MOLECULAR DIVERSITY OF BACTERIA FROM A MUNICIPAL DUMPSITE: IMPLICATIONS TO PUBLIC HEALTH

Kilaza Samson Mwaikono

A Dissertation Submitted in Partial Fulfilment of the Requirements for the Degree of Doctor of Philosophy in Life Sciences and Bioengineering of the Nelson Mandela African Institution of Science and Technology

Arusha, Tanzania

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ABSTRACT

Despite known risks of inappropriate disposal of solid wastes; most cities in developing countries dispose waste in open dumpsite where humans, animals and microbes interact. This study was done in Arusha municipal dumpsite, Tanzania to investigate the abundance and diversity of bacteria, compare the faecal microbiota of pigs scavenging on dumpsite and indoor reared pigs, and also determine the resistance profile of enteric bacteria from the dumpsite. Domestic wastes, solid biomedical wastes, sludge from the river near the dumpsite and faecal materials of pigs were sampled. Total DNA was extracted and the variable region four (v4) of the 16S rRNA gene was sequenced using high throughput Illumina MiSeq platform. The quality control of sequences and the statistical analyses was performed using Mothur platform. A total of 8,469,294 quality sequences were generated. The mean of bacterial species per sample was 8,243. Diversity was high with an average InvSimpson index of 44.2. Thirty-five bacterial phyla dominated by *Firmicutes* (38%), *Proteobacteri*a (35%), *Bacteroidetes* (13%) and *Actinobacteria* (3%) were found. Overall, 76,862 operational taxonomic units (OTUs) dominated by *Acinetobacter* (12.1%), *Clostridium sensu stricto* (4.8%), *Proteinclasticum* and *Lactobacillus* (each 3.4%), *Enterococcus (*2.9%) and *Escherichia/Shigella* (1.7%) were revealed. There was a significant difference in faecal microbiota between scavenging and indoor reared pigs. Pathogenic genera like *Brucella*, *Rickettsia* and *Listeria* were exclusive to scavenging pigs. In solid biomedical waste, 36.2% of OTUs were related to dehalogenation, 11.6% degraders of aromatic hydrocarbons, 8.5% chlorophenol degradation and Atrazine metabolism 8.3%, and bacteria related to pathogens were 34%. *Escherichia/ Shigella*, *Bacilli* and *Proteiniclasticum* were predominant enteric bacteria. Some bacteria in scavenging pigs had 99% sequence similarity to pathogenic *Escherichia furgosonii*, *Shigella sonnei*, *Enterococcus faecium* and *Escherichia coli* O154:H4. Over 50% of the isolates were multidrug resistant. The study provides a comprehensive report on diversity of bacteria in Arusha municipal dumpsite. The high species richness shows the complexity of this man-made ecosystem, and calls for further research to assess for a link between human diseases and the dumpsite. This would provide insight into proper disposal of the waste, as well as, limit the risks to human health associated with the dumpsites.

DECLARATION

I, **Kilaza Samson Mwaikono** do hereby declare to the Senate of the Nelson Mandela African Institution of Science and Technology that this dissertation is my own original work and that it has neither been submitted nor being concurrently submitted for degree award in any other institution.

Kilaza Samson Mwaikono _____ _____

Name and signature of candidate **Date**

The above declaration is confirmed

Prof. Paul S Gwakisa _____ _____

Name and signature of supervisor **Date**

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I humbly acknowledge the government of the United Republic Tanzania through COSTECH and The Nelson Mandela African Institution of Sciences and Technology for sponsoring my PhD studies including this research work, without which this would not have happened.

I am very thankful to the African Biosciences Challenge Fund (ABCF) for offering me a six month research fellowship to access state of the art facilities at the BecA-ILRI hub Nairobi, Kenya, The fellowship made possible to execute part of this work. I also appreciate Dr. Robert Skilton from the same institute, for his logistic guidance, Prof. Morris Agaba for his advice into molecular techniques used in this work, Mr. Solomon Maina as my assistant researcher. It was a great time to work with them and I thank them for their advice and their friendship.

I also acknowledge Mr Edson Rugaimukamu from the Department of Veterinary Microbiology and Parasitology of Sokoine University Agriculture (SUA) for his assistance in biochemical identification of bacterial isolates. Thanks to the Genome Science Centre at SUA for offering me a spacious room right from the designing stage of this research to the final write-up.

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I am indebted to my wife, Edith and our children and my parents for their unwavering support and patience. You may not have "understood" what I have been going through, but your encouragement has been priceless.

Lastly but not the least, I acknowledge my almighty GOD for taking me through.

DEDICATION

To my family

Edith R. Silayo, Sam, Mengo, Sabhogo and Blessing

For patience endured when I was away for this business

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LIST OF ABBREVIATIONS AND SYMBOLS

16S rRNA	16 subunit ribosomal Ribonucleic acid
DNA	Deoxyribonucleic acid
α	alpha
β	beta
γ	Gamma
COSTECH	Commission of Science and Technology
NM-AIST	Nelson Mandela African Institutions of Sciences and Technology
BecA	Biosciences east and central
ILRI	Africa - International Livestock Research Institute
Dom	Domestic waste
Biom	Biomedical waste
FecD	Faecal material from pigs scavenging on municipal dumpsite
FecI	Faecal material of pigs raised under indoor management system
FecIF	Faecal of pigs shifted from indoor to scavenging on dumpsite
Riv	River sludge from Burka River near the municipal dumpsite
API 20E	Analytical Profile Index for analysis of Enterobacteriaceae
CTXM	Cefotaxime
CN	Gentamicin
FOX	Cefoxitin
CAZ	Ceftazidime
AMC	Amoxycillin –clavulanic Acid
NA	Nalidixic acid

CIP	Ciprofloxacin
P	Penicillin G
IPTG -	Isopropyl-beta-D-thiogalactopyranoside
SRA	Sequence Reads Archive
RDP	Ribosomal database Project
NCBI	National Center for Biotechnology Information
OTU	Operational taxonomic unit
GPS	Global Positioning System
OECD	Organization for Economic Cooperation Development
MENA	Middle East and North Africa
ECA	Europe and Central Asia
AFR	Africa
SAR	South Asia
EAP	East Asia and Pacific
LAC	Latin America and carribean

CHAPTER ONE

1.0.Background and literature review

1.1 Global overview of municipal solid waste generation and management

Generation of waste in the form of solid or liquid is a common industrial and community activity. The waste generated is classified as either hazardous or non-hazardous based on the chemical composition or reactive characteristics of the waste and potential adverse effect to man and environment (Palczynski, 2002). The global rate of solid waste generation is projected to reach 6 million tonnes per day by year 2025, while the *associated* management cost will reach $375 billion per year (Hoornweg and Bhada-Tata, 2012). East Asia, mostly China is leading in solid waste generation with expected increase from 520,550 tonnes per day in 2005 to triple in the year 2025.The trend is expected to shift to India by 2025, and then to Sub-Saharan Africa by 2050. Global waste generation per region (Fig.1.1) shows that, countries in the Organisation for Economic Cooperation Development (OECD) contributes almost half of the World's waste, while Africa and South Asia produce the least. Interestingly, OECD countries collects and disposes nearly 100% of their wastes as opposed to least developed countries in Africa where more than 50% of wastes and associated risks remain in their production sites (Fig.1.2).

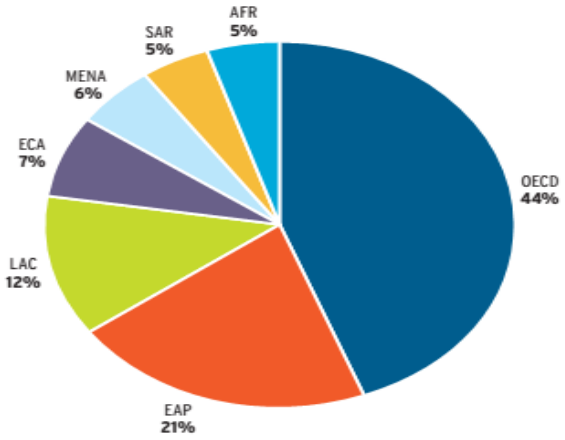

Figure 1.1 Municipal solid waste generation rate by region. EAP - East Asia and Pacific region, LAC - Latin America and Caribbean, ECA – Europe and Central Asia, MENA- Middle East and North Africa, SAR – South Asia, AFR – Africa, OECD – Organization for Economic Cooperation and Development.

Source: World Bank report (Daniel Hoornweg and Perinaz Bhada-Tata, 2012)

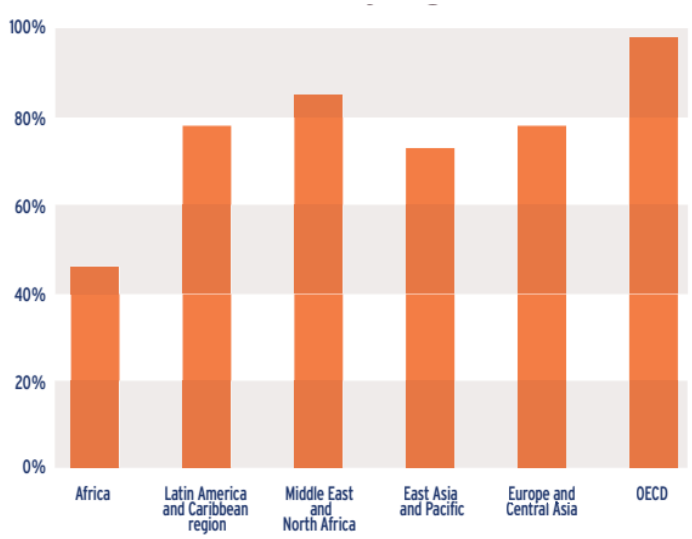

Figure 1.2 Municipal solid waste collection by region

Source: World band report (Daniel Hoornweg and Perinaz Bhada-Tata, 2012)

Waste generated in Sub-Saharan Africa is approximately 62 million tonnes per year. Per capita waste generation is between 0.09 to 3.0 kg per person per day (Karak *et al*., 2012). While the quantity of waste produced in cities continue to increase daily, the effectiveness of the means of handling waste in terms of collection and disposal remain poor. The rapidly growing urban population attributed to immigration of people, mostly youth from rural to urban areas searching for good life is further increasing the challenge to manage solid waste (Achankeng, 2003; Getahun *et al*., 2012). As opposed to integrated waste management approach which advocates a hierarchy of options for solid waste management in the order of "reduce, re-use, recycle, recover, landfill and controlled dumping" of solid waste; in most municipalities in developing countries, waste management is equated to open waste dumping or disposing into water bodies (Cilinskis and Zaloksnis, 1996; Obire *et al*., 2004), further polluting water and endangering both aquatic and terrestrial life.

In Tanzania, it is clearly known that solid waste management is one of the key duties of the urban authorities. However, due to rapid urban growth, together with scarcity of funds many urban authorities are facing a severe challenge in managing solid waste. In most municipalities, less than 45% of solid waste is collected and dumped to designated areas, while the rest remains in vicinity of their generation environment (Mungure, 2008) as shown in (Table 1.1) in four municipals. Many municipal authorities still operate open dumpsites for the final disposal of

solid wastes. Open waste dumping involves indiscriminate disposal of unsorted solid wastes from domestic households, industries, markets and biomedical waste from hospitals/ pharmaceutical/ cosmetics shops in a common dumpsite. With increasing urban population, the previously inaccessible dumpsites are now surrounded by residential areas. It is now common to see domestic animals roaming between dumpsites and residential areas, undoubtedly carrying with them unknown microbes which could be of public health importance.

Table 1.1 Status of solid waste management in four manicipals in Tanzania

Municipality	Generatio (tonnes/day)	Collection (tonnes/day)	% Waste collection
Arusha	375	130 -165	35 - 44
Ilala	650- 670	300 -420	46 - 56
Kinondoni	2016	810	40
Temeke	500 - 600	245 -294	49
Total	3551 - 3751	1485 – 1689	42 -45

Source: Mungure, 2008

Most dumpsites in urban and peri-urban areas bring together unsorted solid waste from diverse sources, and the fact that most of the dumpsites are closer to residential areas, there is high risk of pathogens from the dumpsite to be transmitted to human settings through people, domestic animals and pests scavenging on dumpsites. Interaction between microbes from different sources, presence of variety of chemicals/drugs and interaction between domestic and wild animals on dumpsites render these areas as a possible hotspot for emergence of new pathogens.

Southeast Asia for example, is a hotspot for emerging infectious diseases, including those with pandemic potential such as severe acute respiratory syndrome (SARS) and Influenza A H5N1 (Coker *et al*., 2011). Environmental dynamics in which biological, ecological, and technological processes interconnect in ways that enable microbes to exploit new ecological niches has rendered the region be considered a hotspot. Poor waste management in many municipalities where unsorted garbage is thrown in open dumpsite is a typical creation of new microbial niches, which could lead to microbial changes, hence emergence of new pathogens.

1.2. Study of bacteria of public health importance from the environment

1.2.1. Conventional approaches to study bacteria

Several studies have reported on the presence of microbes of public health importance on dumpsites (Emmanuel *et al*, 2011; Hassen *et al*., 2001; Mangizvo and Wiseman, 2012; Obire, *et al*., 2004; Oviasogie *et al*., 2010). Most studies were based on isolation of bacteria using microbiological culture method followed by either biochemical identification by exposing pure isolates to different sugars, or molecular identification based on 16S rRNA gene sequences. With recent advances in technology, it has been demonstrated that most of the microbes are not yet culturable under laboratory conditions, thus, culture-based methods could miss up to 99% of microbes in a sample under study (Fuhrman, 2012; Handelsman, 2004; Tringe *et al*., 2005).

1.2.2. Application of metagenomics to study bacteria

Metagenomics is the study of genetic material recovered directly from an environmental sample. With advances in the sequencing technology and bioinformatics tools, the metagenomic approach coupled with sequencing, has become the state of the art technology for studying the abundance and diversity of bacteria in environment (Arumugam *et al*., 2013; Fuhrman, 2012; Handelsman, 2004; Tringe *et al.*, 2005). The approach is based on 16S rRNA gene which is universally present in all bacteria. This gene has both conserved and highly variable regions, thus facilitates easy design of primers to be used for identification and diversity study of bacteria. Several researchers have used metagenomics to study bacterial communities (Arumugam *et al.*, 2013; Bibby *et al*., 2010; Caporaso *et al*., 2011; Costa *et al*., 2012; Piterina *et al.*, 2010; Sturgeo *et al*, 2013); more bacteria taxa have been identified far beyond what is known using culture-based approach. With the emergence of next generation sequencing technologies such as Illumina MiSeq, Roche 454 and Ion torrent; sequencing cost is becoming less and less, thus, a sample under study can be scrutinized at varying depth of sequencing to achieve high resolution, hence the possibility of detecting even rare biomes in a sample under study (Shahinas *et al*., 2012).

1.3. Study justification

Dumpsites in Tanzania represent a poorly managed interface where humans, livestock and feral animals interact. This causes a potential health risk to humans and domestic animals scavenging in the dumpsite. Dumpsites harbour a multitude of microbes emanating from diverse sources, and thus pose potential hotspots for evolution and transfer of pathogens to humans, domestic and feral animals interacting at the dumpsite.

1.4. Research question

Does the extensive diversity of bacteria on an open municipal dumpsite contribute to the emergence of new microbes of public health and industrial importance?

1.5. Research Objectives

Main objective

To determine the diversity of bacteria from a municipal dumpsite in Arusha, Tanzania

Specific objectives

i. To carry out a comprehensive qualitative survey of types of solid waste and animals in the Arusha municipal dumpsite,

ii. To determine the abundance and molecular diversity of bacteria from the municipal dumpsite using 16S rRNA amplicons,

iii. To perform comparative analyses of the faecal microbiota from free range pigs scavenging at the dumpsite and indoor reared pigs,

iv. To determine the profile of antimicrobial resistance of enteric bacteria isolated from the dumpsite

1.6. Significance of the study

The study avails knowledge on the nature of interaction between humans, animals and bacteria prevalent in Arusha municipal dumpsite and deduces potential public health risks. It also reveals the abundance, diversity and resistance phenotype of enteric bacteria circulating on the dumpsite and animals scavenging therein which could spill over to the human population.

CHAPTER TWO

High-throughput sequencing of 16S rRNA gene reveals substantial bacterial diversity in Arusha municipal dumpsite*

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Abstract

Multiple types of solid waste in developing countries are disposed of together in dumpsites where there is interaction between humans, animals and the bacteria in the waste. To understand the bacterial diversity, previous studies have focused on culturable bacteria, leaving behind a great number of unculturable. This study focuses on a more comprehensive identification of bacteria associated with the municipal dumpsite in Arusha, Tanzania. A qualitative survey was first performed to ascertain the variety of waste at the dumpsite. Domestic (Dom), biomedical (Biom), river sludge (Riv), and faecal material of pigs scavenging on the dumpsite (FecD) were sampled. Total DNA was extracted from solid wastes and the v4 region of 16S rRNA amplicons was characterized.

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A total of 8,469,294 sequences from 78 samples passed quality control. Catchall analysis predicted a mean of 8,243 species per sample. Diversity of species was high with an average InvSimpson index of 44.21 ± 1.44. Thirty-five phyla were detected, however only 7 accounted for 93% of all sequences. The predominant phyla include *Firmicutes* (38%), *Proteobacteri*a (35%), *Bacteroidetes* (13%) and *Actinobacteria* (3%). Overall 76,862 OTUs were detected, but only 20% were detected more than 10 times. The predominant OTUs were *Acinetobacter* (12.1%), *Clostridium sensu stricto* (4.8%), *Proteinclasticum* and *Lactobacillus* both at (3.4%), *Enterococcus (*2.9%) and *Escherichia/Shigella* (1.7%). *Halomonas, Idiomarina, Tisierella* and *Proteiniclasticum* were significantly associated with Biom; *Enterococcus, Bifidobacterium*, and *Clostridium sensu stricto* with FecD and *Flavobacteria, Lysobacter* and *Commamonas* to Riv ($P \leq 0.05$, indicator value ≥ 70). Taxonomy to metabolism mapping revealed 4.1% of OTUs have the potential to metabolize Atrazine, 12.2% degrade chlorophenol, 14.8% degrade aromatic hydrocarbons and 40.3% are involved in dehalogenation. Taxonomy to pathogen mapping revealed 39% of OUTs were related to human pathogens, and 34.1% of an unknown category, 1.2% rarely human pathogens and 25.6% were not pathogens.

There is an abundant and diverse bacterial community in a municipal dumpsite. The species richness described here shows the complexity of this man-made ecosystem and calls for further research to assess for a link between human diseases and the dumpsite. This would provide insight into proper disposal of the waste, as well as, limit the risks to human health associated with the dumpsite.

Keywords: Municipal dumpsite, Solid waste, Molecular diversity, 16S rRNA, Illumina MiSeq

2.1 Introduction

The amount of waste generated has risen due to the increasing urban population in most developing countries (Getahun, *et al.,* 2012; Guerrero *et al*, 2013; Mungure, 2008). Limited resources and inefficient infrastructure prevent proper waste separation leading to waste remaining in their production sites since it does not get transported to the final dumpsite. The lack of proper waste management systems has also created a dumpsite that includes solid waste, such as plastics, organic waste from households, markets, and abattoirs, agricultural waste, industrial waste, and chemical, pharmaceutical, and biomedical waste. Unattended wastes on

dumpsites attract insects, birds, and rodents, as well as, domestic and wild animals. Livestock, such as pigs, goats, and cattle scavenge on this waste. Dumpsites also provide a source of livelihood that attracts people to scavenge for materials that are sold to recycling industries, and these go about without protective. The unique, man-made ecosystem in the Arusha municipality in Tanzania, representing conditions in other developing cities, has the potential to cause serious impacts to public health.

Studies on the abundance and diversity of bacteria in environments, especially at interfaces like the dumpsite with human and animal activity, would enrich our understanding of the risks from such interfaces. The presence of disease vectors, such as insects, rodents, and several other small wild animals, at dumpsites can potentially spread pathogens from the dumpsite to human habitats. This highlights the importance of a need for a comprehensive study of the abundance and diversity of bacteria in municipal dumpsites.

Previous studies have only focused on the identification of disease vectors and culturable bacteria on the dumpsite. For example, study by (Ahmed, 2012) and (Onyido *et al*., 2011) reports *Periplaneta americanus* (Cockroach), *Musca domestica* (House fly), *Ophyra leucostoma* (Black garbage fly) and *Stomoxys calcitrans* (stable fly) as the most prevalent disease vectors on the dumpsite. (Awisan *et al*., 2013) found *Staphylococcus aureus, Pseudomonas aerugionosa, Klebsiella pneumonia* and *Escherichia coli* as the aerobic and opportunistic pathogens in Irisan dumpsite, (Emmanuel *et al*., 2011) found antibiotic resistant *Salmonella* sp*, Shigella* sp*, Vibrio cholerae, Proteus* sp and *Pseudomonas sp* from the Utisols dumpsite. However, these methods detect less than 1% of bacteria found in a particular environment (Riesenfeld *et al.*, 2004; Simon and Daniel, 2010; Vandecandelaere *et al.,* 2012). Other studies have used molecular approaches to identify culturable and unculturable bacteria in a sample, yet, these techniques, such as Sanger sequencing (Chen *et al.*, 2007; Huang *et al.*, 2005), are tedious and inefficient.

In the current study, total genomic DNA was extracted from samples collected from the different types of solid wastes in the dumpsites and the 16S rRNA amplicons were sequenced using a high throughput Illumina MiSeq platform. To our knowledge, this is the first report using culture independent approaches and high throughput sequencing of the 16S rRNA amplicons to study bacterial abundance and diversity in a municipal dumpsite where interaction between the microbes and animals, disease vectors, and humans is common.

2.2. Materials and methods

2.2.1. Study site and sampling

The site for this study was the Arusha municipal dumpsite, where waste from different urban sources is thrown. Sampling was performed March through June of 2013, whereby prior to sample collection, a qualitative survey was conducted over a period of two weeks to identify types of most common waste on the dumpsite. This comprised waste from households and markets (foods, pampers, clothes, etc.), chemical and biomedical waste (drug containers, used syringes), various plastics and used glassware, waste from abattoirs and brewers, as well as fecal matter from animals scavenging on the dump itself. Detailed descrition of the dumpsite is shown in (Appendix 1.1) Samples for this study were the fresh fecal material of pigs scavenging on the dump (FecD, n = 20), domestic solid waste (Dom, n = 33), solid biomedical waste (Biom, n = 15), and run-off water sludge adjoining a nearby river (Riv, n = 8). The core of fresh fecal materials of pig as well as solid waste and sludge were collected into sterile plastic containers, and, within one hour, the samples were transported on ice to the laboratory, where total DNA was extracted. The number of wild and domestic animals, birds and people found at the dumpsite were counted and recorded for the entire period of the survey.

Ethical statement

This study was approved by the research committee of the Nelson Mandela African Institution of Science and Technology. Permits to sample animals and variety of solid wastes on the dumpsite was granted by the Arusha District Veterinary office and permits to transfer samples between laboratories were given by the Zoosanitary Inspectorate Services of Tanzania, Arusha (VIC/AR/ZIS/0345) and Veterinary Services under the Ministry of Agriculture Livestock and fisheries of Kenya (RES/POL/VOL.XXIV/506).

