period	Number of samples	Region	Reference
Algeria	0.7-1.22 kg/bed/day	75-90	10-25	16 September to 10 October, 2006	10	Africa	Bendjoudi et al., 2009
Libya	1.3 kg/patient/day	72	26		14	Africa	Sawalem et al., 2009
South Africa	0.60 kg/patient/day	60.74	39.26	April and July, 2003	2	Africa	Nemathaga et al., 2008
Taiwan	2.41-3.26 kg/bed/day	N/A	N/A	N/A	150	Asia	Cheng et., 2009
Brazil	2.63 kg/bed/day	80-85	15-20	September 2001 to March 2002	N/A	South America	Da Silva et al., 2005
Jordan	6.10 kg/patient/day*	N/A	N/A	March to September, 2004	14	Asia	Bdour et al., 2009
Ulaanbaatar, Mongolia	1.4-3.0 kg/patient/day	70.67	29.43	January and February 2005	56	Asia	Shinee et al., 2008
Dhaka, Bangladesh	1.71 kg/bed/day	79	21	Over 5 months in 2006	69	Asia	Patwary et al., 2009a
Croatia	2.4 kg per capita	86	14	N/A	151	Europe	Marinkovic et al., 2008
El-Beheira Governorate, Egypt	2.07 kg/bed/day	60.10	38.9	6 month period in 2008	8	Africa	Abd El-Salam, 2010
Sylhet city Bangladesh	0.934 kg/bed/day	63.97	36.03*	July 2003 to June 2004	17	Asia	Kaisar Alam Sarkar, et al., 2006
Binzhou, China	1.22 kg/bed/day	N/A	N/A	December 2006 to January 2007	6	Asia	Ruoyan et al., 2010
Greece	8.4 kg/bed/day	83.33	16.67	N/A	N/A	Europe	Tsakona et al., 2007

* Maximum generation rate cited in literature; N/A: Data is not available

2.5 Risks of Clinical solid waste

The potential microbiological risks associated with the clinical waste are unfamiliar to healthcare workers. This is because of the literature on the role of infectious clinical waste as a reservoir of diseases is extremely limited (Salkin, 2003). Although, there have been a few reports documented on the infectious risks on clinical waste management, but, unfortunately scientifically substantiated evidence of the actual content of microorganisms, survival of microorganisms in clinical waste and the infectious risks to healthcare workers and the general public are extremely rare. Furthermore, the available information is restricted to developing countries, and therefore does not reflect the exposure, practices, and risk situations in developing countries (Salkin, 2003).

The infectious risk posed by clinical solid waste to human health and environment, which needs to be assessed, is the potential presence of pathogenic microorganisms. A great variety of pathogenic microorganisms may present in clinical solid waste (EA, 2003; Patwary et al., 2012; Pruss et al., 1999; Saini et al., 2004). A person involved in the treatment of clinical waste might be exposed to infectious agents through several routes including skin penetration, skin contact, or by the aerogenic route (EA, 2003; Pruss et al., 1999). According to Pruss et al., (1999), the possible microorganisms and their infected routes in the human body are given in Table 2.4.

Table 2.4 The possible microorganisms and the infected routes in the human body (Source: Pruss et al., (1999))

Type of infection	Transmission vehicles	Example of causative organisms
Gastroentic infections	Faeces and/or vomit	Enterobecteria, e.g. *Salmonella*, *Shigella* spp, *Vibrio cholera*, Helminths
Respiratory infections	Inhaled secretions, saliva	*Mycobacterium tuberculosis*, measles virus, *Streptococcus pneumonia*
Ocular infection	Eye secretions	Herpesvirus
Genital infections	Genital secretions	N*eisseria gonorrhoeae*; herpesvirus
Skin infections	Pus	*Streptococcus* spp.
Anthrax	Skin secretions	*Bacillus anthracis*
Meningitis	Cerebro-spinal fluid	Neisseria meningitidis
Acquired immunodeficiency syndrome (AIDS)	Blood,sexual secretions	Human immunodeficiency virus (HIV)
Haemorrhagic fevers	All bloody products and secretions	Junin, Lassa, Ebola, and Marburg viruses
Septicaemia	Blood	*Staphylococcus* spp
Bacteraemia	Blood	Coagulase-negative *Staphylococcus spp.; Staphylococcus aureus*; Enterobacter, Enterococcus, Klebsiella, and *Streptococcus* spp.
Candidaemia	Blood	Candida albicans
Viral hepatitis A	Faeces	Hepatitis A virus
Viral hepatitis B and C	Blood and body fluids	Hepatitis B and C viruses