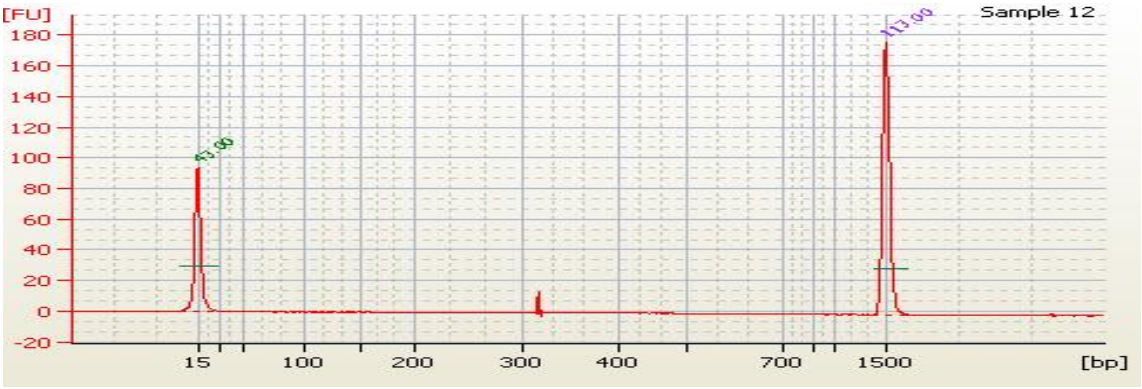
2.2.2. Extraction of total genomic DNA

Total genomic DNA was extracted from about 250 mg of solid waste samples using PowerSoil™ DNA extraction kit (MOBIO Laboratories, Carlsbad, California, USA) according to the manufacturer's protocol (Appendix 2.1). Quality and quantity of total DNA was verified with a NanoDrop ND-2000c spectrophotometer (Thermo Scientific) and gel electrophoresis run in 0.8

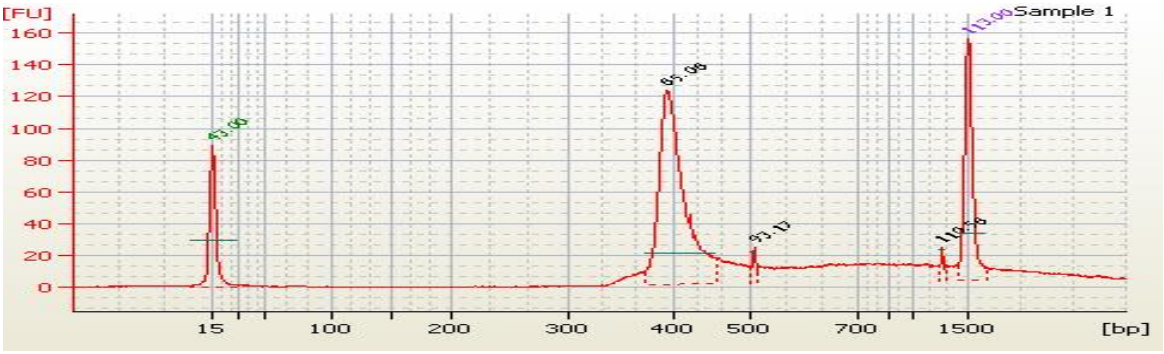
% agarose and visualized by ultraviolet illumination after staining with gel red^{TM}. The DNA was stored at -20^{o}C until further processing.

2.2.3. 16S r RNA amplification, library construction and sequencing

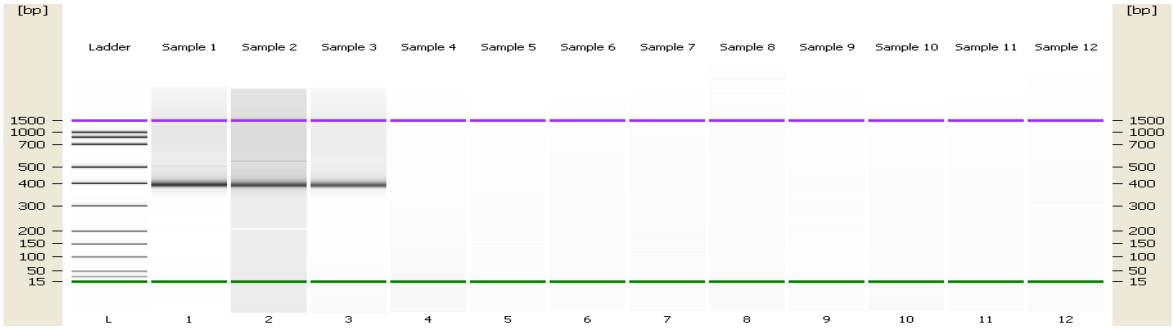
The Illumina sequencing preparation guide (Illumina, 2013) was used to prepare a pooled amplicon of the v4 region of 16S rRNA gene for sequencing. Primers (515F 5'-GTGCCAGCMGCCGCGGTAA-3' and 806R 5'-GGACTACHVGGGTWTCTAAT-3') designed for v4 region of 16S rRNA and protocols were adapted from (Caporaso *et al*., 2011) The reverse primer was linked to Golay barcode with varying sequences to be able to identify amplicons of different samples in pool (Appendix 2.2). Duplicate reactions were done in PCR master mix reaction in 20 µl AccuPower® Taq PCR PreMix composed of 0.5 µl of 10pmol/µl each for the forward and reverse primers, 17 µl molecular grade water, and 2 µl DNA template. The PCR program was run on GeneAMP™ PCR system 9700 set at 95 ^{o}C for 3 min, 35 cycles of 94 ^{o}C for 45 s, 50 ^{o}C for 60 s and 72 ^{o}C for 90 s and a final extension at 72 ^{o}C for 10 min. Amplicons quality was assessed using gel electrophoresis, and then pooled and purified using QIAquick® PCR purification kit (Qiagen, German) following manufacturer's protocol. Purified PCR products were normalized to 120 ng. DNA was quantified using Qubit® dsDNA assay kit in Qubit fluorometer 2.0 (Invitrogen, Life Technologies) and the quality was assessed using Agilent DNA 1000 Chip in Agilent 2100 Bioanalyzer (Agilent Technologies, Waldbronn, Germany) (Fig.2.1).



(a)



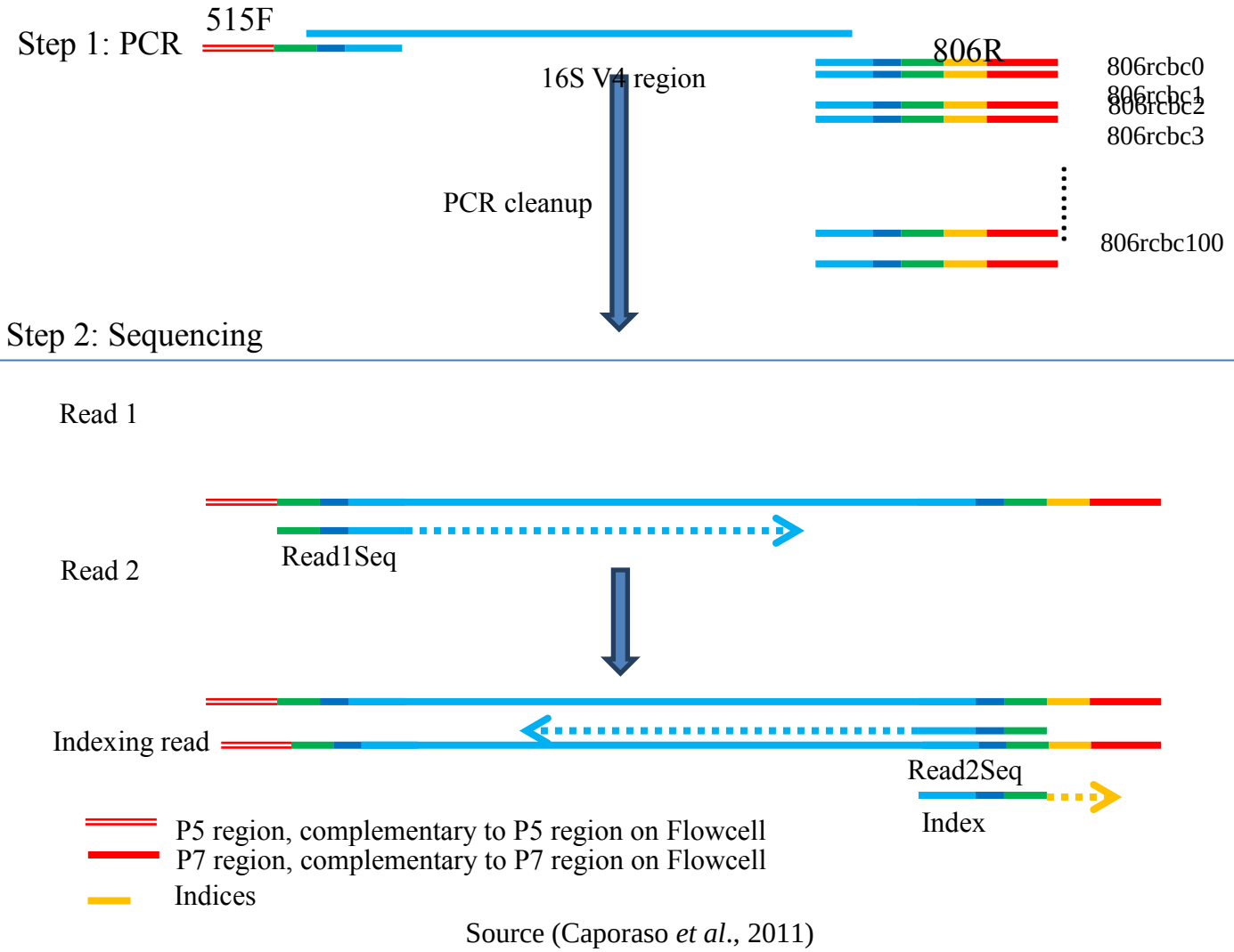
(b)



(c)

Figure 2.2.1 Quality assessment of DNA library using bioanalyser and DNA chip before sequencing. Fig.2.1 (a) shows two peaks of controls (DNA ladder) at 15bp and 1500bp within which the sample amplicon size is estimated. Fig.2.1(b) shows the peak of the DNA sample at size ~ 400bp and retention time of 85.06min. Fig.2.1(c) is an electropherogram for the three repricates of a pool of DNA samples which also shows that the DNA was ~ 400bp as expected.

Library denaturing, dilution, and PhiX control preparation was done as described in the 16S metagenomic sequencing library preparation guide (Illumina, 2013). Libraries were denatured and primers were used according to the method described in (Caporaso *et al*., 2011). Sequencing of the library was performed with the Illumina MiSeq platform (San Diego, USA) using 2×250 paired- end chemistry at the BecA - ILRI Hub genomic platform, Nairobi, Kenya, as shown in (Fig.2.2).



Source (Caporaso *et al*., 2011)

Figure 2.2 Amplification of the V4 region of the 16S rRNA gene and sequencing flow chart. Step1 indicates the amplification process where the forward primer 515F and reverse primers 806R are used. The reverse primer has varying barcodes that facilitate pooling of amplicons of 100 samples and also for tracking sample source after sequencing. Step 2 shows the forward (Read1) and reverse (Read2) sequencing. Indices are also sequenced; these will be removed to ensure that only sequences of v4 region of 16S rRNA remain.

2.2.4. Sequence data analysis and statistics

The Mothur package algorithms (v1.34.1) were used for both quality control and sequence data analysis (Schloss *et al*., 2009). After paired end reads were assembled, sequences were aligned with the Silva 16S rRNA reference database (www.arb-silva.de) (Quast *et al.,* 2013). Sequences that were < 239 bp and > 260 bp in length, contained >2 ambiguous base calls or long runs (>8 bp) of homopolymers, or did not align with the correct region were removed. Chimeras were identified using Uchime (Edgar *et al.,* 2011) and eliminated. Taxonomy was assigned using the RDP taxonomy database (http://rdp.cme.msu.edu/index.jsp) (Cole *et al.,* 2014). Sequences were binned into operational taxonomic units (OTUs) at 97% sequence similarity cut-off.

Species richness was assessed with Chao1 (Chao, 1984) and abundance based coverage estimator ACE (Anne Chao & Lee, 1992) while evenness and diversity of species were estimated by Shannon (Anne *et al.,* 2003), jackknife (Heltshe and Forrester, 1983) and inverse Simpson (Hunter and Gaston, 1988) indices as well as catchall analysis (Bunge, 2012). All analyses were performed using built-in commands in Mothur v1.34.1. Rarefaction analyses were done at a maximum of 97% sequence similarity cut-off, and were plotted using Phyloseq package (McMurdie and Holmes, 2013) in R version 3.1.2. In order to compare bacterial populations between different solid wastes in the same municipal dumpsite, subsampling of 9,159 sequences from different wastes was done to normalize them for efficient comparison (Gihring *et al*., 2012). This consisted of random selection of a number of sequences from each sample consistent to the lowest abundance in all samples. The community membership was compared using the traditional Jaccard index, while population structure was assessed using the Yue & Clayton measure of dissimilarity. Dendrograms were created using Mothur to compare the similarity of bacterial populations among all sample types using both Jaccard index and Yue & Clayton measure which account for the relative abundances in each sample. Figures were generated by FigTree v1.4.2 (Rambaut, 2009).

To check if the bacterial communities differed significantly between solid wastes, the parsimony (Schloss and Handelsman, 2006) and Unweighted UniFrac (Lozupone, Hamady, Kelley, & Knight, 2007) tests were done. The MOTHUR commands "parsimony" and "Unifrac.Unweighted", respectively, were applied to the Jaccard and the Yue & Clayton OTU based phylogenetic tree. The statistical significance of the difference in genetic diversity of

bacteria community within each solid waste type from the average genetic diversity of both communities pooled together was also assessed using Analysis of Molecular Variance (AMOVA) (Michalakis and Excoffier, 1996).

The core microbiota analysis was performed in Mothur with command "get.coremicrobiome" and it consisted of identification of OTUs present in at least 75% of solid waste samples. Due to heterogeneity of solid waste types on the dumpsite, we considered an OTU present in at least 50% of all samples to constitute the core microbiota for a given sample type. Indicator analysis (Dufrêne & Legendre, 1997) was used to test for possible OTUs affiliated to different types of solid waste. Indicator values (IV) ranged from 1 -100 with higher values for stronger indicators. Though literature considers indicator values > 30 and P-value ≤ 0.05 as good indicators (Dufrêne & Legendre, 1997), in this study only OTUs with indicator values ≥ 70 and P ≤ 0.05 were judged as having strong affiliation to particular solid waste. The Metastats program (White *et al*., 2009) was used to identify statistically different OTUs among solid wastes. The shared OTUs file, consensus taxonomy file, and metadata file generated in Mothur v.1.34.1 were imported into METAGENassist (Arndt *et al*., 2012) where further visualization using a heatmap and taxonomic to phenotype mapping, such as taxonomy to metabolism and taxonomy to human pathogens, were explored to get more insights on the nature of bacteria present on dumpsite. The BIOM file generated in Mothur was imported into MEGAN5 v5.5.3 (Mitra *et al.,* 2011) where Principal coordinate analysis and relative abundance of different taxa were visualized. A p-value of ≤ 0.05 was considered significant for all comparisons. Details of the bioinformatics analysis steps are shown in (Appendix 2.3). All sequence data discussed in this work have been deposited in sequence reads archive of the National Center for Biotechnology Information (NCBI), and assigned with accession numbers SRP046216, SRP045926, SRP046287, SRP047436, SRP046287 and SRP045810.

2.3. Results

2.3.1. Description of Arusha municipal solid waste dumpsite

The Arusha municipal dumpsite is located in peri-urban at about 12 km in south east of the Arusha city, at Latitude 03°25' S, Longitude 36°40' E, and at an elevation of 1260 m above the sea level. The dumpsite was established in 2003 with an approximate size of 20 hectares of land.

It serves a population of about 800,000 people from both Arusha municipal and part of Arumeru district. The dumpsite is surrounded with residential areas and also close to river Burka in the northern part. During rain reason, wastes from the dumpsite drains to this river. The dumpsite is characterized with heaps of garbage varying from the time of its establishment to the fresh ones. There are swampy areas within the dumpsite due to the collection of water between heaps of garbage following rain, thus making it a good breeding place for pathogens and vectors. Until when this study was conducted there were no facilities for solid waste processing and disinfection.

Types of animals, insects and birds including their population size, as well as variety of solid waste on dumpsite as recored over a period of two weeks are shown in (Table 2.1). Solid biomedical waste such as swabs, used syringes and needles, as well as drug containers from hospitals and pharmaceuticals carry microbes from infected people and drug residues on dumpsite, respectively. Domestic waste and industrial waste had a significant contribution on the variety of solid waste on the dumpsite. Solid waste sorting prior to disposal is not practiced. There were high interactions between wild and domestic animals, birds, insects and people picking recyclable materials. It was also noted that most people working on dumpsite did not wear any protective gear; thus being exposed to a variety of microbes circulating in the dumpsite. The survey found that the dumpsite is located within residential area, where scavenging animals were freely moving between the dumpsite and residential areas

Table 2.1 Types of organisms and solid waste in municipal dumpsite in Arusha, Tanzania

Animals interaction		Variety of solid waste on dumpsite		
Name	Estimated /day	Biomedical	Domestic	Industrial
Humans	20 - 40	used syringes	food remains	bottles
Animals		used swabs	Diapers	tetra packs
pigs	150 - 200	used needles	worn clothes	cardboard boxes
cattle	10 - 15	expired medicine	fruits and	broken bottles
Goats	5 - 10	drug containers	vegetables peels	brewery filter mash
Donkeys	2 - 4	oxygen masks	worn utensils	skin/hides
Dogs	5 -10	catheters	expired cosmetics	
Sheep	3 -7		dead animals	
Rodents	8 -15			
Mosquitoes				
Cockroaches				
Flies				
Crows	10 -20			
Storks	7 -14			
Chicken	20 -30			
Ducks	2 -4			

2.3.2. Diversities of bacteria communities on the dumpsite

A total of 8,469,294 v4 region of 16S rRNA gene sequences of bacteria from 78 solid wastes samples passed all quality control filters. The number of high quality sequences per sample ranged from 329 to 291,482 (median 109,718, SD 56,553). Catchall analysis of richness predicted an overall mean of 8,243 ± 759 species per sample (range 349 – 21,092, SD 4,804). Good's coverage ranged from 0.9766 – 0.9934 (mean 0.9837, SD 0.0083). Rarefaction curves for different types of solid waste are shown in (Fig.2.3) The overall diversity of bacteria populations was high with an average inverse Simpson index of 44.21 ± 1.44, Shannon's evenness average 4.95 ± 0.02, Chao1 richness estimator of 5,926 ± 239, and an abundance based coverage estimator (ACE) of 7,202 ± 228.

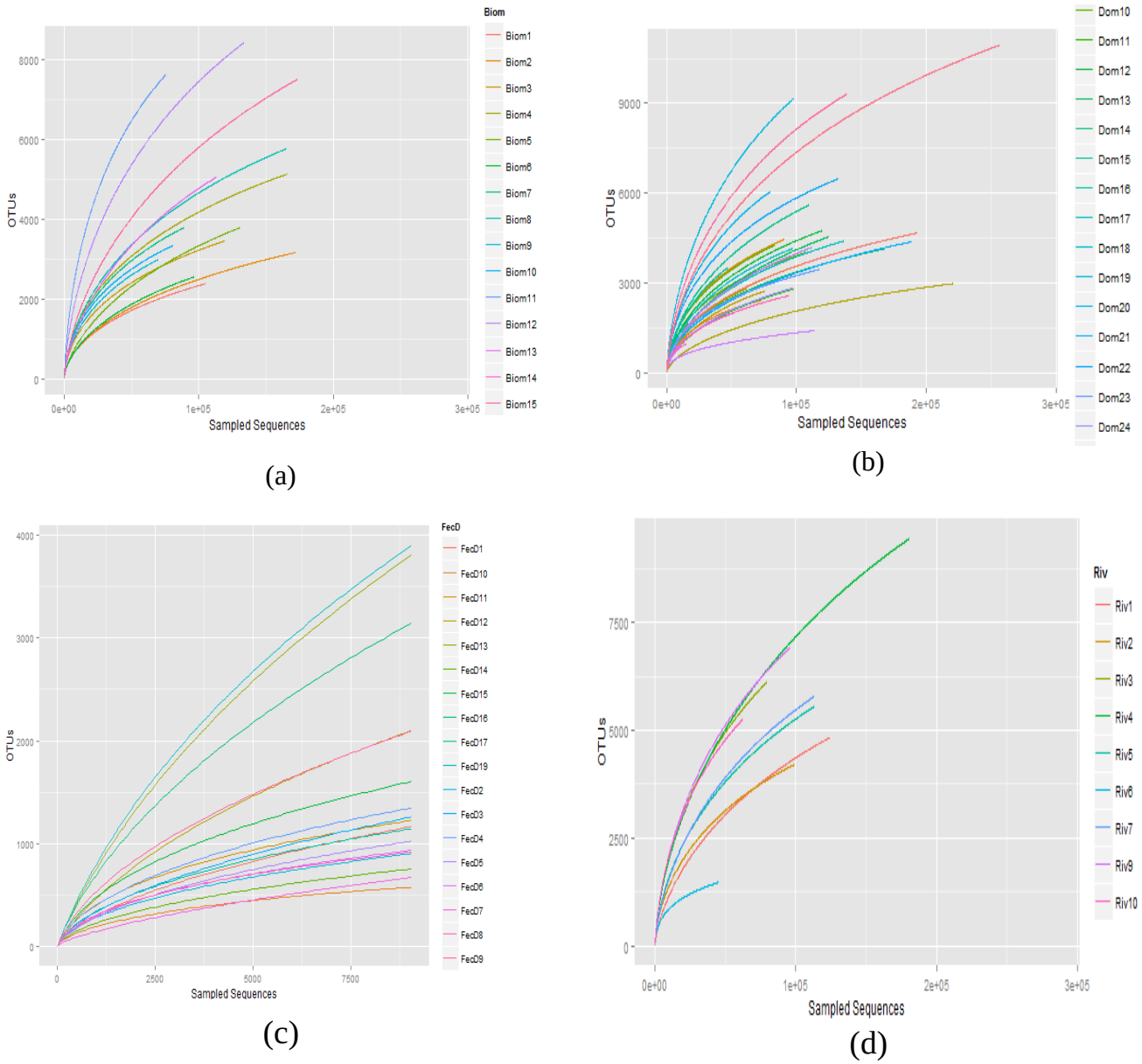


Figure 2.3 Rarefaction curves of bacteria from different solid wastes on the dumpsite. Estimation of OTUs was done at > 97% sequence similarity cut-off. Fig.2.3(a) represents solid biomedical waste, Fig.2.3(b) domestic waste, Fig.2.3(c) faecal material of pigs scavenging on dump and Fig.2.3(d) represents river sludge near the municipal dumpsite. No subsampling was done to any type of solid waste**.**

Thirty-five bacterial phyla were detected, however, only seven of these accounted for more than 93% of all sequences. The predominant phyla were *Firmicutes* (38%), *Proteobacteria* (35%), *Bacteroidetes* (13 %), *Actinobacteria* (3%), *Acidobacteria* (2%), *Chloroflexi* (2%) and *Spirochaetes* (1%). A large number of phyla were rare and had < 1% of all sequences (Fig.2.4). The unclassified bacteria at phylum level accounted for 2.4% of all sequences.

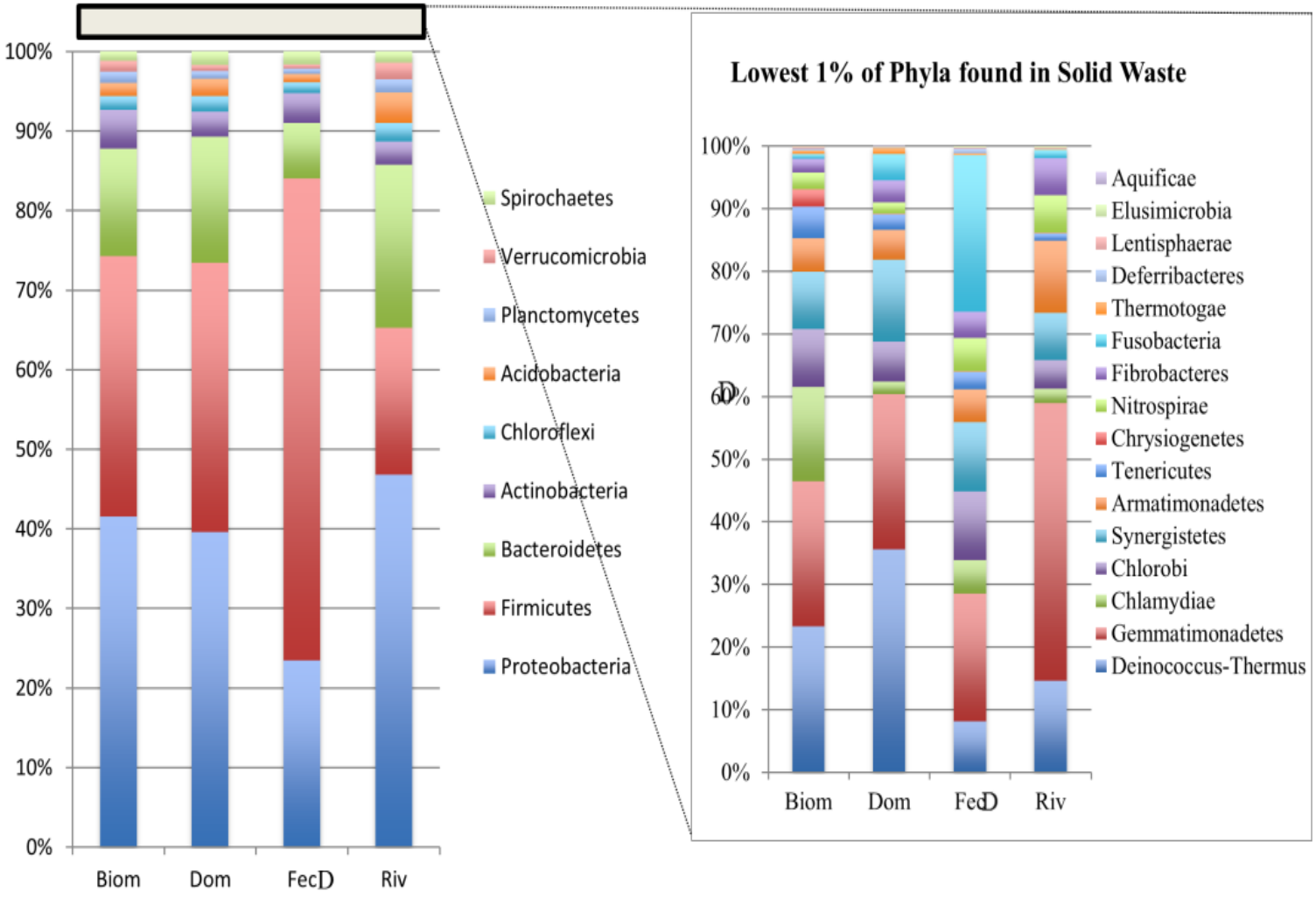


Figure 2.4 Abundance of predominant bacterial phyla on dumpsite. Different colours represent different types of bacterial taxa and their relative abundance. Bacterial taxa were assigned at 97% sequence similarity cut-off level.

Two hundred and eighty eight bacterial taxa were detected at family level, with *Moraxellaceae* (12%), *Clostridiaceae-1* (8%), *Ruminococcacea*e (5%), *Lachnospraceae* (3.7%) and *Lactobacillaceae* (3.4%) being the most prominent. Unclassified bacteria at family level accounted for about 14% of all sequences. A total of 1,350 bacterial genera were detected. *Acinetobacter* was the most abundant accounting for (12.1%) of all sequences followed by *Clostridium sensu stricto* (4.8%), *Proteiniclasticum* (3.4%), *Lactobacillus* (3.4%), *Prevotella* (2.6%), *Enterococcus (*2.9%) and *Escherichia/Shigella* (1.7%) (Fig.2.5). Overall, a total of 76,862 OTUs were detected, however, only 20% (15,272/76,864) were identified more than ten times.

The core microbiota analyses revealed that none of the OTUs were present across all 78 samples at relative abundance of 1% or more. Only one OTU, *Acinetobacter* (assigned sequences 688,875) was found in 62% (48/78) of samples and *Clostridium sensu stricto* (assigned sequences 202,494) in 49% (38/78) of samples. When each type of solid waste was analysed separately, *Proteiniclasticum* and *Acinetobacter* were found in 67% (10/15) of solid biomedical waste samples; *Clostridium_sensu_stricto* in 80% (16/20) of faecal samples of pigs scavenging on dumpsite; *Acinetobacter* and *Proteiniclasticum* were found in 73% (24/33) and 55% (18/33) of domestic solid waste, respectively, and in river sludge, 80% (8/10) of samples had *Acinetobacter.* Indicator analysis clearly revealed higher affiliation of some bacterial OTUs to specific solid waste on the dumpsite.

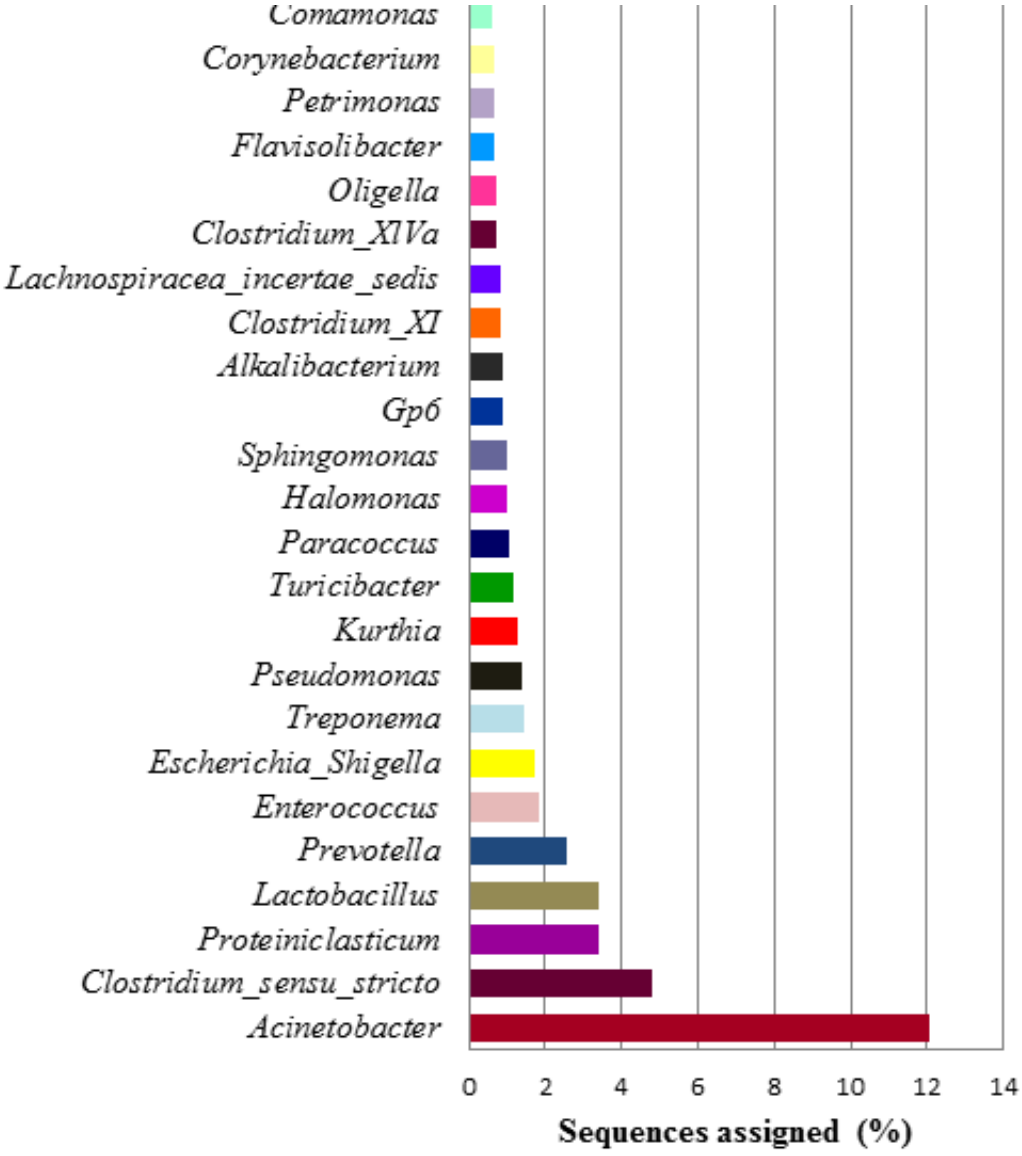


Figure 2.5 Abundance of predominant bacteria at genus level in the municipal dumpsite. Bar graph depicts the percentage of sequence reads assigned to each taxon at 97% sequence similarity cut-off.

Thirteen OTUs were significantly associated with Biom (Indicator value ≥ 70 and P ≤ 0.05) amongst which are *Halomonas*, *Alishewanella*, and *Proteiniclasticum*; five were associated FecD, for example *Enterococcus, Bifidobacterium, Clostridium sensu stricto* and *Cellulosilyticum* and nine were associated with Riv, such as *Commamonas, Lysobacter* and *Flavobacterium* (Table 2.2). None of the OTUs were significantly affiliated with Dom at indicator value > 70 and P ≤ 0.05.

Table 2.2 Indicator analyses of bacterial OTUs associated to different solid waste

OTU	Description (Genus)	#sequences	Indicator value	P-value
	Biomedical solid waste (Biom)			
Otu000140	*Idiomarina*	6078	89	0.001
Otu000011	*Halomonas*	83211	86	0.022
Otu000116	*Sporolactobacillaceae_incertae_sedis*	7683	82	0.023
Otu000035	*Tissierella*	18780	80	0.007
Otu000080	*Alkaliflexus*	16213	79	0.004
Otu000136	*Saccharofermentans*	5945	78	0.005
Otu000124	*Alishewanella*	13449	78	0.053
Otu000003	*Proteiniclasticum*	269626	78	0.001
Otu000141	*Unclassified Bacteroidetes*	7998	75	0.001
Otu000411	*Proteiniclasticum*	3303	74	0.006
Otu000525	*Unclassified Bacteroidetes*	346	73	0.001
Otu000567	*Pseudomonas*	2187	71	0.013
Otu000069	*Corynebacterium*	18152	70	0.046
	Faecal material of pigs scavenging on dumpsite (FecD)			
Otu000114	*Clostridium_sensu_stricto*	12444	78	0.039
Otu000477	*Unclassified Lachnospraceae*	2394	78	0.008
Otu000310	*Clostridium_sensu_stricto*	2749	77	0.009
Otu000005	*Enterococcus*	159372	77	0.005
Otu000104	*Bifidobacterium*	11495	73	0.007
	River sludge (Riv)			
Otu000054	*Cloacibacterium*	30207	84	0.051
Otu000807	*Flavobacterium*	2218	84	0.003
Otu000037	*Lysobacter*	35128	81	0.013
Otu001247	*Unclassified Xanthomonadaceae*	849	77	0.001
Otu001672	*Lacibacter*	479	77	0.001
Otu000017	*Commamonas*	54114	77	0.039
Otu001307	*Ferruginibacter*	613	75	0.001
Otu000133	*Dechloromonas*	7673	75	0.039
Otu001398	*Novosphingobium*	571	74	0.001
Otu000596	*Flavobacterium*	2057	73	0.002
Otu001167	*Niabella*	931	72	0.005

Of the 35 bacterial phyla detected in this study, Metastats revealed 11 phyla significantly different between Dom and FecD. Further, at genus level, out of 1,428 differentially abundant genera, 173 genera were significantly different between Dom and FecD. When Biom and FecD were compared, 8 phyla and 144 genera were significantly different. The Biom and Dom comparison revealed no difference in bacteria community at phylum level ($P > 0.05$), but 9 bacterial family and 39 genera were significantly different. Of the 1,428 genera found in Biom and Dom, 16% (227/1428) were unclassified. The phylum *Lentisphaerae,* 66 bacterial family and 180 genera were significantly different between Dom and Riv solid waste (Appendix 2.4).

2.3.3. Comparison of bacteria community structure and membership

Phylogenetic tree generated using the Yue & Clayton measure as well as the Jaccard index is presented in Fig 2.6 (a) and Fig 2.6 (b), respectively. Results of the Parsimony test obtained after the phylogenetic analysis of the Yue and Clayton tree ignoring the branch length revealed a significant difference in bacterial community structure between Dom-FecD ($P = 0.011$) and Dom-Riv ($P = 0.028$). There was no difference in community structure between Biom-Dom ($P= 0.111$), Biom-FecD ($P = 0.068$), Biom-Riv ($P = 0.5240$ and FecD-Riv ($P = 0.100$). When branch was considered, significantly different structures were found between Dom-Riv ($P = 0.001$) and FecD-Riv ($P=0.034$), while none was detected between Biom-Dom, Biom-FecD, Dom-FecD and Biom-Riv ($P > 0.05$) using Unweighted UniFrac (Table 2.3). Further, comparison of the community membership using the phylogenetic tree based on the Jaccard index; the parsimony test revealed that Biom-Dom, Dom-FecD and Biom-Riv ($P > 0.05$) (Table 2.4) have the same community membership, while in Biom-FecD ($P = 0.016$), Dom-Riv ($P = 0.002$) and FecD-Riv ($P = 0.002$) were different. When Unweighted UniFrac analysis was performed, only Dom-FecD ($P= 0.039$) had significantly different community membership. Analysis of molecular variance revealed a significant similarity in bacterial community between Biom-Dom (Yue & Clayton, $P = 0.475$, Jaccard index, $P = 0.012$), while the rest of the groups were statistically different ($P < 0.008$) (Table 2.4).

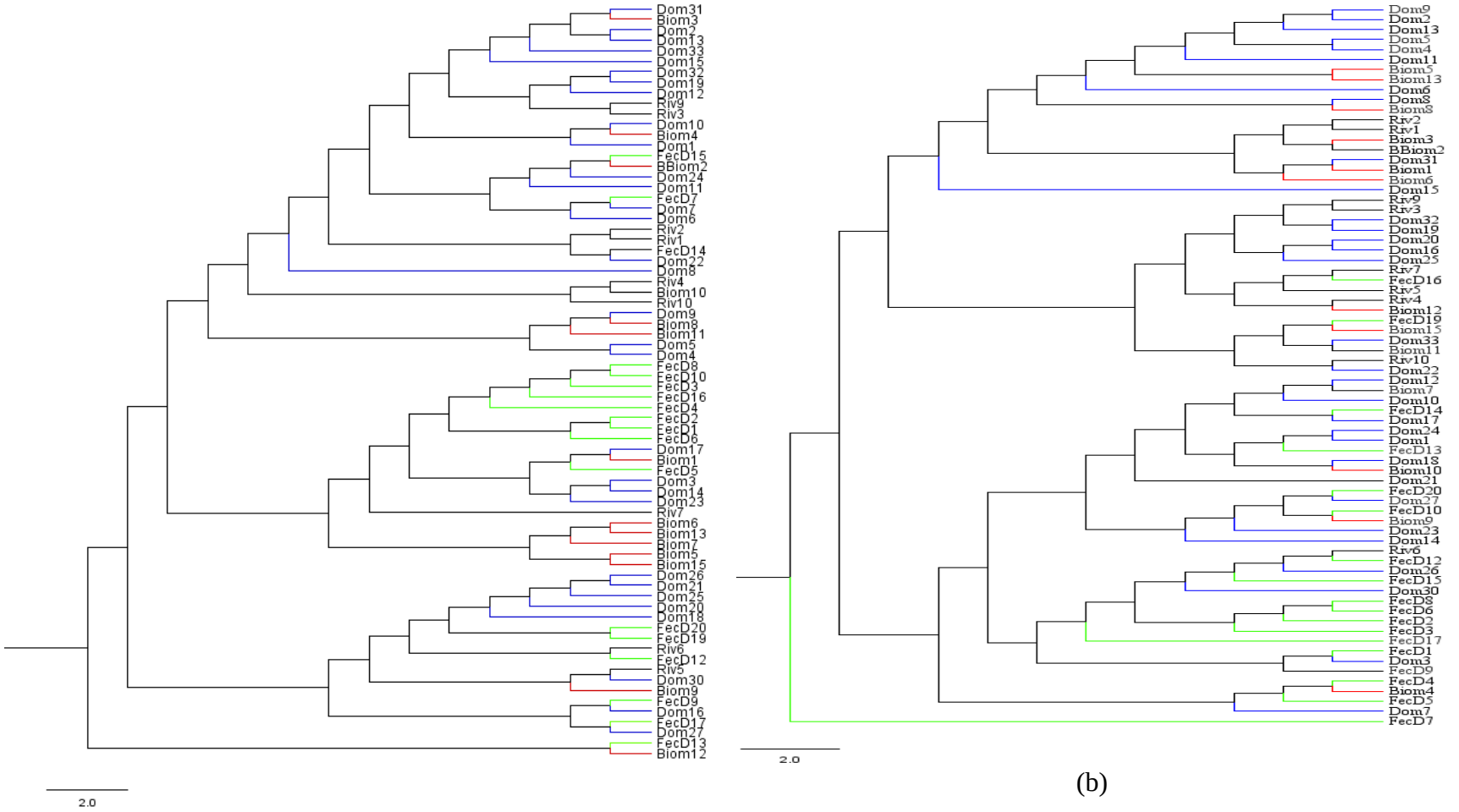


Figure 2.6 OTU based dendrogram demonstrating the similarity and differences of the bacterial communities from different solid wastes.

Fig.2.3 (a) is a Yue & Clayton indises based dendrogram showing dissimilarities between the structures of bacterial community from different solid wastes. Fig.2.3 (b) is based on traditional Jaccard index which shows community membership of bacteria from different

Table 2.3. Comparison of bacterial community structure between different solid wastes

	Parsimony		Unweighted UniFrac		Amova	
Groups	ParsScore	ParsSig	UWscore	UWSig	FScore	P* - value
Biom-Dom	10	0.111	0.894454	0.052009	2.28212	0.012
Biom-FecD	8	0.068	0.910399	0.085009	3.14737	< 0.001
Dom-FecD	10	0.011	0.853535	0.127009	3.38992	0.002
Biom-Riv	7	0.524	0.934552	0.197003	1.92136	0.016
Dom-Riv	6	0.028	0.959495	0.001009	1.80755	0.028
FecD-Riv	6	0.1	0.967847	0.034006	2.46059	0.004

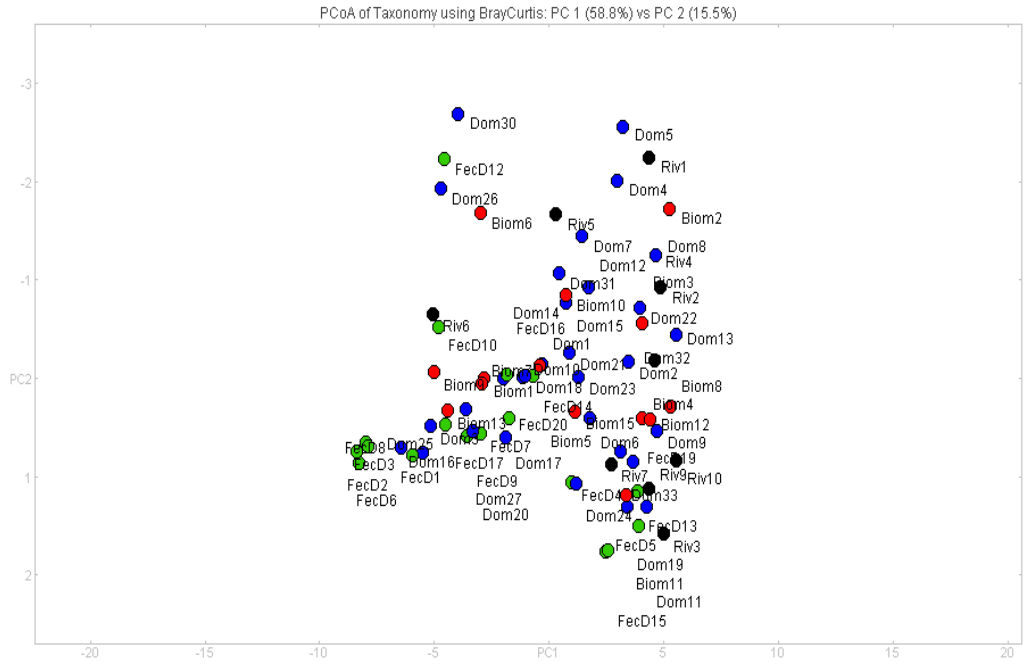
P* = Correction for multiple comparisons (Bonferroni): Significance P- value ≤ 0.0083. Analysis was based on phylogenetic tree generated using Yue & Clayton measure.

Table 2.4 Comparison of bacteria community membership between different solid wastes

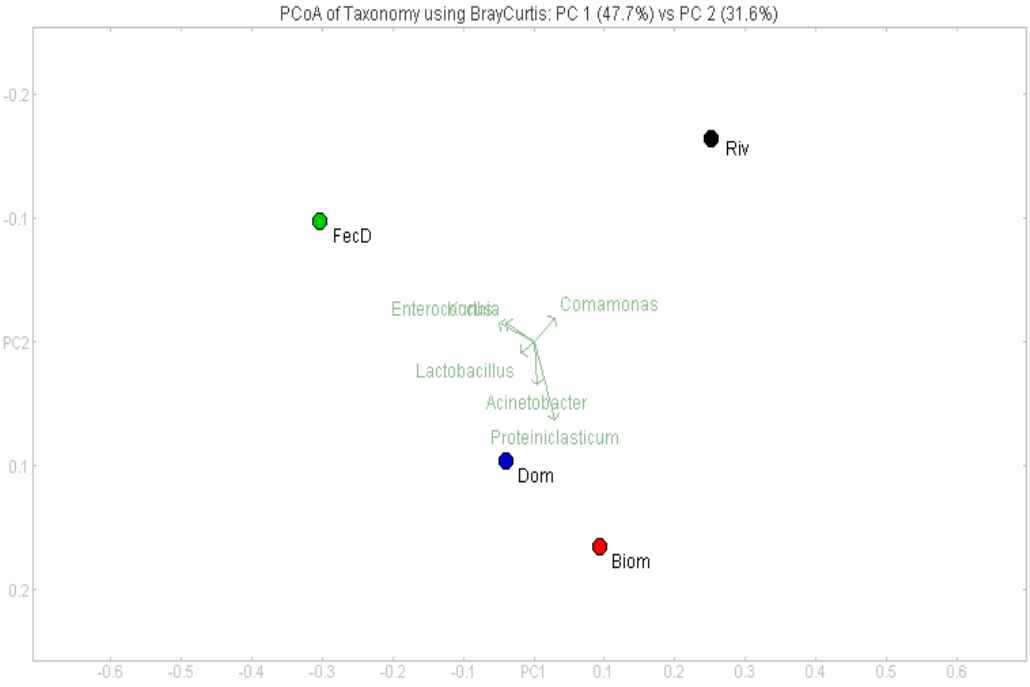
	Parsimony		Unweighted UniFrac		Amova	
Groups	ParsScore	ParsSig	UWScore	UWSig	FScore	P* - value
Biom-Dom	11	0.297	0.982725	0.149009	0.988888	0.475
Biom-FecD	7	0.016	0.972863	0.235009	2.24675	< 0.001
Dom-FecD	12	0.08	0.981599	0.039009	2.39594	< 0.001
Biom-Riv	5	0.061	0.98467	0.055007	1.37949	< 0.005
Dom-Riv	5	0.002	0.98451	0.060009	1.57885	0.004
FecD-Riv	4	0.003	0.976693	0.159006	2.25111	0.001

Analysis was based on phylogenetic tree generated using Jaccard index.

Further, Fig.2.7 (a) and Fig.2.7 (b) are the graphic representation of the principal coordinate analyses plot based on Bray-Curtis distances. The spatial separation between centers of the clouds of the bacteria community structure of different solid waste using Amova have shown statistical difference between Biom-FecD ($P < 0.001$), Dom-FecD ($P = 0.002$) and FecD-Riv ($P = 0.004$), but the same community structure between Biom-Dom ($P = 0.012$) which is clearly depicted in a PCoA plot constructed from a pool of bacteria community of the same waste type Fig.2.7 (b). Bacterial OTUs responsible for the difference in clustering of solid waste types were *Halomonas, Acinetobacter* and *Lactobacillu*s from Biom and Dom solid waste; *Enterococcus* and *Kurthia* in FecD, and *Lysobacte*r in Riv.



(a)



(b)

Figure 2.7. PCoA plots showing clustering of bacterial community from different solid wastes. The PCoA plot was built based on Bray-Curtis dissimilarity distances. (Fig.2.4a) was generated from individual samples, while (Fig.2.4b) was from the pool of the same types of waste.. Distances between symbols on the ordination plot reflect relative dissimilarities between different solid waste types.

2.3.4. Analysis of individual solid waste types

When each type of solid waste was analysed separately, more than 65% of sequences from each type passed all quality control filters. Bacterial species richness, abundance and diversity were found to be high in all types of solid wastes as shown in (Table 2.5) for ACE, Chao 1, Shannon, jackknife and InvSimpson indices as well as number of OTUs. Riv had the highest bacteria community diversity and evenness (InvSimpson = 58.5 ± 1.2, Shannon = 5.6 ± 0.01) followed by Biom (InvSimpson = 54.2 ± 1.3 and Shannon = 5.1 ± 0.01). Despite the higher number of quality reads and OTUs detected therein, Dom solid waste had the least bacteria community evenness and diversity followed by FecD (InvSimpson, Dom = 26.9 ± 0.7, FecD = 31.2 ± 0.9; Shannon index, Dom = 5 ± 1.0, FecD = 4.5 ± 0.01).

Table 2.5 Summary of good quality sequences and diversity indices of different solid waste at species level

*Samples	Valid reads	OTUs	ACE[a]	Chao1[a]	Shannon[a]	InvSimpson[a]
Dom	3,466,427	26,243	7,338 ± 230	6,788 ± 244	5 ± 1.0	26.9 ± 0.7
Biom	1,706,442	18,994	8,218 ± 249	6,756 ± 257	5.1 ± 0.01	54.2 ± 1.3
Riv	926,648	15,025	10,422 ± 283	8,219 ± 287	5.6 ± 0.01	58.5 ± 1.2
FecD	2,369,595	16,697	6,183 ± 214	5,073 ± 226	4.5 ± 0.01	31.2 ± 0.9

OTUs - Operational taxonomic units, ACE - abundance based coverage estimator. [a]Calculations were performed using Mothur package with an OTU definition at > 97% sequence similarity.*Samples: Dom – Domestic solid waste; Biom- Solid biomedical waste; Riv – River sludge; FecD – Faecal material of pigs scavenging on dumpsite

Proteobacteria was the most predominant phylum in Biom (37.4%), Dom (35.7%) and Riv (42.5%) solid wastes, while *Firmicutes* predominated in FecD (59.1%) only. Majority of the bacterial phyla in all solid waste were rare and contributed < 0.1% of all sequences. Biom and FecD had only 11 phyla accounted for 98.2% and 99.7% all sequences, respectively. Likewise, in Dom and Riv, 12 phyla accounted for 99.6% and 99.4% of all sequences, respectively (Fig.2.8).

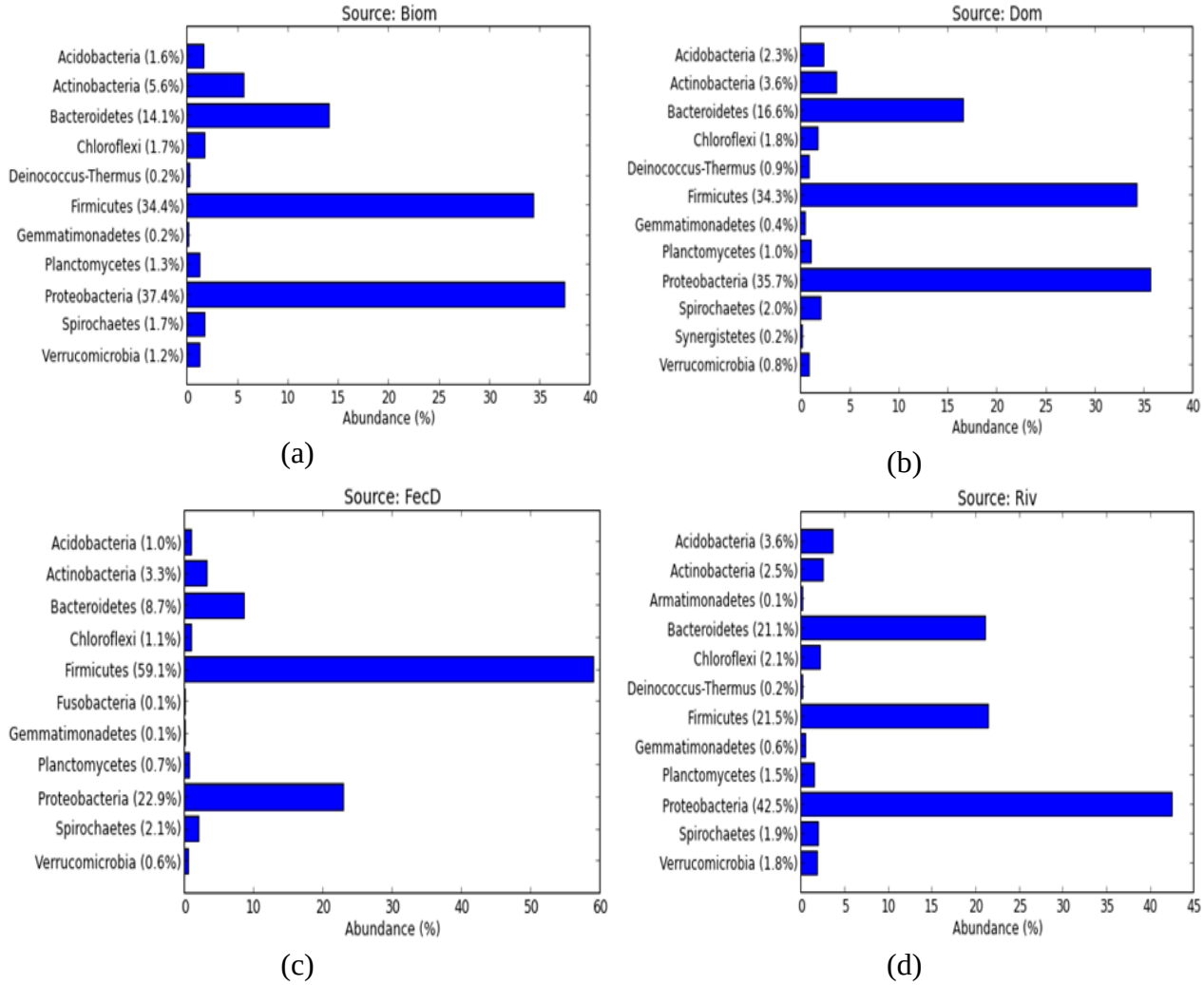
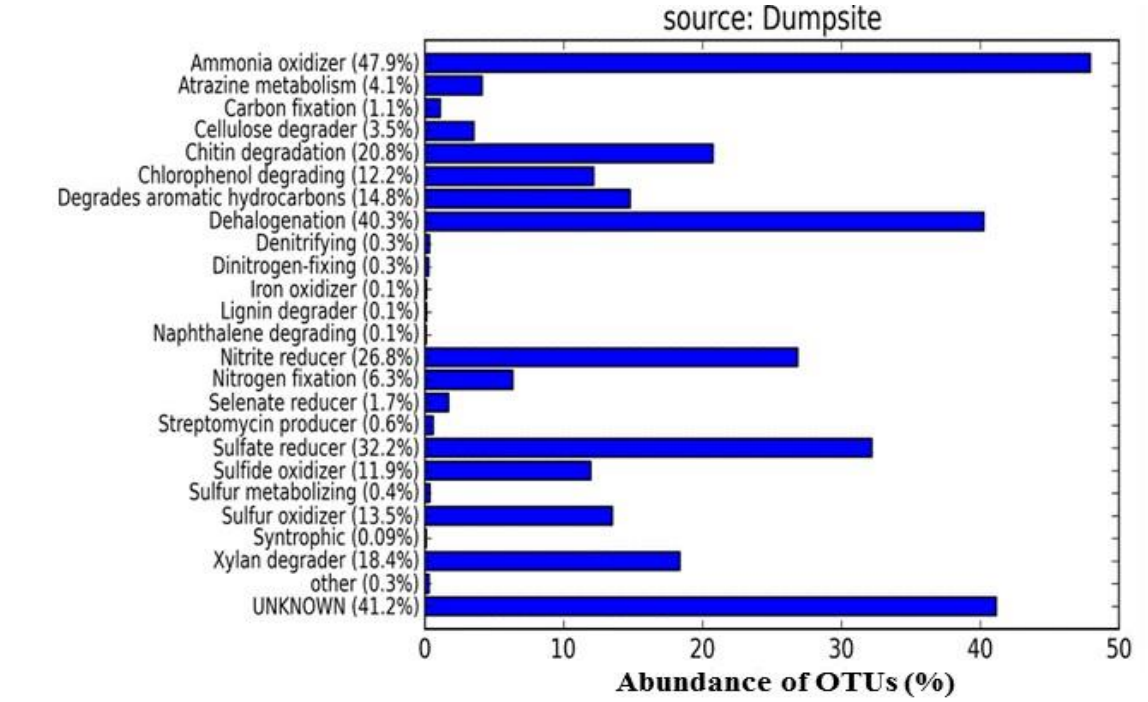


Figure 2.8 Abundance of predominant bacterial phyla in different solid waste. Solid biomedical waste (Biom, *n* =15 Fig.5a), domestic solid waste (Dom, *n* = 33, Fig.2.5 b), faecal material of pigs on scavenging on dumpsite (FecD, *n* = 20 Fig.2.5 c) and in river sludge (Riv, *n* =8 Fig.2.5 d). Only phyla at abundance ≥ 0.1% are shown. The bar graph depicts the percent of sequences assigned to each taxonomic group at cut-off of 97% sequence similarity.

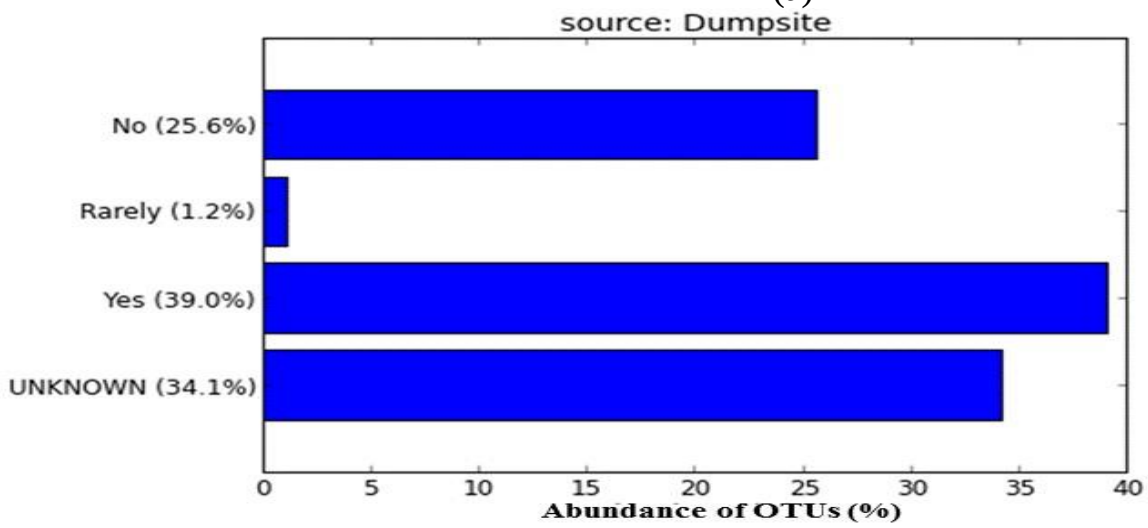
2.3.5. Taxonomy to phenotype mapping of OTUs from the municipal dumpsite

Taxonomy to phenotype mapping revealed diverse metabolic processes on the dumpsite. Fig.2.9 is the taxonomic to metabolism and taxonomic to human pathogens mapping, respectively, of the bacteria OTUs detected in this study. Twenty-three different metabolic processes were revealed, and 41.2% of the OTUs belonged to the bacteria with unknown metabolic processes. The processes revealed include ammonium oxidation, dehalogenation, chitin degradation, aromatic hydrocarbon degradation, chlorophenol degradation and atrazine metabolism.

Similarly, taxonomy to human pathogens mapping revealed that 39.0% of OTUs were related to human pathogens, 25.6% are not human pathogens, 1.2% rarely human pathogens, and again 34.1% of the OTUs fell in group of tunknown pathogenicity. Detailed mapping of OTUs from different waste type is shown in (Appendix 2.5).



(a)



(b)

Figure 2.9. Taxonomy to phenotype mapping of bacterial OTUs from the dumpsite. Fig.2.6 (a) show the abundance of OTUs for different metabolic process and Fig.2.6 (b) show the abundance of OTUs based on whether they are related to human pathogen, not human pathogen, rarely pathogens and unknown pathogenicity.

2.4. Discussion

This study has identified a substantial abundance and diversity of bacteria in a municipal dumpsite in Arusha, Tanzania. The estimated richness (8,243 species) per sample and high diversity (InvSimpson index = 44.21) of bacteria on the dumpsite surpasses many of the previous culture based studies. The detection of 76,862 OTUs from 35 bacterial phyla using high throughput sequencing technique gave a more comprehensive look at the bacterial community on the dumpsite. Of the four predominant phyla, *Firmicutes* was the overall most abundant and includes a variety of gram-positive bacteria. It's particularly dominance in fecal material of pigs is consistent with previous reports by (Pajarillo *et al*., 2015) as well as in horse fecal microbiota by (Costa *et al*., 2012 and Shepherd *et al*., 2012). The predominance of *Firmicutes* in FecD, especially the genus *Clostridium sensu stricto* may be due to the feeding habits of pigs on fibre from a variety of unsorted solid waste as reported by (Middelbos *et al*., 2010 and Yildirim *et al*., 2010).

The presence of some bacterial genera exclusively to some types of solid waste, justifies a need to sort and treat solid wastes differently. This would deter a possible genetic material exchange, a process which can result in the emergence and re-emergence of new bacteria of public health importance. The revealed affiliation of *Enterococcus* to faecal material of pigs scavenging on dumpsite is consistent with previous findings in faecal material of pigs (Dang *et al*., 2011; Novais *et al*., 2013 and Freitas *et al*, 2011). The potential of *Enterococcus* in nosocomial infections and multidrug resistance (Arias *et al*, 2012; Lebreton *et al*., 2013 and Marcadé *et al*., 2013) suggests a need to further examine the antibacterial resistance of isolates from pigs scavenging on dumpsites, and their relation to human and animal pathogens.

The affiliation of *Halomonas* (1%) to solid biomedical waste is consistent with findings in gold mines (Lin *et al*, 2012) and in contaminated heavy metals wastes (Dziewit *et al*, 2013), as both environments are rich in chemicals. *Halomonas* metabolize cyanide (Khamar et al., 2015) and some are reported to carry plasmid *pZM3H* which confers resistance to different chemicals (Dziewit *et al*, 2013), leading to their application in soil remediation (Makhdoumi *et al*., 2015; Achal *et al.*, 2012). The fact that these bacteria are predominant, it would be worthwhile to study their roles in the dumpsite, and examining if they contain plasmids that confer resistance to

different chemicals. This could lead into their application in the control of chemical pollutants, especially cyanide at the dumpsite.

Proteobacteria was the predominant second overall phylum and the most abundant in solid biomedical waste, domestic solid waste and river sludge. Several studies have linked the predominance of this phylum with human and animals diseases (Costa *et al*, 2012; Suchodolski *et al*, 2012; Suchodolski *et al*, 2010). At the genus level, the overall predominance of *Acinetobacter* (12%) on the dumpsite is consistent with report by (Saini *et al*., 2004 and Hossein *et al*., 2013) in solid waste. Previous studies report some species of *Acinetobacter* associated with human and animal's diseases. A case example is the existence of *Acinetobacter* in clinical isolates from intensive care unit (Omer *et al.*, 2015), animal with urinary tract infections (Pomba *et al.*, 2014) and as a causative agent of the nosocomial outbreak in Spain (Mosqueda *et al*, 2013). Its predominance in such extreme environment may be linked to their re-counted capacity to detoxify chemicals as well as their multidrug resistance (Franzetti *et al*., 2014; Zarrilli *et al*., 2013 and Mosqueda *et al.*, 2013) which would support their survival in an environment with diverse chemicals from unsorted solid wastes.

Escherichia and *Shigella* spp. were among the predominant genera. These bacteria were also reported in several culture based studies (Rastogi *et al*., 2011; Anitha *et al*., 2012 and Oyeleke *et al.*, 2009). Their capacity to acquire multidrug resistance and their association to human and animal pathogens is well known (Doumith *et al*., 2012; García *et al.*, 2010 and Fritah *et al.*, 2014). The abundance of these bacteria within the dumpsite with such extraordinary interaction between animals, humans and microbes may be causing potential health risks to human and animals interacting on the dumpsite. The predominance of these bacteria at the dumpsite underscores a need to study on whether a link exists between bacteria on the dumpsite and the known pathogens. Understanding the potential health risks associated with bacteria from the dumpsite would improve the health of not only the humans interacting with the dumpsite, but with all others that may come in contact with them or other animals.

The significant similarity in community structure and membership of bacteria between solid biomedical waste and domestic solid waste; solid biomedical waste and river sludge, and faecal materials of pigs scavenging on dumpsite and river sludge, implies that similar types of bacteria are found in multiple types of solid wastes. This may be attributed to by the disposal of unsorted

wastes in the same open dumpsite as well as presence of high interaction between animals, dumpsite workers and microbes, hence exchange of bacteria between them; including possibility of people working on dumpsite to carry these bacteria to their family members and to other people who might come into contact with. Despite the fact that all samples were from the same dumpsite, distinct affiliation of some bacteria OTUs exclusively to some waste, implies that some bacteria have specific nutritional and environmental requirements to flourish. It would be interesting to study the bacterial community of sorted solid wastes to examine changes in abundance and diversity of the associated bacterial community.

2.5. Conclusion

This study reports a rich and diverse bacterial community in Arusha municipal dumpsite. The species richness reported here shows the complexity of this man-made ecosystem and calls for further research to assess for a link between human diseases and the dumpsite. Understanding the role of the bacteria within the dumpsite and bacteria found within different types of waste will provide insight into proper disposal of the waste, as well as, limit the risks to human health associated with the dumpsite.

CHAPTER THREE

The faecal microbiota of free-range pigs (*Sus scrofa domesticus*) scavenging on a municipal dumpsite[†]

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Abstract

Pigs scavenging in dumpsites are exposed to diverse microbes of public health importance, yet little is known of their faecal microbiota. We characterized the faecal microbiota of pigs scavenging in Arusha municipal dumpsite as compared with conventionally reared pigs. Thirty-nine faecal samples were collected from pigs under three management systems: Pigs scavenging on a municipal dumpsite (FecD), indoor-reared pigs (FecI) and pigs transferred from indoor to free-range on the dumpsite (FecIF). Total DNA was extracted; v4 region of 16S rRNA gene amplified and sequenced using an Illumina MiSeq platform. A total of 4,364,507 sequences with an average of 114,852 reads per sample passed quality control. The predicted mean of species per sample was 5,979. There was significant difference in bacteria community membership and structure between FecD and FecI (Yue and Clayton, P = 0.001; Jaccard, P = 0.032), respectively.

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Two clusters of faecal microbiota were found; the first comprised pigs scavenging at dumpsites only, while the second had a combination of all management systems. Thirty one phyla were identified and the predominant were *Firmicutes* 51%, *Proteobacteria* 26%, *Bacteroidetes* 12%, *Spirochaetes* 3% and *Actinobacteria* 3%. Generally, 40803 Operational taxonomic units (OTUs) at 97% sequence similarity were found; however, only 19% were detected more than ten times. Out of 830 genera, 74 were significantly different ($P \leq 0.05$) between FecD and FecI amongst which 27 (36%) were exclusive to FecD. Some pathogenic genera like *Brucella, Rickettsia, Afipia, Ignavigranum* and *Listeria* were exclusive to FecD. There is a unique faecal microbiota in pigs scavenging on the dumpsite. Some bacteria exclusively to FecD are of public health importance. These findings suggest a controlled grazing of livestock to mitigate possible health risks linked to free range food animals. Further study of the gut microflora of free-range pigs at dumpsites and their clinical significance is warranted.

Keywords: Pigs, Faecal microbiota, 16S rRNA, Municipal dumpsites, Free-range pigs

3.1. Introduction

Studies of faecal microbiota of animals have received increasing attention due to the complexity of gut microflora as well as its importance for public health and disease. Despite the known importance of fecal microbiota (Round and Mazmanian, 2009; Sokol *et al*., 2006; Tlaskalová-Hogenová *et al*., 2011), there is limited information on its composition in free-range animals and its impact on the environment. The current rising trend of urban and peri-urban Agriculture in Africa has resulted into pig production under free range management to become a viable livestock farming system playing a major role in meat production either for home consumption or income generation (Hamill *et al.,* 2013; Jacobson *et al.,* 2010; Karimuribo *et al.,* 2001; Lekule and Kyvsgaard, 2003; Mutua *et al*., 2011; Wabacha *et al.,* 2004; Wilson and Swai, 2013). One advantage of free range farming system, especially for pigs includes low capital investments, which allow smallholder farmers the opportunity to enter into livestock keeping. Despite the benefits which pig production can bring to a household, keeping pigs under a free range system increases the risk of the pigs of acquiring diseases, either production-limiting or zoonotic in nature (Kagira *et al*., 2010; Nwanta *et al.,* 2011; Thomas *et al.,* 2013).

In urban and peri-urban farming settings with high human-animal interaction free-ranging pigs may be a cause of transmission of zoonotic diseases (Cavaco *et al*., 2008; Hamill *et al.,* 2013; Horton *et al.,* 2011; Jørgensen *et al.,* 2007; Ocaido *et al.,* 2013). Some reported diseases in Tanzania include Leptospirosis (Kessy *et al*., 2010; Mgode *et al.,* 2014; Wilson and Swai, 2013), Campylobacterosis (Komba *et al.,* 2013; Mdegela *et al.,* 2011; Ocaido *et al.,* 2013) which have serious health implications for humans and other animals. Of interest to this study was the management environment of free-range pigs, which scavenged on dumpsites in a peri-urban area. Dumpsite composition included solid waste, typically organic waste from households, markets and abattoirs, waste from agriculture and industries as well as chemical/ pharmaceutical/ biomedical waste, on which the pigs scavenged. Since free-range management of pigs would presumably have a profound effect on the composition of gut flora, detailed study of fecal microbiota of pigs scavenging on dumpsite would help in surveillance of pathogens of potential public health importance. Despite the known importance of fecal microbiota in different animal species (Costa *et al.,* 2012; Costa et al., 2014; Round and Mazmanian, 2009; Sokol *et al.,* 2006; Weese *et al.,* 2014), there are no reports on microflora of pigs free-ranging on dumpsite.

Studies of microbiota have traditionally used culture-based methods (Moore *et al*., Kornegay 1987; Robinson *et al.,* 1981; Robinson *et al.,* 1984), but recently it has become evident that such methods provide a very superficial understanding of the microbiota because of its complexity and large percentage of the microbiota currently unculturable (Abriouel *et al*., 2011; R. Arumugam *et al.,* 2013; Fuhrman, 2012; Handelsman, 2004; Hirsch *et al.,* 2010; Lowe *et al*., 2011; Vandecandelaere *et al.,* 2012). With the advent of culture-independent methods, particularly next generation sequencing, assessment of complex polymicrobial environments has now become much more informative.

In the current study, we compared fecal microbiota of free range pigs, which continuously scavenged on the dump with that of pigs reared indoors using v4 region of 16S rRNA gene sequenced using Illumina MiSeq platform. To our knowledge, this is the first study in Tanzania reporting the influence of free-range management of pigs in an urban dumpsite on composition of enteric microbiota.

3.1. Materials and methods

3.1.1. Study site and fecal samples

Site for this study was the Arusha municipal dumpsite in Tanzania which is in close proximity to the household of smallholder farmers, who allow their livestock, mostly pigs, to scavenge on solid wastes. A detailed description of the dumpsite is shown in (Appendix 1.1). Animals for this study were pigs, aged 4 - 10 months. Group 1 pigs continuously scavenged on the dumpsite for their entire life (FecD, *n*=19) and did not receive any supplemental feeding. Group 2 pigs (FecI, *n* = 8) were reared indoors at a Livestock Training farm (LITA, Tengeru, Arusha) at about 40 km away from the dumpsite, while the third group (FecIF, *n* = 13) comprised of FecI pigs, which were shifted from indoor rearing to the free ranging on the municipal dumpsite. This group was sampled within the first month after shifting from indoor to free range management. Samples were the core of fresh fecal material. Faecal materials from FecD and FecIF were sampled concurrently.

3.1.2. Extraction of total genomic DNA

Total genomic DNA was extracted from about 250 mg of faecal samples of pigs as shown in chapter two, section 2.2.2.

3.1.3. 16S rRNA amplification, library construction and sequencing

Amplification of v4-16S rRNA gene, library construction and sequencing was as shown in chapter 2 section 2.2.3

3.1.4. Quality control and statistical analysis

The Mothur package algorithms (v1.34.1) were used for both quality control and sequence data analysis (Schloss *et al.,* 2009). After paired end reads were assembled, sequences were aligned with the Silva 16S rRNA reference database (www.arb-silva.de) (Quast *et al.,* 2013). Sequences that were < 239 bp and > 260 bp in length or contained > 2 ambiguous base calls or long runs (>8 bp) of homopolymers or did not align with the correct region were removed. Chimeras were identified using Uchime (Edgar *et al*., 2011) and eliminated. Catchall analysis was used to assess

species richness (Bunge, 2012). Taxonomy was assigned using the RDP taxonomy database (http://rdp.cme.msu.edu/index.jsp) (Cole *et al.,* 2014). Sequences were binned into operational taxonomic units (OTUs) at 97% sequence similarity level.

Subsampling was done to normalize sequence numbers for further comparison (Gihring *et al.,* 2012) and it consisted of random selection of a number of sequences from each sample consistent to the lowest abundance of all samples. Population diversity, richness estimation and coverage were established by generating collector's curves of the Chao1 richness estimator (Chao, 1984), the inverse Simpson diversity index (Hunter and Gaston, 1988) and Shannon weaver index for bacterial population evenness (Chao and Shen, 2003). Rarefaction curves at 0.03 distances which were plotted using Phyloseq package (McMurdie and Holmes, 2013) in R version 3.1.2.

Community membership was compared using the traditional Jaccard index, while community structure was assessed using the Yue & Clayton measure of dissimilarity. The core microbiota was assessed through identification of OTUs present in all samples at a minimum abundance of 1%.

Dendrograms were created using Mothur to compare the similarity of faecal microbiota among all samples using both Jaccard index and Yue & Clayton measure which account for the relative abundances in each sample. Figures were generated by FigTree v1.4.2. Parsimony (unifrac unweighted and unifrac weighted) tests were applied to the Jaccard and Yue & Clayton OTU based trees to determine significance of clustering between the groups. The statistical significance of the separation was also assessed using Analysis of Molecular Variance (AMOVA), and Homogeneity of Molecular Variance (HOMOVA). The Metastats (Meadow *et al.,* 2014) and Indicator analysis (Dufrêne & Legendre, 1997) were used to identify specific OTUs that characterize each of the pig's management system. Indicator values (IV) ranged from 1 -100 with higher values for stronger indicators. Only OTUs with stronger indicator value (IV > 30 and P-value ≤ 0.05 were considered good indicators. Shared OTUs files generated in Mothur v.1.34.1 were converted into biom file and imported into MEGAN5 v5.5.3 (Mitra *et al.,* 2011) where further taxonomic profile comparison for the relative abundance, principal coordinate analysis (PCoA) based on Bray-Curtis distances (Beals, 1984) and heat-map of faecal microbiota were analysed. A p-value of ≤ 0.05 was considered significant for all comparisons.

3.2. Results

A total of 4,364,507 v4 region of 16S rRNA gene sequences of bacteria from 39 faecal samples of pigs passed all quality control filters. The number of sequences per sample ranged from 551 to 291467 (mean 114852, SD 56720). Only 37 samples each with sequences more than 16,900 were considered for the downstream analyses. Catchall analysis of richness predicted a mean of 5979 species per sample (median 4478, SD 4011). Good's coverage ranged from 0.9876 – 0.9962 (mean 0.9876, SD 0.00617). Rarefaction curve based on subsampling of 9016 sequences per sample is displayed in (Appendix.3.1). Population diversity was high with an average inverse Simpson index of 37.75 (SD 27.93, range 2.9514 – 99.8023), Shannon's evenness values were on average 5.8138 (SD 1.05, range 3.35 – 7.80) and Chao1 richness estimator was on average 3193.9 (SD 2076.4, range 823 -8318). Summary of quality sequence reads and diversity indices of faecal microbiota of pigs from different management systems is shown in (Appendix 3.2).

Comparison of diversity of faecal microbiota revealed that there were no significant differences between the three management systems; FecD-FecI (InvSimpson index 23.68 vs 38.06, p = 0.1091), FecD-FecIF (23.69 vs 41.19, p = 0.062) as well as between FecI-FecIF (38.06 vs 41, p = 0.42). It was also noted that there was no difference in evenness of bacterial communities between FecD-FecI (Shannon index 5.60 vs 6.41, p = 0.053) as well as between FecD –FecIF (5.60 vs 5.73, p = 0.37). However bacteria community evenness was significantly different between FecI-FecIF groups (Shannon index 6.41 vs 5.73, p = 0.04).

Two main clusters of faecal microbiota were evident (Fig.3.2). The first cluster (A) comprised exclusively of FecD pigs, while cluster B had a combination of FecD, FecI and FecIF faecal microbiota. A similar pattern was visualized in a PCoA plot, in which clearly a cluster of FecD microbiota is separated from the rest

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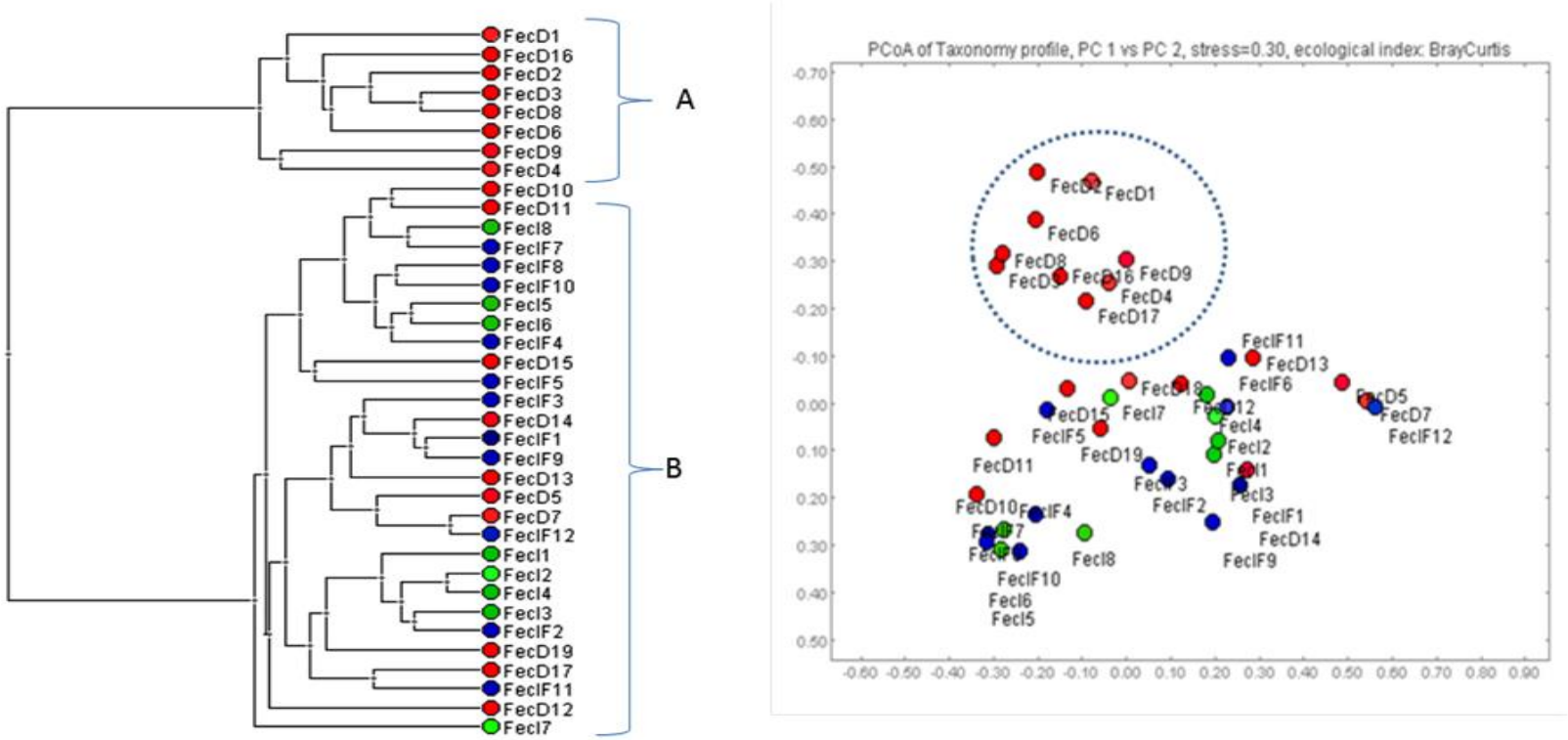

Figure 3.1 Dendrogram and PCoA plots show clustering of faecal microbiota of pigs from different management systems. Two main clusters are evident. Cluster A is comprised of faecal microbiota exclusive to FecD while cluster B comprises of faecal microbiota from all the three management systems. The PCoA plot built on Bray-Curtis dissimilarity distances shows a similar clustering pattern where some FecD groups are away from the rest. Distances between symbols on the ordination plot reflect relative dissimilarities between different pig management systems. The OTUs are estimated at 97% 16S rRNA sequence similarity. Different color represents different management systems, red is for pigs scavenging on dumpsite (FecD), Green is for pigs reared indoor and blue is for pigs shifted from indoor to free range on the dumpsite.

Community membership and population structure were significantly different based on parsimony test applied to both Yue and Clayton (P = 0.001) and Jaccard (P = 0.014) between FecD and FecI pigs as well as between FecD and FecIF pigs, Yue and Clayton (P = 0.001) and Jaccard (P = 0.032). There was no difference in community membership and population structure for faecal microbiota from FecI and FecIF (Yue and Clayton P = 0.19, Jaccard P = 0.18). Significant differences in community structure were also observed with AMOVA (FecD - FecI, P < 0.001; FecD-FecIF, P < 0.004) and HOMOVA (FecD – FecI, P = 0.03, FecD – FecIF, P = 0.035); likewise there was no significant difference in community structure between faecal microbiota from FecI and FecIF (AMOVA, P= 0.227, HOMOVA, P = 0.522).

Thirty-one bacterial phyla were identified (Table 3.1, Fig.3.3), however, only six phyla (*Firmicutes* 51%, *Proteobacteria* 26%, *Bacteroidetes* 12%, *Spirochaetes* 3%, *Actinobacteria* 3% and *Acidobacteria* 1%) accounted for 96% of all sequences. When each management system was individually analysed, a similar pattern was noted whereby the phyla *Firmicutes*, *Proteobacteria* and *Bacteroidetes* in that order, were the most abundant. Interestingly, *Firmicutes* accounted for 63% of bacteria sequences of pigs scavenging on dumpsite, while in indoor reared pigs and pigs shifted from indoor to scavenging on dumpsite it was 46% and 43%, respectively. The abundance of *Bacteroidetes* was higher in indoor reared pigs and pigs shifted to scavenging on dumpsite (in each case accounting for 21% of sequences in each group) compared to 7.5% in pigs permanently scavenging on dumpsite. There were few significant differences in relative abundance between bacterial phyla from scavenging pigs and indoor reared pigs (Table 3.1). The most pronounced difference was the *Bacteroidetes* (FecD vs FecI, 0.084 vs 0.20; p = 0.0036).

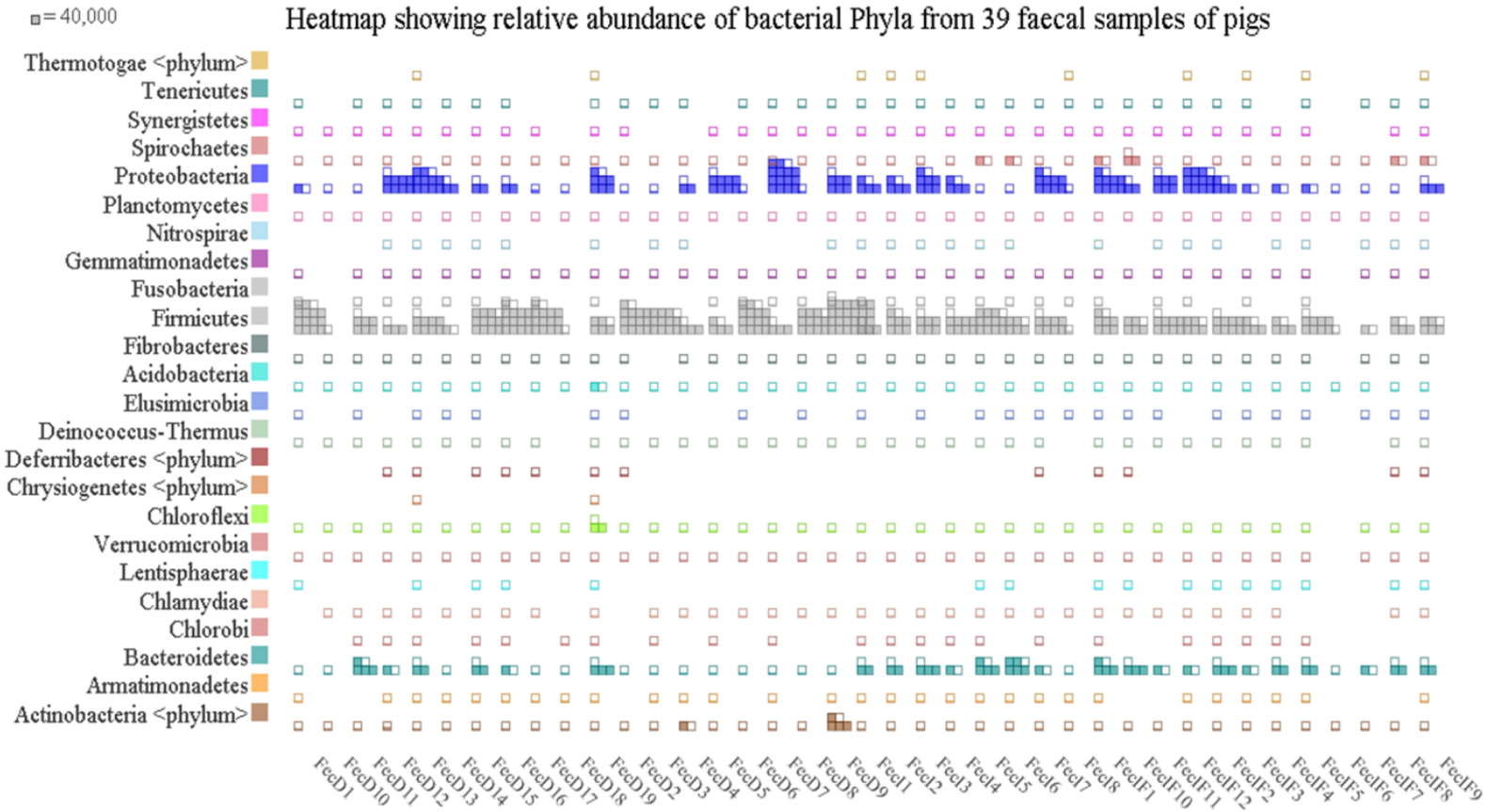

Figure 3.2. Heatmap showing abundance of predominant bacterial phyla from 39 faecal samples of pigs from different management systems. Identification of bacterial phyla was done at 97% 16S rRNA sequence similarity. Colours represent different bacterial phyla. Each square represents 40,000 sequences assigned to particular phylum. Rare phyla are not included. A fully shaded square represent 40,000 sequences.

Table 3.1 Comparison of the relative abundance of faecal microbiota of free-range and indoor reared pigs at phylum level

Phyla	FecD Relative abundance (%)	FecI Relative abundance (%)	P-value
Acidobacteria	1.005	1.739	0.171
Actinobacteria	3.261	1.552	0.088
Armatimonadetes	0.032	0.058	0.140
Bacteroidetes	8.418	20.055	0.004
BRC1	**0.005**	**0.020**	**0.015**
Chlamydiae	0.042	0.018	0.259
Chlorobi	0.051	0.020	0.298
Chloroflexi	1.022	0.675	0.356
Chrysiogenetes	0.000	0.000	0.226
Deferribacteres	0.003	0.001	0.227
Deinococcus-Thermus	**0.040**	**0.122**	**0.029**
Elusimicrobia	0.002	0.016	0.052
Fibrobacteres	**0.037**	**0.245**	**0.005**
Firmicutes	56.385	42.440	0.079
Fusobacteria	0.133	0.016	0.249
Gemmatimonadetes	**0.130**	**0.329**	**0.041**
lentisphaerae	0.001	0.002	0.197
Nitrospira	0.021	0.040	0.234
OD1	0.006	0.002	0.311
OP11	0.003	0.010	0.161
Planctomycetes	0.678	0.775	0.355
Proteobacteria	23.366	24.661	0.444
Spirochaetes	2.031	3.696	0.069
SR1	0.002	0.001	0.302
Synergistetes	**0.026**	**0.189**	**0.005**
Tenericutes	0.012	0.008	0.304
Thermotogae	**0.000**	**0.008**	**0.000**
TM7	0.016	0.024	0.124
Verrucomicrobia	0.569	0.497	0.370
WS3	0.003	0.003	0.468
Cyanobacteria_Chloroplast	**0.250**	**0.035**	**0.020**

A total of 40,803 OTUs were identified, but only 30 OTUs accounted for 47% (2,027,180/ 4,364,507) of all sequences. It was also revealed that only 19% (7,748/4,083) of OTUS were found more than ten times, thus majority of the OTUs were rare. Individual group analysis revealed 28,261 OTUs in FecD, amongst which *Clostridium_sensu_stricto* (11%), *Acinetobacter* (9%), *Enterococcus* (5%), *Kurthia* (4%), *Lactobacillus, Turicibacter and Escherichia_Shigella* 3%, respectively were the most abundant. From FecI, 17038 OTUs were found and it was dominated by *Lactobacillus (*9%), *Comamonas* (7%), *Acinetobacter* (7%), *Prevotella 4% and Clostridium_sensu_stricto* (3%); *while in FecIF, out of 14343 OTUs Acinetobacter* (19%) *Unclassified Planococcaceae* (6%), *Lactobacillus* (4%), *Treponema* (4%), *Escherichia_Shigella* (3%) *and Prevotella* (2%) were the most abundant (Appendix 3.3).

When faecal microbiota from pigs permanently scavenging on dumpsite and indoor reared pigs were compared, of the 830 genera found, only 15 accounted for 74% of all sequences. Seventy four genera were significantly different between scavenging pigs and indoor reared pigs ($P \leq 0.05$) amongst which 27 (36%) were exclusive to pigs permanently scavenging on dumpsite. Likewise, when the faecal microbiota of scavenging pigs was compared to that of pigs shifted from indoor to free range, out of 125 significantly different genera 27% (34/125) were exclusive to pigs permanently scavenging on dumpsite.

Indicator analysis further revealed that there some OTUs highly associated with a particular pig management system. It was shown that 51 OTUs were significantly associated with pigs scavenging on dumpsite (Indicator value > 30, p-value ≤ 0.05), 335 OTUs with indoor reared pigs (Indicator value > 32, p-value ≤ 0.05) and 72 OTUs were associated with pigs shifted from indoor to free range on the dumpsite (Indicator value > 31, p-value ≤ 0.05). Taxonomic profiling using Megan5 normalized sequences (FecI= 928930, FecIF = 928926 and FecD = 928954) revealed *Clostridia* and *Bacilli* to be the most abundant classes in all three pig management systems, however free range management system was shown to have a higher contribution to the abundance of *Clostridia* and *Bacilli*. Other predominant bacterial classes were *Gammaproteobacteria, Betaproteobacteria*, *Alphaproteobacteria* and *Bacteroidia* (Fig. 3.4).

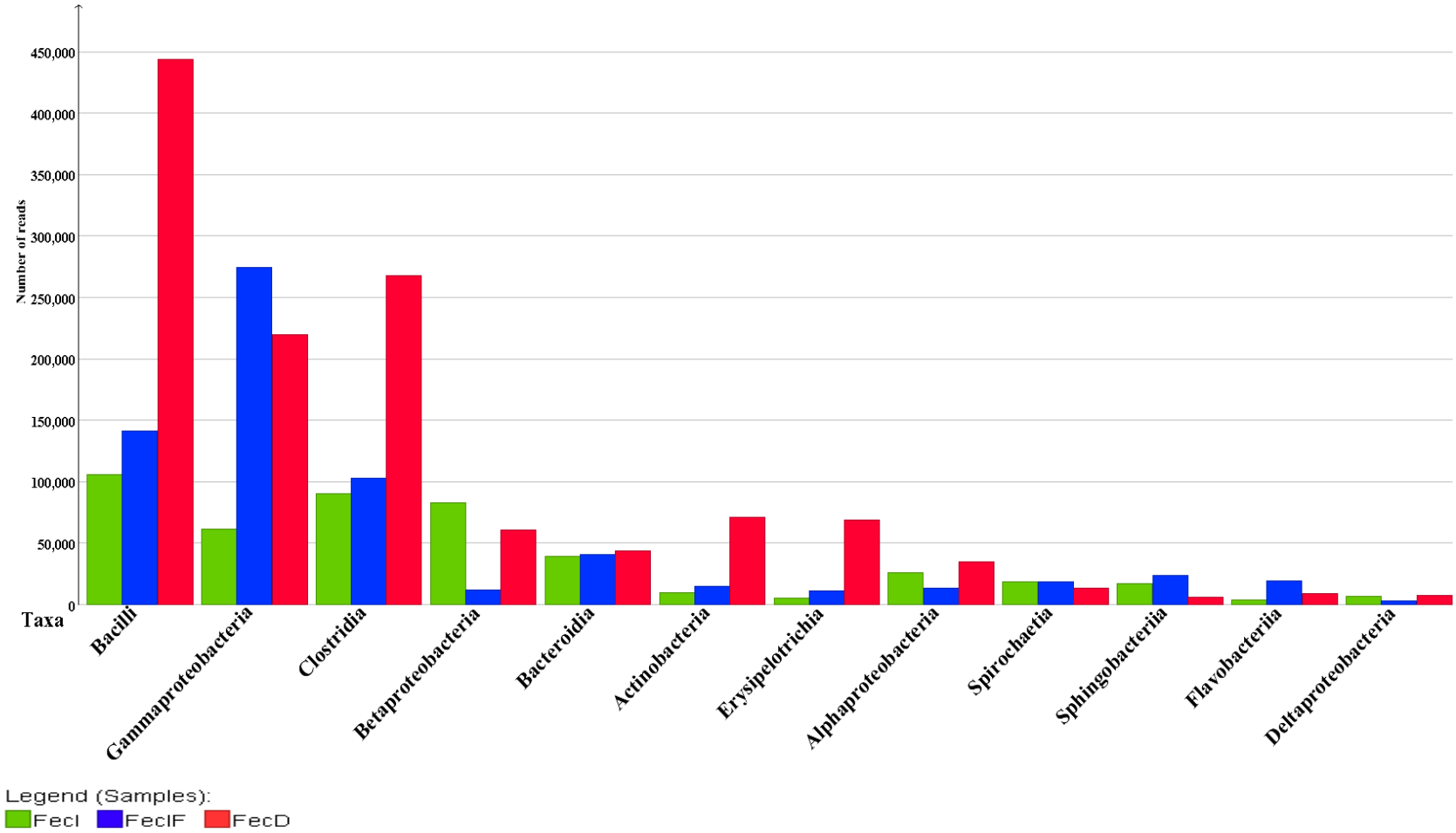

Figure 3.3. Abundance of faecal microbiota from pigs under different management systems at class level. Taxonomic profiling was done using normalized sequence counts from different management systems. Each peak shows the number of sequences assigned to particular class, the higher the peak, the most abundant is the respective class

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A closer look at the taxonomic distribution of numerically abundant bacterial classes revealed unequal abundance of bacterial genera in different pig management systems. Four genera from *Bacilli (Allofustis, Ignaviragnam, Listeria, Tetragenococcus)* and 3 from *Clostridia* (*Desulfovirgula, Lachnobacterium and Moorella)* were exclusive to pigs scavenging on dumpsite, moreover, as pigs were shifted from indoor to free-range management, some bacterial genera were acquired. Thus, 6 genera under *Bacilli* class were found in pigs shifted from indoor to scavenging on dumpsite and pigs permanently scavenginhg on dumpsite (*Alicyclobacillus, Alloicoccus, Atopococcus, Atopostipes, Brochothrix* and *Solibacillus*) but not in indoor reared pigs (Appendix 3.4). A similar pattern was shown for class *Gammaproteobacteria,* where 17 bacterial genera were exclusive to scavenging pigs, while 12 were only found in pigs shifted from indoor to scavenging and pigs permanently scavenging (Appendix 3.5). Class *Alphaproteobacteria* and *Deltaproteobacteria* had, respectively 26 and 6 genera, being exclusive to permanent scavenging pigs (Table 3.2). Some important pathogenic genera like *Brucella, Rickettsia, Afipia, Ignavigranum* and *Listeria* were among the bacterial genera exclusively found in pigs permanently scavenging on dumpsite.

When each management system was individually analysed for core microbiota, none of the OTU was found across all samples. Clostridium sensu stricto constituted the core microbiota in FecD and was found in 80% (14/20) of samples followed by Turicibacter (70%) of samples. FecIF had Acinetobacter found in (69%) of samples, while in FecI Lactobacillus was found in 100% of samples. Unique bacterial genera were predominant in FecD pigs which are *Enterococcus, Kurthia, Acinetobacter, Lactobacillus* and *Turicibacter*. Insignificant difference in predominant genera was found between FecI and FecIF. In FecI *Lactobacillus, Prevotella, Acinetobacter and Comamonas* were predominant, while in FecIF were *Acinetobacter, Prevotella* and *Lactobacillus*

Table 3.2 Bacterial genera exclusively found in free range pig management system

Proteobacteria	*Firmicutes*
Class *Alphaproteobacteria*	Class *Bacilli*
Brucella, Rickettsia, Oligotropha, Rhodocista,	*Listeria,*
Blastomonas, Rhodoblastus, Rhodopseudomonas,	*Ignavigranum,*
Labrenzia, Ancylobacter, Pseudoxanthobacter,	*Dolosigranulum, Tetragenococcus,*
Loktanella, Roseococcus, Acidocella, Acidiphilium, Seohaeicola, Oceanicola, Maribius, Roseibium,	*Fructobacillus,*
Roseivivax, Oceanibaculum, Methylopilla, Afipia,	*Pisciglobus, Kurthia, Ignavigranum, Planomicrobium, Salinicoccus, Aerococcus, Alkalibacterium, Atopococcus, Desemzia, Dolosigranulum, Isobaculum, Marinilactibacillus, Enterococcus, Pisciglobus*
Class *Gammaproteobacteria*	Class *Clostridia*
Acidithiobacillus, Bowmanella, angrovibacter	*Lachnobacterium, Lachnospira,*
Pectobacterium, Perlucidibaca, Salinisphaera,	*Desulfovirgula*
Alkanibacter, Methylosarcina, Sinobacter,	*Selemonadales*
Kangiella, Salinicola, Chromohalobacter,	*Allobaculum*
Marinomonas, Ectothiorhodosinus, Aggregibacter, Rhodanobacter, Arsenophonus, Hahella.	*Anaeroarcus, Desulfosporomusa*
Class *Deltaproteobacteria*	
Desulfarculus, Desulfatibacillum, Desulfobacula	
Desulfocella, Desulfurivibrio, Thermodesulforhabdus	
Class *Betaproteobacteria*	
Albidiferax, Sulfuricella, Chitinibacter,	
Denitratisoma, Chitinilyticum	

3.4.Discussion

This study compared the faecal microbiota of pigs under different management regimes using high throughput sequencing technology. A remarkable microbial richness and diversity in the faecal microbiota of pigs scavenging on dumpsite has been detected. The estimated richness of 5,979 species per sample and a total of 40,803 OTUs found in this study exceed estimates by previous studies. For example, a study of faecal microbiota of pigs by (Kim *et al*., 2011) reported

18,711 OTUs, a chaperonin (*cpn*60) based analysis of faecal microbiota (Costa *et al.,* 2014) reported 1,141 OTUs and also a study of nasal microbiota in pigs (Weese *et al.,* 2014) reported 1,749 species. However, it is also important to point out that neither of the previous studies investigated pigs under free-range scavenging on dumpsite nor used a similar sequencing depth like in the current study.

Most previous studies of pig faecal microbiota have reported *Firmicutes* and *Bacteroidetes* as the predominant phyla (Isaacson & Kim, 2012; Pajarillo, *et al*., 2015). Several reports of faecal microbiota in other animals have also shown a predominance of *Bacteroidetes*. For example in wild wolves feeding on raw meat (16.9%) (H. Zhang & Chen, 2010), dogs fed on experimental diet 31 - 34% (Middelbos, Boler, *et al.,* 2010) and feline microbiota studies reported *Bacteroidetes* as one of the major phyla (0.45% - 10%) (Handl *et al.,* 2011; Ritchie *et al.,* 2010). In the current study, the abundance of bacterial phyla in faecal microbiota of the pig was in the order of *Firmicutes*, *Proteobacteria*, *Bacteroidetes* and *Spirochaetes*. The higher predominance of *Proteobacteri*a than *Bacteroidetes* in faecal microbiota of pigs was not unexpected. This is consistent with the findings in captive cheetah where there was underrepresentation of *Bacteroidetes* in faecal microbiota (Becker *et al.,* 2014). An appealing explanation for these findings may be the management regime and diet, which have been reported to have influence on gut microbiota (Isaacson and Kim, 2012; Wu *et al.,* 2011; Yang *et al.,* 2014).

Based on phylogeny and principla coordinate analysis, this study has shown that management regimes have a major influence on the population structure and community membership of faecal microbiota of pigs. The data show the presence of two clusters of bacterial populations and the existence of some bacterial genera exclusively to pigs scavenging on dumpsite. These findings therefore strongly suggest the existence of two distinct clusters of faecal microbiota, presumably, enterotypes as previously reported for humans. The enterotypes concept has been reported in humans whereby each group is identifiable by the predominance of different bacterial groups: *Bacteroides* (enterotype 1), *Prevotella* (enterotype 2) and *Ruminococcus* (enterotype 3) (Arumugam *et al*., 2011). Diet is a defining criterion for a particular enterotype. *Bacteroides* enterotype is found in humans whose diet is rich in animal protein and saturated fat, *Prevotella* enterotype is found in humans whose diet is rich in carbohydrate and simple sugars, while *Ruminococcus* are found in diet rich of polyunsaturated fat and alcohol.

Gut microbiome has been associated with the selection of bacterial population that optimally degrades the available substrate (Sonnenburg *et al.,* 2010). In spite of the fact that *Bacteroidetes* comprise important degraders of complex and otherwise indigestible dietary polysaccharides (Thomas *et al.,* 2011; Tremaroli and Bäckhed, 2012), the predominance of *Enterococcus, Kurthia, Alkalibacteria* and *Turicibacter* genera instead of *Bacteroidetes* in free-range pigs feeding on variety of garbage from food industries, abattoirs, domestic, market and biomedical waste on dumpsite was interesting. The fact that none of the predominant genera of pigs scavenging on dumpsite has been reported as part of the known enterotypes, warrants further study on their functional genomics to tell their roles in both animals and public health. The predominance of *Prevotella* in indoor reared pigs which are fed on a diet rich in carbohydrate as revealed in this study, further support the possible existence of this enterotype in indoor reared pigs. Similarly, existence of enterotypes in animals was speculated in free ranging wood bison by (Weese *et al.,* 2014).

While indoor reared pigs were highly dominated with *Lactobacillus* genera which is well known in inhibiting colonization of the gastrointestinal tract with pathogenic bacteria (Amin *et al.,* 2011; Neal-McKinney *et al.,* 2012), free-range pigs were dominated with *Enterococcus*. This group of bacteria constitutes some of the important nosocomial multidrug resistant bacteria (Arias and Murray, 2012; Freitas *et al*., 2011; Lebreton *et al.,* 2013) some of which are shared between animals and humans. The fact that during study period pigs scavenging on dumpsite were seen to be clinically healthy, it could be that bacteria unique to these pigs confer some benefits these animals; thus further research to assess potential of these bacteria as source of probiotics is warranted.

The significant difference in faecal microbiota of indoor reared pigs and pigs scavenging on dumpsite as revealed at genus level and the presence of some pathogenic genera such *as Brucella, Rickettsia, Afipia, Ignavigranum* and *Listeria* exclusive to pigs scavenging on dumpsite, reflects the nature and composition of bacterial population on the dumpsite. It also confirms the influence of dumpsite environment on diversity of microbiota circulating between free-range animals and points to potential health hazards for people handling these animals and garbage pickers. Further study would be necessary to relate changes happening in pigs

scavenging on dumpsite to what is happening to people handling these animals, including members of their families.

It was interesting to find that in both management systems, the phylum *Proteobacteria* was the second to *Firmicutes* in predominance contrary to previous reports on faecal microbiota of pigs (Isaacson and Kim, 2012; Pajarillo *et al.*, 2015; Park *et al*, 2014) where Bacteroidetes dominated. Previous studies on the intestinal microbiota of animals including humans have associated high abundance of *Proteobacteria* with the presence of gastrointestinal tract diseases (Costa *et al.*, 2012; Kaakoush *et al*., 2012; Suchodolski *et al*., 2012). High abundance and diversity of *Proteobacteria* in these animals probes further study, first, on the roles of these bacteria in the gastrointestinal tract and secondly, the safety of food derived from these animals.

3.5. Conclusion

There is a significant difference in the composition of bacteria between pigs reared under different management systems, as well as the presence of faecal bacteria of public health importance in free-range pigs scavenging at an urban dumpsite in Arusha Tanzania. The findings suggest a need for controlled grazing of livestock in urban and peri-urban areas to mitigate possible public health risks associated with free-range food animals. Further studies of the gut microflora of free-range pigs in urban dumpsites and their clinical significance to animal and human health warrant further research.

CHAPTER FOUR

16S rRNA amplicons survey revealed unprecedented bacterial community in solid biomedical wastes[‡]

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Abstract

Despite known risks of inappropriate disposal of biomedical solid waste; most cities in developing countries are still disposing unsorted and untreated solid biomedical waste in public open dumpsites. While many studies reported the presence of pathogens in fresh biomedical waste from hospitals, none has reported on the abundance and diversity of bacterial community in aged solid biomedical waste from open dumpsite. A qualitative survey was done to identify types of solid biomedical waste on the Arusha municipal dumpsite. Soils, sludge or washings of biomedical wastes were sampled. Total DNA was extracted and v4 region of 16S rRNA amplicons were sequenced using an Illumina MiSeq platform. A total of 1,706,442 sequences from 15 samples passed quality control. The number of sequences per sample ranged from 70664 to 174456 (mean 121765, SD 35853). Diversity was high with an InvSimpson index of

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63 (Range 5 – 496, SD 121). Thirty five phyla were identified, but only 9 accounted for 96% of all sequences. The dominant phyla were *Proteobacteria* (37.4%), *Firmicutes (*34.4%), *Bacteroidetes* (14.1%), *Actinobacteria* (5.6%) and *Chloroflexi* (1.7%). Catchall analysis predicted a mean of 9,399 species per sample. Overall, 31,402 operational taxonomic units (OTUs) were detected, however, only 19.8% (6,202) OTUs were found more than ten times. The most predominant OTUs were *Proteinclasticum* (10.4%), Acinetobacter (6.9 %), *Halomonas* (3.9 %), *Pseudomonas* (1.7%), *Escherichia/Shigella* 1.5% and *Planococcus* (1.3%). *Proteiniclasticum* sp and *Acinetobacter* sp were found in 67% (10/15) of all samples at relative abundance of 1%. Taxonomic-to-phenotype mapping revealed the presence of 36.2% related to bacteria involved in dehalogenation, 11.6% degraders of aromatic hydrocarbons, 14.8% chitin degraders, 8.5% chlorophenol degradation and Atrazine metabolism 8.3%. Taxonomy-to human pathogen mapping found 34% related to human pathogens and 39.4% were of unknown pathogenicity.

There's rich and diverse bacterial community in aged solid biomedical waste. Some of the predominant OTUs are related to bacteria of industrial use. We found a good number of OTUs mapping to human pathogens. Most of OTUs mapped to unknown metabolism and also to group unknown whether they human pathogens or not. To our knowledge, this is the first reports on bacteria related to industrial use from solid biomedical waste. This finding will facilitate to design further research using functional metagenomics to better understand the potential of bacteria from aged solid biomedical waste in industrial application and soil remediation.

4.1. Introduction

Biomedical waste is defined as any solids, liquids, sharps, laboratory waste, and drug containers which are generated as a result of healthcare activities for both humans and animals. Hospitals, research institutions, health care, teaching institutes, clinics, laboratories, blood banks, animal houses and veterinary institutes are the main source of biomedical waste. Despite known biomedical health hazards to people, animals and environment (Acharya *et al.*, 2014; DeRoos, 1974; Gidarakos *et al.*, 2009; Hossain *et al.*, 2011); their disposal have remained a serious challenge in most cities of developing countries even to-date (Boss *et al.*, 2009; Hassan *et al.*,

2008; Rastogi *et al*., 2011; Yadavannavar *et al.*, 2010). Several studies have attempted to identify risks associated with biomedical waste with an objective of understanding their microbial composition and concomitant risks that could guide in designing appropriate methods that curtails associated risks. For this reason, biomedical waste sterilization has become an important regulatory requirement designed to eliminate any risks associated with biomedical wastes prior to their permanent disposal.

According to (DeRoos, 1974) and (Wallace *et al.*, 1972), biomedical waste can contain higher concentration of pathogenic organisms. For example, several researchers reported bacteria of public health importance in fresh biomedical waste from hospital environments (Anitha and Jayraaj, 2012; Rastogi *et al.*, 2011; Saini *et al.*, 2004), where *Escherichia* sp, *Pseudomonas* sp, *Klebsiella* sp, *Salmonella* sp, *Staphylococcus* sp, *Serratia* sp, *Acinetobacter* sp, *Enterococcus* sp and *Streptococcus* sp were detected. It is important to note that most of the studies used culture-based methods followed by biochemical or molecular identification. Such approaches are nowadays known to underestimate true bacterial composition by more than 99% due to the presence of the not yet culturable bacteria (Riesenfeld, *et al*., 2004; Vandecandelaere *et al.*, 2012), thus missing a true microbial composition of solid biomedical waste and associated risks.

Previous studies on microbial composition of biomedical waste have dealt with, mostly fresh biomedical waste from hospital environments and have focused on culturable bacteria of public health importance (Anitha and Jayraaj, 2012; Oyeleke and Istifanus, 2009; Rastogi *et al*., 2011). Unfortunately, none of these studies have reported microbial composition in dumpsites with high accumulation of aged and untreated biomedical waste. In African urban settings, unsorted solid waste including biomedical waste is continuously thrown in common municipal dumps and the waste is left unattended for a long period. The effect of continuous dumping and aging on microbial composition as well as on the potential exchange of genetic material between microbial populations in such an environment is not well established. Improper disposal of biomedical waste can create opportunity for bacterial proliferation as well as bacterial acquisition of new genotypes.

Owed to the fact that bacteria (including pathogens) present in untreated biomedical waste can leach out and contaminate the environment; this poses a potential health risk to humans and animals. The objective of this study was to determine the abundance, taxonomic diversity and

composition of the bacterial community in aged solid biomedical waste in a dumpsite in Arusha, Tanzania using culture independent high throughput sequencing of v4 region of the 16S rRNA gene. We communicate unprecedented abundance and diversity of bacterial community in aged solid biomedical waste dominated by Operational Taxonomic Units (OTUs) of industrial importance.

4.2. Materials and methods

4.2.1. Study site

Site for this study was the Arusha municipal dumpsite, where unsorted waste from different urban sources is thrown. Detaiked description of the study site is shown in (Appendix 1.1). Sampling was done during March to June 2013 whereby prior to sample collection, a qualitative survey was conducted to identify types of most common wastes on the dumpsite. This comprised waste from households and markets (foods, pampers, clothes, etc.), chemical and biomedical waste (drug containers, used syringes), various plastics and used glassware, waste from abattoirs and brewers as well as fecal matter from animals scavenging on the dumpsite itself. Samples for this study were the different aged biomedical solid waste (Biom n = 15) from various sources. Soils, sludge or washings of solid biomedical waste were collected into sterile plastic containers and within one hour transported on ice to the laboratory where total DNA extraction was done and then used in the downstream processes.

4.2.2. Ethical Consideration

This study was approved by the research committee of The Nelson Mandela African Institution of Science and Technology, in Arusha, Tanzania. Permits to sample the dumpsite was granted by the Arusha District Veterinary office and to transfer samples between laboratories permits were given by the Zoosanitary inspectorate services of Tanzania, Arusha (VIC/AR/ZIS/0345) and Veterinary Services under the Ministry of Agriculture Livestock and fisheries of Kenya (RES/POL/VOL.XXIV/506).

4.2.3. Extraction of total genomic DNA from solid biomedical waste

Total genomic DNA was extracted from about 250 mg of solid biomedical waste using PowerSoil™ DNA extraction kit (MOBIO Laboratories, Carlsbad, California) as shown in chapter two, section 2.2.2.

4.2.4. 16S rRNA amplification, library construction and sequencing

Amplification of v4 region of 16S rRNA gene, library construction and sequencing was performed as shown in chapter 2, section 2.2.2

4.2.5. Sequence Data Analysis and Statistics

The Mothur package algorithm (v1.34.1) was used for both quality control and sequence data analysis (Schloss *et al*., 2009). After paired end reads were assembled, sequences were aligned with the Silva 16S rRNA reference database (www.arb-silva.de) (Quast *et al*., 2013). Sequences that were < 239 bp and > 260 bp in length or contained > 2 ambiguous base calls or long runs (> 8 bp) of homopolymers or did not align with the correct region were removed. Chimeras were identified using Uchime (Edgar *et al.,* 2011) and eliminated. Taxonomy was assigned using the RDP taxonomy database (http://rdp.cme.msu.edu/index.jsp) (Cole *et al.,* 2014). Sequences were binned into operational taxonomic units (OTUs) at 97% sequence similarity level.

Species richness was assessed with Chao1 richness estimator (Chao, 1984), abundance based coverage estimator ACE (Chao *et al.,* 1992), Shannon weaver (Chao and Shen, 2003) and InvSimpson (Hunter & Gaston, 1988) indices as well as with CatchAll analysis (Bunge, 2012). All analyses were performed using built-in commands in Mothur v1.34.1 and CatchAll v4. Rarefaction analysis was done at maximum of 3% cut-off of sequence differences and was plotted using Phyloseq package (McMurdie and Holmes, 2013) in R version 3.1.2. The core microbiota was assessed through identification of OTUs present in at least 50% of samples at a minimum abundance of 1%. The Metastats programme (Meadow *et al.*, 2014) was used to assess differentially abundant and significantly different bacterial taxa between biomedical wastes. Shared OTUs files generated in Mothur was converted into biom file and imported into

MEGAN5 v5.5.3 (Mitra *et al.*, 2011) where further taxonomic profile comparison were performed. A p-value of ≤ 0.05 was considered significant for all comparisons.

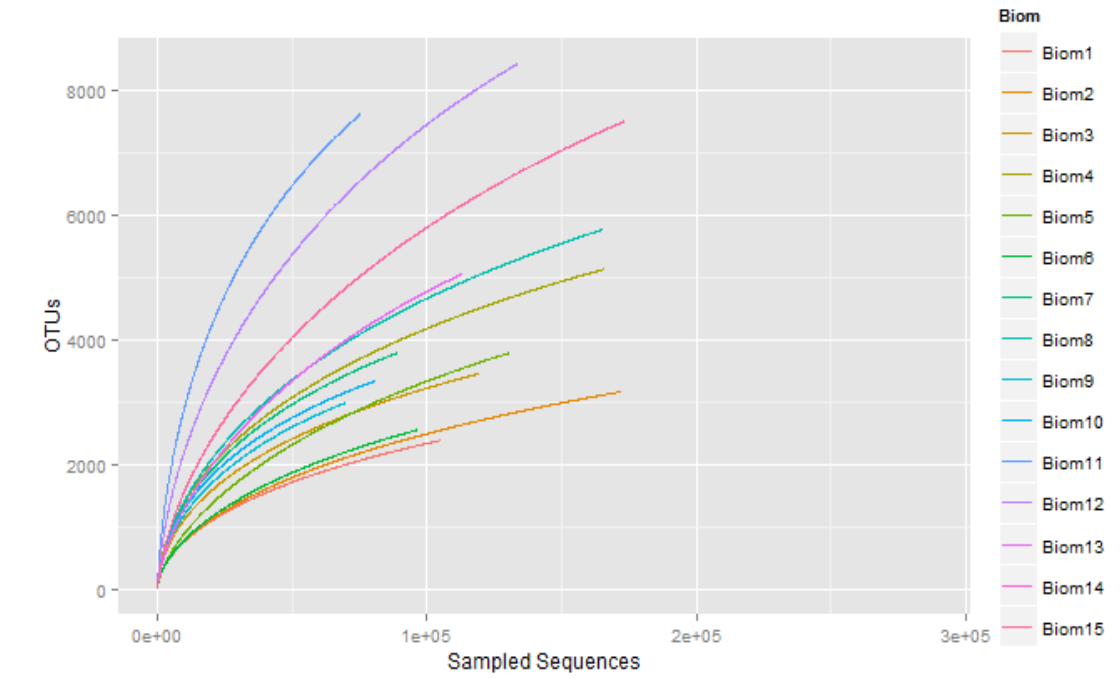
4.2.6. Availability of supporting data

Sequences of bacteria from solid biomedical waste generated in this study were deposited at NCBI Sequence reads archive (SRA) and assigned with accession number [SRP045926].

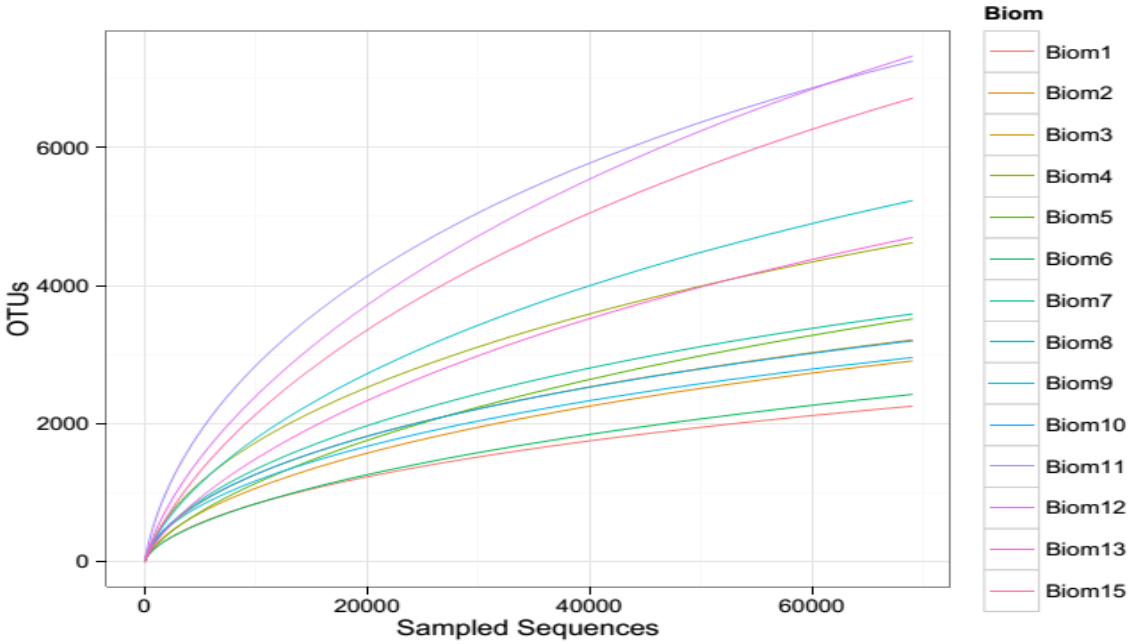
4.3.Results

4.3.1. Sequencing depth, taxonomic assigning, diversities and core microbiome analysis

A total of 1,706,442 v4 region of 16S rRNA gene sequences from 15 solid biomedical waste samples passed all quality control filters. The number of sequences per sample ranged from 70,664 to 174,456 (mean 121,765, SD 35,853). Catchall analysis of richness predicted a mean of 9,399 species per sample (range 544 – 16,621, SD 3,678). Good's coverage ranged from 0.9625 – 0.9926 (mean 0.9835, SD 0.0074). Rarefaction curves showing sampling efficiency is displayed in (Fig.4.1).



(a)



(b)

Figure 4.1 Rarefaction curves of v4 region of 16S rRNA gene sequences from 15 samples. Fig.4.2 (a) show rarefaction curves as per sequences generated from each sample and Fig.4.2 (b) is a rarefaction curve after subsampling of 69,000 sequences from each sample. Figure shows that with more sampling more OTUs would be detected.

The overall bacterial diversity was high with an average Chao1 richness of 6,330 (range 3,728 – 12,287, SD 2760); ACE 7,682 (range 4476 – 15,167, SD 3,383); Shannon weaver index 5.0 (range 3.8 – 7.3, SD 0.976) and an average InvSimpson index of 63 (Range 5 – 496, SD 121). Summary of per sample good quality sequences, estimated OTUs and diversity indices are shown in Table 1.

Thirty-five bacterial phyla were identified, however only nine were most predominant and accounted for 96% of all sequences. *Proteobacteria* was the most abundant phylum accounting for 37.4% of all sequences. Other predominant phyla were *Firmicutes* 34.4%, *Bacteroidetes* 14.1%, *Actinobacteria* 5.6%, *Chloroflexi* 1.7%, *Acidobacteria* 1.7% and *Planctomycetes* 1.3%. Fig.4.2 summarizes predominant bacterial phyla in solid biomedical waste.

Table 4.1 Summary of good quality sequence data and diversity indices of different samples at species level

Sample	Quality reads	OTUs	Chao1	Inv Simpson	Shannon
Biom1	106,046	2,369	3,728	19	4.3
Biom2	172,105	3,101	4,627	7	3.8
Biom3	119,512	3,426	4,970	22	4.9
Biom4	167,643	5,075	7,284	83	5.8
Biom5	130,218	3,670	5,613	12	4.0
Biom6	96,878	2,533	3,847	9	4.0
Biom7	90,308	3,788	5,402	25	5.2
Biom8	167,215	5,781	8,568	45	5.5
Biom9	70,664	2,973	4,336	54	5.4
Biom10	81,145	3,352	4,752	54	5.5
Biom11	7,7074	7,615	10,590	496	7.4
Biom12	137,828	8,353	12,287	37	6.2
Biom13	113,620	4,952	7,464	5	4.1
Biom14	1,730	285	420	30	4.5
Biom15	174,456	7,357	11,110	16	4.9

OTUs - Operational taxonomic units (97% sequence identity), Calculation was performed with an OTU definition at > 97% sequence identity cut-off.

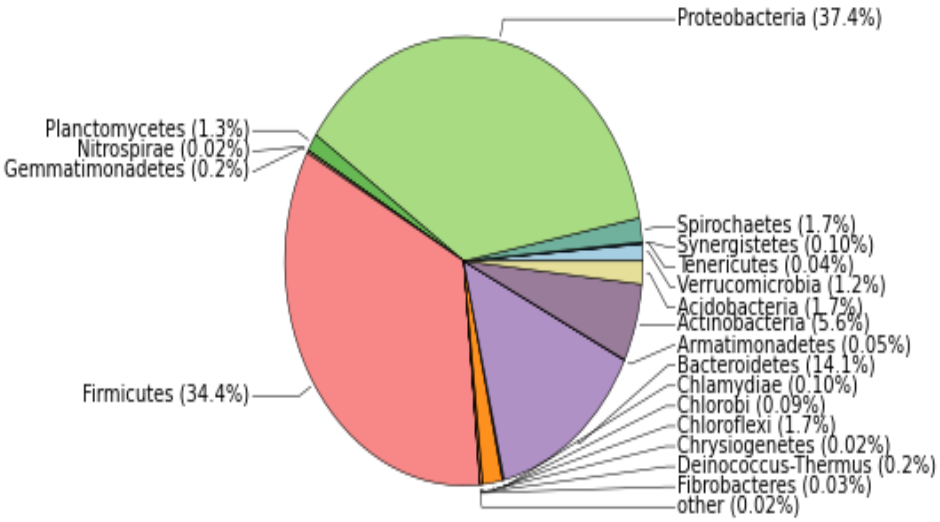


Figure 4.2 Predominant bacteria phyla in solid biomedical wastes. OTUs were assigned at 97% sequence similarity cut-off.

A total of 31,402 OTUs were found, however only 6,201 (19.8%) were identified more than 10 times, thus majority of OTUs were rare. The most predominant OTUs were *Proteiniclasticum* (10.4%), *Acinetobacter* (6.9%), *Halomonas* (3.9%), *Pseudomonas* (1.7%), *Planococcus* (1.3%), *Oligella* (1.2%) and *Paracoccus* (1%) (Table 4.2). Generally most OTUs were rare and only 9 were found to have abundance of 1% or higher as shown. The core microbiome analysis revealed that none of the OTU was found across all samples tested. Only *Proteiniclasticum* and *Acinetobacter* were detected in 67% (10/15) of samples at relative abundance of 1% or more.

Table 4.2 Abundance of predominant OTUs in solid biomedical waste

OTU	Reads	%	Phylum	Genus
1	175,640	10.4	*Firmicutes*	*Proteiniclasticum*
2	116,657	6.9	*Proteobacteria*	*Acinetobacter*
3	65,854	3.9	*Proteobacteria*	*Halomonas*
4	38,308	2.3	*Proteobacteria*	*Acinetobacter*
5	28,125	1.7	*Proteobacteria*	*Pseudomonas*
6	26,140	1.5	*Proteobacteria*	*Escherichia/Shigella*
7	22,324	1.3	*Firmicutes*	*Planococcus*
8	20,506	1.2	*Proteobacteria*	*Oligella*
9	17,020	1.0	*Proteobacteria*	*Paracoccus*

Despite the fact that all samples were collected from the same dumpsite, the dendrogram established using UPGMA (Unweighted Pair Group Method Arithmetic mean) clearly grouped sequences into two clusters (Fig.4.3). Cluster "Biom A" comprised sequences from 10 samples while cluster "Biom B" comprised sequences from 5 samples. Further, it was also revealed predominance of *Firmicutes*, *Proteobacteria* and *Bacteroidetes* phyla in cluster A and *Proteobacteria, Bacteroidetes* and *Firmicutes* in cluster "Biom B" in that order.

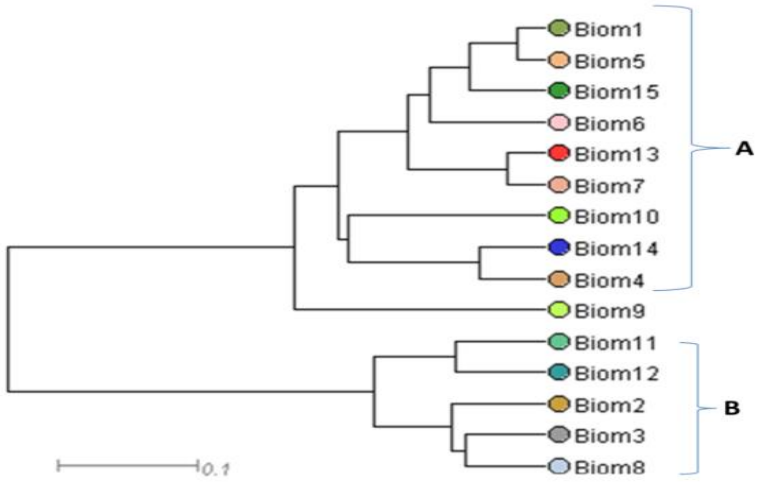


Figure 4.3 Population structure of bacteria from solid biomedical waste. Phylogenetic tree was established using UPGMA (Unweighted Pair Group Method with Arithmetic Mean) based on Bray Curtis distances of dissimilarity. 16S rRNA sequence similarity was established at 97% cut-off level.

Further, comparison of the two clusters revealed that, of the 31 differentially abundant bacterial phyla, six (*Firmicutes, BRC1, Proteobacteria, Verrucomicrobia, Planctomycetes, Armatimonadetes* and *Deinococcus-Thermus*) were significantly different between clusters A and B (Table 4.3). *Firmicutes* was found to be most abundant in cluster A than in cluster B (0.3059 vs 0.1527, p = 0.0000278) while *Proteobacteria* dominated in cluster B than in cluster A (0.3094 vs 0.2424, p = 0.01203). All significantly different phyla between the two clusters are displayed in bold.

Further, scrutiny of the two clusters at genus level revealed that, of the 1,092 bacterial genera, 88 genera and 26 unclassified genera were significantly different between the two clusters (Appendix 4.1). *Proteiniclasticum, Halomonas, Acinetobacter, Clostridium sensu stricto* and *Prevotella* were the dominant genera in cluster Biom_A, while cluster Biom_B was dominated by *Acinetobacter, Pseudomonas, Oligella* and *Paracoccus* (Fig.4.4).

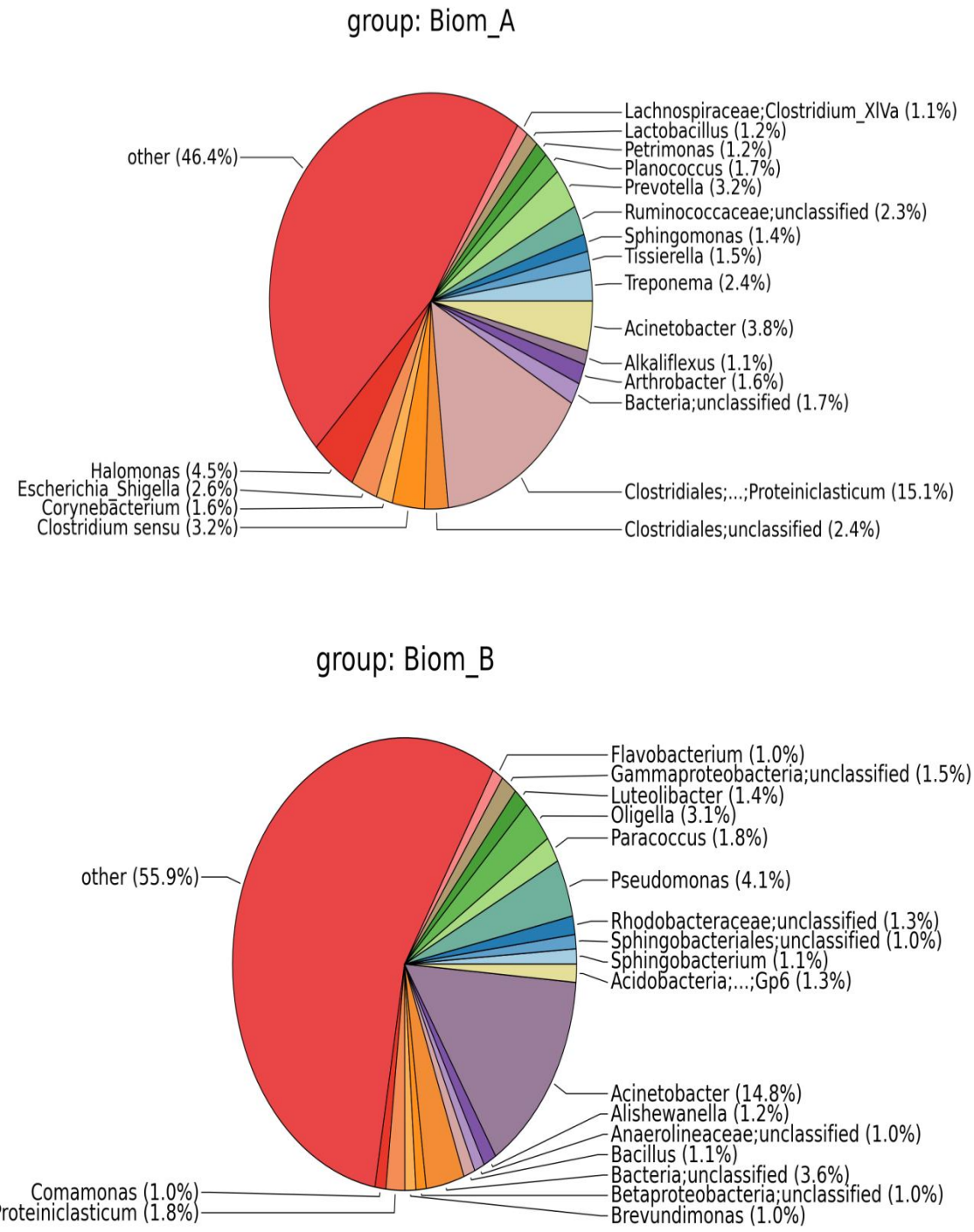


Figure 4.4 Abundance of predominant bacterial genera in in different clusters of solid biomedical waste. Group Biom_A comprised of samples collected in wet areas closer to soilid wastes from abbatoirs, group Biom_B comprised of samples from dryf solid biomedical wastes. Normalized sequence counts from each cluster were used in the comparison.

Table 4.3 Differential abundance of bacterial phyla in two clusters of biomedical waste

S/N	Taxa	Mean Biom A	Variance Biom A	Mean Biom B	Variance Biom B	P value
1	***Firmicutes***	**0.305923**	**4.59E-03**	**0.152768**	**1.30E-03**	**2.78E-05**
2	***BRC1***	**0.000999**	**5.01E-07**	**0.002457**	**9.75E-07**	**0.007861**
3	***Proteobacteria***	**0.242448**	**2.84E-03**	**0.309438**	**1.56E-03**	**0.012028**
4	***Verrucomicrobia***	**0.017403**	**1.98E-05**	**0.026937**	**5.61E-05**	**0.015972**
5	***Planctomycetes***	**3.40E-02**	**1.51E-04**	**0.07181**	**9.98E-04**	**0.01725**
6	***Armatimonadetes***	**2.53E-03**	**1.58E-06**	**0.005066**	**4.58E-06**	**0.022833**
7	***Deinococcus-Thermus***	**3.96E-03**	**1.85E-06**	**0.002813**	**2.73E-07**	**0.028806**
8	*Gemmatimonadetes*	8.68E-03	2.05E-05	0.005329	5.45E-06	0.074972
9	*Fibrobacteres*	9.33E-04	1.03E-06	0.000291	9.66E-08	0.084389
10	*Acidobacteria*	0.030651	9.77E-05	4.18E-02	1.56E-04	0.100611
11	*OD1*	0.000426	9.43E-07	0.002339	7.55E-06	0.154306
12	*Chlorobi*	7.78E-04	2.00E-06	0.002064	2.81E-06	0.161917
13	*WS3*	0.000135	8.88E-08	0.000592	4.55E-07	0.166972
14	*Synergistetes*	0.001385	7.37E-07	0.00097	6.79E-08	0.177667
15	*Spirochaetes*	0.006286	1.47E-05	0.003774	9.17E-06	0.193194
16	*Lentisphaerae*	3.99E-05	7.96E-09	3.53E-04	3.42E-07	0.274389
17	*Chloroflexi*	3.03E-02	1.69E-04	0.045982	7.94E-04	0.279194
18	*OD1*	0.002724	4.40E-05	0.000264	1.76E-07	0.282389
19	*OP11*	0.000176	3.11E-07	0.000654	6.89E-07	0.285417
20	*Fusobacteria*	5.89E-04	2.20E-07	0.000268	2.79E-07	0.290972
21	*Chrysiogenetes*	1.36E-05	1.84E-09	0.000164	8.97E-08	0.306889
22	*Deferribacteres*	6.07E-05	1.66E-08	0.000227	1.21E-07	0.342444
23	*Actinobacteria*	8.00E-02	5.69E-04	6.89E-02	4.86E-04	0.432667
24	*Bacteroidetes*	1.30E-01	1.83E-03	1.16E-01	1.09E-03	0.569167
25	*SR1*	0.000316	2.69E-07	0.000198	5.33E-08	0.608222
26	*TM7*	0.001534	2.15E-06	0.001212	1.38E-06	0.693528
27	*Thermotogae*	0.00018	6.95E-08	1.45E-04	2.41E-08	0.775917
28	*Tenericutes*	0.001475	3.53E-06	0.001615	1.96E-06	0.872
29	*Nitrospira*	0.000975	4.88E-07	0.000946	2.22E-07	0.911194
30	*Chlamydiae*	3.61E-03	3.70E-05	3.61E-03	2.53E-06	0.972
31	*Elusimicrobia*	0.000133	1.41E-07	8.70E-05	1.52E-08	1

4.3.2. Taxonomy to phenotype mapping of OTUs from biomedical waste

Taxonomy to metabolism mapping of the OTUs revealed presence of bacteria capable of degrading environmental pollutants. Bacteria involved with dehalogenation 36.2%, degrader of aromatic hydrocarbons 11.6%, chitin degradation 14.8%, and chlorophenol degradation 8.5% and Atrazine metabolism 8.3% (Fig.4.5). Surprisingly, 46.5% of OTUs had unknown metabolism.

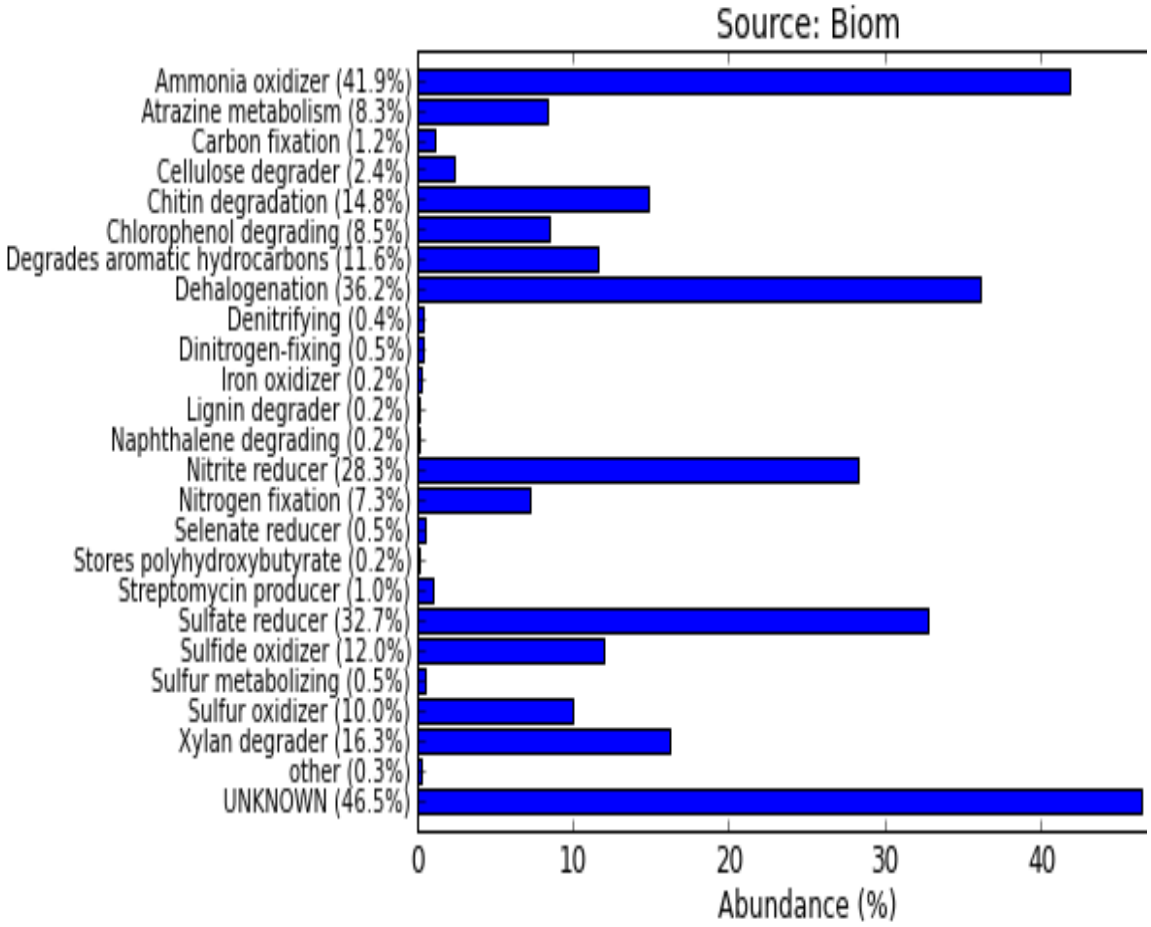


Figure 4.5 Taxonomy to metabolism mapping of bacteria OTUs from solid biomedical waste.

Likewise, taxonomy to human pathogens mapping revealed that the community of bacteria in solid biomedical waste comprised 34% related to human pathogens, 2.1% in category of rarely pathogens and 24.5% non-pathogens (Fig.4.6). Interestingly, 39.4% of the bacteria community were not known whether they are pathogens of not.

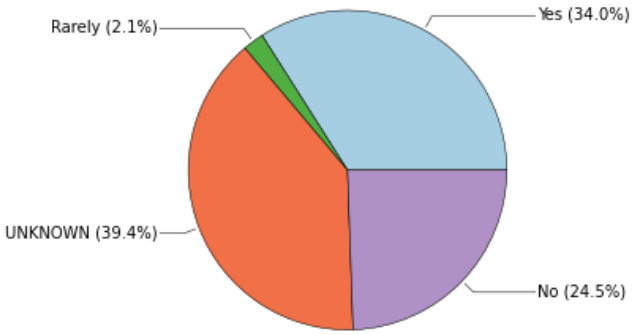


Figure 4.6 Taxonomy to human pathogens mapping of bacteria from solid biomedical waste

4.4. Discussion

This study has identified abundant and diverse of bacteria in solid biomedical waste in a municipal dumpsite in Arusha, Tanzania. The estimated mean of 9,399 bacterial species per sample and the InvSimpson index of diversity are the highest being reported to-date compared to any of the previous studies related to solid biomedical waste (Anitha and Jayraaj, 2012; Hossain *et al.,* 2013; Rastogi *et al.,* 2011). Most previous studies were based on culture methods followed by either biochemical or molecular identification using 16S rRNA gene sequencing; an approach which has proved to underestimate true bacteria diversity to the extent of missing up to 99% of the not yet cultured bacteria (Riesenfeld *et al.,* 2004; Vandecandelaere *et al.,* 2012). The molecular approach and high throughput sequencing reported in this study have shown outstanding difference in microbial diversity compared to previous studies. For example, bacteriological profiling of biomedical waste by (Rastogi *et al*., 2011; Anitha and Jayraaj, 2012; Oyeleke and Istifanus, 2009) using culture method, all together could only isolate and identify *Pseudomonas* sp, *Escherichia coli, Staphylococcus* sp, *Proteus* sp, *Enterococcus* sp, *Citrobacter* sp, *Bacillus* sp, *Corynebacterium* sp, *Micrococcus* sp, *Actinomyces* sp and *Klebsiella* sp. Similarly, based on culture method followed by molecular identification (Hossain *et al*., 2013) found mostly the same groups of bacteria: *Pseudomonas aeruginosa, Salmonella* sp, *Klebsiella pneumoniae, Serratia marcescens, Acinetobacter baumannii, Staphylococcus aureus, Staphylococcus epidermidis, Enterococcus faecalis, and Streptococcus pyogenes*. Such approaches clearly underestimate true bacterial diversity compared to 31,402 OTUs detected in the current study.

This study revealed two clusters of bacterial populations as shown by the phylogenetic tree and the significantly different taxa at both phylum and genus levels. The reason for existence of two bacterial populations could be due to different types and sources of solid biomedical waste. Metadata information revealed that all samples from group Biom-A were from wet areas and closer to solid wastes from abbatoirs. Findings of the qualitative survey indicated that some wastes had pharmaceutical origin, while others were from hospitals, veterinary or medical laboratories as well as domestic households. While all these wastes are mixed up in the dumpsite, it is possible that different biomedicals exert varying selective pressure, hence influencing the differential abundance of bacteria in different samples as shown by the two clusters.

Proteobacteria was the most predominant phylum in solid biomedical wastes. The fact that previous studies have associated prevalence of *Proteobacteria* with diseases of humans and animals (Costa *et al.,* 2012; Suchodolski *et al*., 2012; Suchodolski *et al*., 2010), their abundance in solid biomedical waste suggests that either, such wastes are rich in nutrients that support *Proteobacteria* proliferation or solid wastes from infected animals and humans brings along such bacteria in a common dumpsite. It is further important to note that majority of predominant OTUs in our solid biomedical waste were from *Proteobacteria* previously reported to have diverse industrial applications. For example, *Halomonas* spp accounting for 2% of all sequences is reported to produce exopolysaccharides capable of emulsifying most of hydrocarbons (Bouchotroch et al., 2000); industrially used in synthesis of ectoine which is used as a stabilizer for enzymes and cell protectant in skin and health care (Schwibbert *et al.,* 2011).

Predominance of *Acinetobacter* sp (9.2% of all sequences) is in agreement with findings of (Saini *et al.,* 2004; Hossain *et al*., 2013; Omer *et al*., 2015) in solid biomedical waste. This group of bacteria resists and detoxify chromium VI thus having bioremediation potential in removal of heavy metal from industrial waste and contaminated sites (Bhattacharya and Gupta, 2013; Franzetti *et al*., 2014). The detection of *Pseudomonas* spp (1.7%) in solid biomedical waste is also reported by (Hossain *et al.,* 2013; Hossain *et al.,* 2011; Oviasogie *et al.,* 2010; Rastogi *et al.,* 2011) based on culture methods. Apart from this bacteria being associated with multidrug resistance (Jensen *et al.,* 2001; Ndugulile *et al.,* 2005; Odjadjare *et al.,* 2012; Pfeifer *et al*., 2010) with impact to humans and animals health, *Pseudomonas* spp has been reported to be the first

bacterium to degrade chloroaromatic compounds and also a novel *amylase* and *lipase* producer of industrial application (Khannous *et al*., 2014; Miyazaki *et al.*, 2015). *Pseudomonas* is used in bioremediations of environmental pollutants (Wasi *et al.*, 2013) and importantly to degrade polyethylene materials (Nandi *et al.*, 2013). *Paracoccus* sp (1%) was another predominant genus. This bacteria is reported to have plasmid that carries genes for degradation of toxic solvent used in chemical industry (N, N-dimethylformamide) and also in biodegradation of chlorpyrifos and 3, 5, 6-trichloro-2-pyridino (Dziewit *et al.*, 2010; Xu *et al*., 2008).

The fact that taxonomy to metabolism mapping revealed presence of bacteria capable of metabolizing environmental pollutants; similarity of bacteria found in solid biomedical waste with those reported in various industrial application suggests the likely potential of aged solid biomedical waste as a source useful bacteria.

Escherichia / Shigella spp was among the predominant OTU accounting for 1.5% of all sequences. This group has been reported in solid waste by several culture based studies (Anitha and Jayraaj, 2012; Oyeleke and Istifanus, 2009; Rastogi *et al*., 2011). The importance of this genus to animal and human health as well as its applications in biotechnology is well known (Huang *et al*., 2012; Na *et al*., 2013; Thakker *et al.*, 2012). In either case, predominance of *Escherichia /Shigella* in solid biomedical wastes suggests a possible acquisition of unique features that qualify their survival in such extreme environment. The survival of these bacteria in aged solid biomedical waste could lead into development of multidrug resistance and hence pose health risks, such as emerging infectious diseases, or inversely, their adaptation under such harsh environments could lead into acquiring features with potential application in biotechnology. The presence of a big number of OTUs mapping to human pathogens (34%) and OTUs with unknown status as to whether they are human pathogens or not (39.4%) suggests that there is a lot yet to be known of the bacterial composition of solid biomedical waste and their economic importance.

*Firmicute*s was the second most abundant phylum and comprised *Proteiniclasticum* and *Planococcus* as the most predominant genera. *Proteiniclasticum* accounted for 10.4% of all sequences and was found in 67% (10/15) of all samples at relative abundance of 1% or more. Since this genus has been reported in ruminants (Zhang *et al.*, 2010) and tannery waste water (Desta *et al*., 2014), its overall predominance in solid biomedical waste could be attributed in

part by ruminant animals scavenging on the dumpsite and also by solid biomedical waste from various sources. The fact that *Proteiniclasticum* has been reported in uranium mines (Khan *et al*., 2013) and in cold and alkaline environment from which novel enzymes with industrial potential were discovered (Vester *et al.,* 2014); the predominance of this genera in solid biomedical waste suggests that they might have acquired features to adapt in solid biomedical waste which could be of industrial potential.

The second predominant genus of *Firmicutes* was *Planococcus* spp (1.3%). This group of bacteria has been associated with the reduction of Chromium VI under high salt conditions (Subramanian *et al*., 2015), bioremediation of petroleum refinery effluents (Vennila and Kannan, 2013) and in production of *chitinase* enzymes of potential use in biotechnology and agro-industry (Essghaier *et al.,* 2010). These genera might have developed unique features to adapt extreme dumpsite environment. It is important to point out that, the current study used aged solid biomedical waste from open dumpsite. The predominance of industrially important bacteria in solid biomedical waste in an urban dumpsite calls for further research in order to gain a deeper understanding of public health and industrial importance of these bacteria.

4.5. Conclusion

There is rich and diverse bacterial community in aged solid biomedical waste. Some of the predominant OTUs are related to bacteria of industrial use while others mapped to human pathogens. There's high percentage of bacteria with unknown metabolic processes and a good number of bacteria were not known whether they are human pathogens or not. To our knowledge, this is the first report which anticipates presence of bacteria of industrial value in solid biomedical waste. This finding will help to design further research using functional metagenomics to better understand the potential of bacteria from aged solid biomedical waste to public health and also in industrial application

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CHAPTER FIVE

Prevalence and antimicrobial resistance phenotype of enteric bacteria from a municipal dumpsite[§§]

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Abstract

The objective of the study was to determine the prevalence and antibiotic resistance phenotype of enteric bacteria from the municipal dumpsite. Samples were collected from different type of solid waste, including domestic waste (Dom), solid biomedical waste (Biom), river sludge near the dumpsite (Riv) and faecal material of pigs scavenging on the dumpsite (FecD). A control sample was collected from faecal material of pigs initially reared indoor (FecI) and shifted to scavenging on the dumpsite (FecIF). Total genomic DNA was extracted, and the 16S rRNA gene was amplified, sequences and used to study prevalence of enteric bacteria. The same sample was used to isolate enteric bacteria that were later tested to 8 different antibiotics for their susceptibility phenotype. Solid wastes are not sorted in Arusha municipal. There was high interaction between animals and humans on the dumpsite. A total of 219 enteric bacteria from 75 genera were identified. *Escherichia* sp and *Shigella* sp (12%), *Bacillus* sp (11%) and *Proteiniclasticum* (4%) were the predominant genera. Most of the *Escherichia* sp, *Shigella* sp and *Bacillus* were from FecD, while *Proteiniclasticum* sp was from solid biomedical waste. Some isolates from FecD had 99% sequence similarity to pathogenic *Escherichia furgosonii*, *Shigella sonnei*, *Enterococcus faecium* and *Escherichia coli O154:H4*. Over 50% of the isolates

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were resistant to Penicillin G, Ceftazidime and Nalidixic Acid. Ciprofloxacin and Gentamycin were the most effective antibiotics with 81% and 79% susceptible isolates, respectively. Of all the isolates, 56% (45/80) were resistant to more than one antiobiotic. *Escherichia* sp and *Bacillus* sp (12 isolates each) constituted a large group of multidrug resistant bacteria. All *Pseudomonas* sp from Biom and FecD were multidrug resistant. There is high prevalence of antibiotic resistant enteric bacteria on the dumpsite. We report possible risks of spreading antibiotic resistant bacteria/genes from the dumpsite to clinical settings through animals and humans interacting on the dumpsite. This finding calls for a comprehensive research to study the shared resistome in bacteria from the environment, humans and animals using PCR and metagenomic based approaches to identify prevalence of known and capture new resistant genes

Keywords*:* Enteric bacteria, pigs, antibiotic resistance, municipal dumpsite, solid wastes

5.1. Introduction

Antibiotic resistant bacteria are extremely important to human and animal health, as it has become a major public health challenge globally (Broens *et al.,* 2012; Edelstein *et al.,* 2003; Heritage *et al*., 1999; Mshana *et al*., 2013; Sayah *et al.,* 2005; Soge *et al*., 2006). Microbes have developed a mechanism to evade our drugs and the trend is worrisome as day's go by. The knowledge on the origin of antibiotic resistance in the environment is key to public health owing to the growing importance of zoonotic diseases as well as the necessity for predicting emerging resistant pathogens (Allen *et al.,* 2010)**.** Inappropriate use of antibiotics has been pointed out as one of the reasons which leads to selection and hence development of drug resistant microbes (Gómez *et al*., 2014; Sözen *et al*., 2013; You *et al*., 2008).

Poor solid waste management in many municipalities in developing countries (Getahun *et al.,* 2012; Karak *et al.,* 2012; Marchand *et al.,* 1995; Sharholy *et al.,* 2008) is associated with the accumulation of unsorted garbage in both undesignated areas and in open dumpsite. In African settings it is normal to find biomedical / pharmaceutical / antibiotic residues thrown into common dumpsites. The diverse microbes from domestic, biomedical and industrial wastes create a complex interface on dumpsites that favours bacterial evolution. The variety of chemicals and drug residues on dumpsites are likely to create a selection pressure to microbes,

hence generating resistant groups that could easily be carried by feral and domestic animals as well as humans often times interacting on dumpsite.

Several studies have reported on the prevalence of bacteria of public health importance on municipal dumpsite (Achudume & Olawale, 2007; Emmanuel *et al.,* 2011; Semrau, 2011). Enteric bacterial isolates from the dumpsite were reported to be resistant to commonly used antibiotics (Emmanuel *et al.,* 2011). The fact that geographical conditions and types of waste generated in one location varies from any other; and since microbial proliferation depends on the geographical conditions and available nutrients; it is logical that public health risks caused by one municipal dumpsite cannot be the same elsewhere.

Despite the poor solid waste management in most municipalities in Tanzania (Matiko, 2012; Mungure *et al.,* 1995), no study has been done to screen for antimicrobial resistant bacteria from dumpsites. Only few studies on antimicrobial resistant bacteria have been reported in hospital settings. For example, a report on antimicrobial resistant bacteria in diabetic women by Lyamuya *et al*., (Lyamuya *et al.,* 2011), multiple resistant bacteria causing surgical site infection by (Manyahi *et al.,* 2014), nasal carriage of methicillin resistant *Staphylococcus* by under-five in Tanzania (Sabrina *et al*., 2014) and antimicrobial resistant bacteria from urinary isolate. All of these studies were conducted in hospital settings.

In this study, culture independent approach was used to identify enteric bacteria on the dumpsite and culture based method was used for isolation and study antimicrobial resistance phenotype. We communicate high prevalence of antibiotic resistant bacteria amidst a complex interaction of domestic and feral animals as well as humans on a municipal dumpsite

5.2. Materials and methods

5.2.1. Study site and sampling

Site for this study was the Arusha municipal dumpsite in Tanzania, where waste from different urban sources is dumped. Sampling was done during March to June 2013 whereby prior to sample collection, a qualitative survey was conducted to identify types of most common solid waste on the dumpsite. This comprised waste from households and markets (foods, pampers, clothes, etc.), chemical and biomedical waste (drug containers, used syringes), various plastics and used glassware, waste from abattoirs and brewers as well as fecal matter from animals

scavenging on the dumpsite itself. Samples for this study were fresh droppings of pigs continuously scavenging on the dumpsite (FecD, *n* = 20), solid waste from different sources (domestic waste – Dom, *n* = 22; solid biomedical waste – Biom, *n* = 15) and run-off water sludge from adjoining a nearby river (Riv, *n* = 10). About 5 g of the core of fresh droppings of pig as well as solid waste and sludge from the dumpsite were aseptically collected into sterile plastic containers and within one hour transported on ice to the molecular biology laboratory of the Nelson Mandela African Institution of Science and Technology, and stored at -20 ^{o}C until further processing. As a control sample, fresh fecal materials collected from indoor reared pigs (FecI, *n* = 10) which were later shifted from indoor to free range on dumpsite (FecIF, *n* =15) were incorporated in this study.

5.2.2. Ethical statement

This study was approved by the research committee of The Nelson Mandela African Institution of Science and Technology, in Arusha, Tanzania. Permits to sample the dumpsite was granted by the Arusha District Veterinary office and to transfer samples between laboratories permits were given by the Zoosanitary inspectorate services of Tanzania, Arusha (VIC/AR/ZIS/0345), and Veterinary Services under the Ministry of Agriculture Livestock and fisheries of Kenya (RES/POL/VOL.XXIV/506).

5.2.3. Extraction of total genomic DNA and PCR amplification

Total genomic DNA was extracted from about 250 mg of sample using PowerSoil™ DNA extraction kit (MOBIO Laboratories, Carlsbad, California, USA) as per manufacturer's protocol. Quality of DNA; A260/A280 and A260/A230) was verified with NanoDrop ND-2000c spectrophotometer (Thermo Scientific) and electrophoresis in 0.8 % agarose gel stained with GelRed (Biotium) and run in 0.5X TBE buffer and electrophoresis run at 80V for 30 minutes. Bacterial 16S rRNA gene fragments were amplified using universal primers 27F (5'-agagtttgatcctggctcag -3') and 1492R (5'-ggttaccttgttacgactt-3') (Bourne & Munn, 2005; Calheiros *et al*., 2010; Galkiewicz & Kellogg, 2008). PCR reaction in 20 µl AccuPower® Taq PCR PreMix (Bioneer Corporation, Korea) composed of 0.8 µl of 10 pmol/µl each for the forward and reverse primers, 16.4 µl molecular grade water and 2 µl DNA template. Amplification was done in TC-PLUS PCR machine (TECHNE Scientific, UK) programme set at 94 ^{o}C for 5 min (initial denaturation), 35 cycles of 94 ^{o}C for 30s, 57 ^{o}C for 30 s (annealing), 68

^{o}C for 1min (initial extension) and final extension at 68^{o}C for 7 min. Amplicons were verified with gel electrophoresis in 1.5% agarose at 100 V, 45 min and visualized using Gel documentation system (DIGIDOC-IT System, UK) (Appendix 5.1). The PCR products were purified using Qiagen kit (Qiagen, Valencia, CA) following manufacturer's protocol.

5.2.4. 16S rRNA gene library construction and Sequencing

Five libraries corresponding to five sample sources, FecI, FecD, FecIF, Biom and Dom were constructed. Pure PCR product from the same sample source were pooled in equal concentration, ligated to vector *pTZ57R/T* (Fermentas, Lithuania) and then transformed DH5α™ strain of *E. coli* (Invitrogen, Life Technologies) as per manufacturer's instructions. Transformed bacteria cells (150µl) were inoculated in LB agar composed of 100 mg/l Ampicillin, 40 µl of 20 mg/ml X-gal and 60 µl of 100 mM IPTG (Thermal Scientific) then incubated at 37 ^{o}C for 24hrs (J.P Selecta, Spain). To ascertain presence and correct orientation of insert DNA, screening of recombinant clone was done using colony PCR (Appendix 5.2 and Appendix 5.3). Briefly, individual white clones (90 – 100 per library) were resuspended into 20µl PCR master mix composed of 0.5 µl each of the universal vector specific primers M13F (5'-CGCCAGGGTTTCCCAGTCA-3') and M13R (5'-CAGGAAACAGCTATGAC-3') (Kleinsteuber, Riis, Fetzer, Harms, & Müller, 2006) and the AccuPower® Taq PCR PreMix as explained above. PCR programme run in GeneAMP™ PCR system 9700 (Applied Biosystems) set at 95^{o}C for 3 min (initial denaturation) and 35 cycles of 94^{o}C for 1 min, 55^{o}C for 1 min, 72 ^{o}C for 2 min and final extension at 72 ^{o}C for 15 min. Amplicons, along with *p*TZ57R positive controls were visualized using 1.5 % agarose gel electrophoresis. Colony PCR products were purified using QiAquick® PCR kit as previously explained. The quality of DNA was further verified with NanoDrop reading and agarose gel electrophoresis. Clones with a single band (ninety from each library) and at a minimum of 25 ng/µl concentration were selected for sequencing. Bidirectional sequencing of 16S rRNA nucleotide was done using Automatic BigDye® terminator cycle chemistry (Applied Biosystems, USA). Forward and reverse M13 primers were independently used to generate forward and reverse sequences. Plasmid *p*GEM® (Promega, USA) was used as a control. Electrophoresis and data collection were performed on ABI 3730 DNA analyser (Applied Biosystems, USA).

5.2.5. Sequence data analysis and statistics

The 16S rRNA sequences were edited, trimmed and assembled using CLC Main Workbench (v7.0.3, CLC Bio Aarhus, Denmark). Quality control was done using default setting (quality limit = 0.05, and residue ambiguous = 2). Trimmed sequences were assembled with minimum aligned read length of 50 at stringency = medium and conflict vote (A, C, G, T). Conflicts were resolved to generate consensus sequences. Mothur algorithm v1.34 (Schloss *et al*., 2009) was used for sequence alignment, chimera detection, and distance calculation and clustering of sequences. Sequence identification was done using Naive Bayesian classification method in the Ribosomal Database Project (RDP) http://rdp.cme.msu.edu/ (Cole *et al.*, 2014). The differences in bacteria community between solid wastes were determined using the Parsimony, Libshuff and Unifrac analysis using the built-in commands in Mothur. A p value ≤ 0.05 was considered significant for all comparisons. High quality representative sequences were deposited at the NCBI database and assigned with the GenBank accession numbers KM244771 – KM244905

5.2.6. Phylogeny of enteric bacteria from the dumpsite and similarity to known pathogens

The MEGA6 software (Tamura *et al*., 2013) was used to build phylogenetic tree of enteric bacteria from different solid wastes. The 16S rRNA gene sequences of pathogenic bacteria gi|210063436| and gi|444439579| for *Enterococcus faecium* and *Shigella sonnei*, respectively were incorporated in the analysis. The 16S rRNA sequence of *Methanosarcina* sp (gi|37222667|) from Archaea was used as an out-group. Sequence alignment was done using ClustalW (Thompson *et al.*, 2002) and the evolutionary history was inferred using the Neighbor-Joining method (Saitou and Nei, 1987). The evolutionary distances were computed using the Jukes-Cantor method (Jukes and Cantor). Sequence similarity of enteric bacteria isolate from the dumpsite to known pathogens was assessed using the BLASTN v2.2.31 at the NCBI GenBank database. All sequences with identity of $\geq 99\%$ were considered highly similar to particular known bacteria**.**

5.2.7. Isolation and identification of enteric bacteria from the dumpsite

The same sample used for total genomic DNA extraction was used to isolate enteric bacteria. Briefly, a sterile loop was used to inoculate sample onto MacConkey agar (HiMedia Laboratories Ltd, India) that was previously sterilized at 115 ^{o}C for 15 min and casted into a sterile petri dish

followed with sterility check by incubating overnight at 37^{o}C. The media was incubated at 37 ^{o}C for 24 hours. Based on morphology and colony characteristics, individual colonies were sub-cultured onto MacConkey agar to generate individual pure colonies. Isolation of gram positive fastidious bacteria was done using blood agar media constituting Tryptone Soy Agar (HiMedia Laboratories Ltd, India) and 8% sheep blood. Based on the nature of hemolysis (α, β or γ); individual colonies from primary culture were further sub-cultured to generate pure colonies.

Initially, pure isolates were identified based on colony morphology and Gram staining according to Cowan and Steel method (Cowan and Steel, 1974). Further, identification was done using Analytical Profile Index kit (API 20E) specific for *Enterobacteriacea* and other non-fastidious gram negative rods (bioMerieux, France) as per manufacturer's instructions. Briefly, young pure colonies of about 8 - 24 hours were emulsified in 5 ml of 0.85% normal saline and then distributed into API 20E strips. Strips were incubated at 37 ^{o}C for 24 hours after which colour changes was recorded (Appendix 5.4). Apiweb™ V4.1 software (bioMerieux, France) was used to interpret results. None *Enterobacteriaceae* isolates were identified based on their 16S rRNA sequences. Briefly, genomic DNA of pure isolate was extracted using ZR-Bacteria DNA kit™ (Zymo Research, USA) as per manufacturer's instructions. The quality of DNA, amplification of 16S rRNA, purification amplicons, sequencing, identification isolates through sequence similarity was done as previously explained in section 5.2.6.

5.2.8. Antimicrobial susceptibility testing

The Kirby-Bauer disk diffusion technique (Bauer *et al.*, 1966) was used to study the antimicrobial susceptibility of bacteria isolates from the dumpsite. The commercially prepared antibiotic discs, Cefotaxime (CTXM, 30ug), Cefoxitin (FOX, 30ug), Penicillin G (P, 10ug), Amoxycillin / Clavulanic acid (AMC, 20/10ug) and Ceftazidime (CAZ, 30ug) in group of β-lactam antibiotics; and Ciprofloxacin (CIP, 5ug) and Nalidixic acid (NA, 30ug) in group of quinolones; and Gentamicin (CN, 10ug) in aminoglycoside antibiotics were used in this study. All antibiotic discs were purchased from (Oxoid, Basingstoke UK). An overnight culture of pure isolates in Tryptone Soy Broth (TSB) (HiMedia Laboratories Pvt, India) was suspended into sterile Peptone water (HiMedia Laboratories Pvt, India). Freshly prepared culture of 0.5 McFarland was inoculated on Mueller-Hinton agar media (HiMedia Laboratories Pvt, India). Sterile swabs were used to spread the suspension evenly on the plate. The plates were allowed to

dry for few minutes and then, the disks impregnated with antibiotics were applied on the surface. The plates were incubated at 37^{o}C for 24 hours, after which the diameter of the zone of inhibition was measured. Interpretation of the susceptibility test was done according to the Clinical Laboratory Standard Institute (CLSI, 2013). Isolates were categorized as resistant (R), intermediate resistant (IR) or Susceptible (S). Interpretation of antimicrobial resistance phenotype was performed as per Clinical Laboratory Standards Institute guide (CLSI, 2013). Excel program was used to prepare summary plots of resistance profile of different enteric bacteria isolates.

5.3. Results

5.3.1. Prevalence of enteric bacteria, phylogeny and similarity to known pathogens

A total of 218 enteric bacteria from both isolates and cloned amplicons of 16S rRNA were identified. These bacteria were from 75 different genera. *Escherichia/Shigella* (12%), *Bacillus* (11%) and *Proteiniclasticum* (4%) were the most abundant genera. It was also noted that *Escherichia/Shigella* and *Bacillus* were mostly contributed by faecal materials of pigs scavenging on dumpsite (FecD) (8% and 4%, respectively) while *Proteiniclasticum* dominated in Biom waste

Due to the importance of *Escherichia* and *Shigella* to public health; further analysis of enteric bacteria from pigs scavenging on the dumpsite was performed. In this analysis phylogenetic relationship of sequences of enteric bacteria from pigs scavenging on the dumpsite was compared to those from indoor reared, and pigs shifted from indoor to free range on the dumpsite. The phylogenetic tree (Fig.5.1) revealed three major clusters of bacteria. The first cluster (A) was composed of bacterial sequences exclusively found in indoor reared pigs (FecI). The second and third clusters (B and C) comprised of sequences originating from indoor, pigs shifted from indoor to free range as well as pigs permanently under free range. In these clusters at least two bacterial sequences from the same source clustered together. Of interest, sequences of both *Enterococcus faecium* and *Shigella sonnei;* well-known human pathogens fell into cluster B, and moreover, fell closer to sequences originating from FecD pigs.

Further, implication of sequence similarities shown between the two reference pathogenic bacteria (*Enterococcus faecium* and *Shigella sonnei*) with enteric bacteria from the FecD pigs was investigated. On interrogation of the 16S rRNA gene sequences at NCBI database with bacteria sequences generated in this study, 17 sequences of bacteria with high similarity to *Shigella sonnei, Escherichia furgosonii*, *Escherichia faecium* and *Escherichia coli 0157:H7* (Table 5.1) all of them known as important human and animal pathogens.

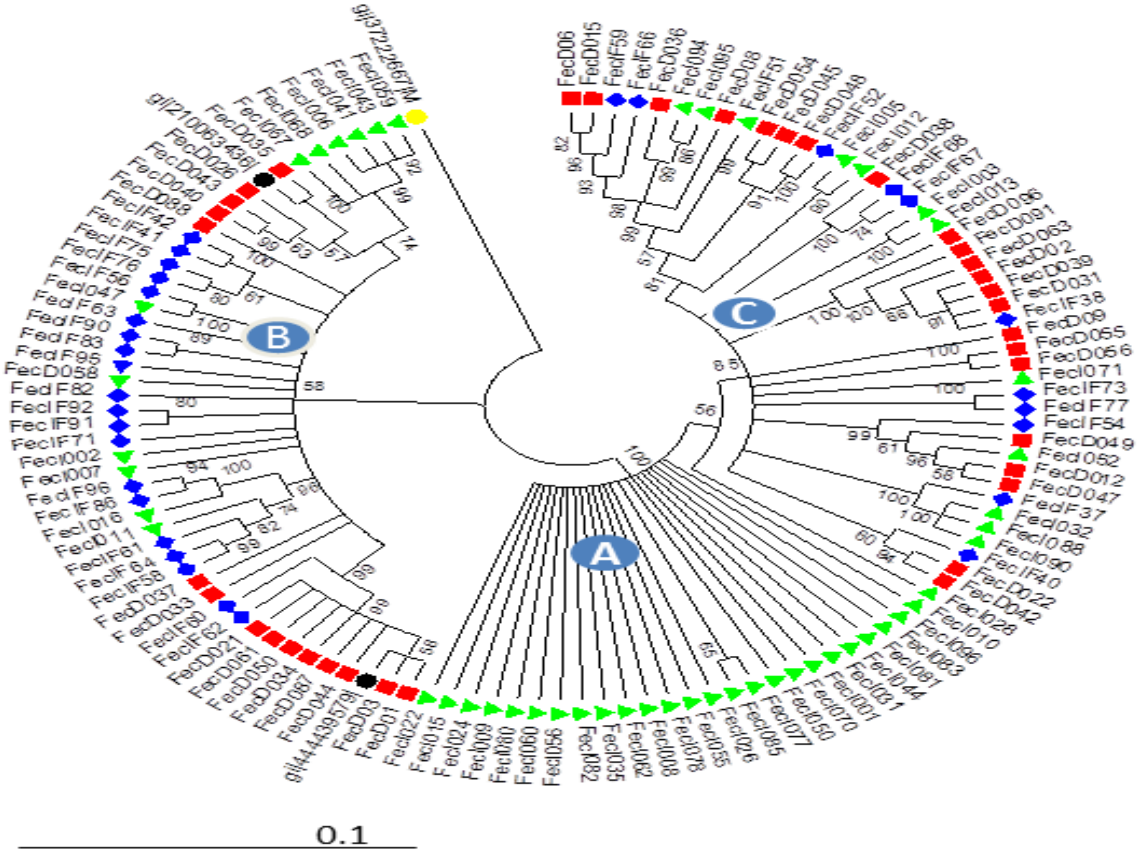


Figure 5.1 Phylogenetic trees of faecal bacteria from pigs under different management system. Evolutionary relationship of faecal bacteria of pigs under different management system was established using Mega6 software. The bootstrap values (expressed as percentages of 100 replications) are shown at branch points; only values above 50% are indicated. The scale bar represents substitutions per 100 nucleotides. Green triangles are bacteria sequences from indoor reared pigs; Blue-diamond are bacteria sequences from pigs recently shifted from indoor to free range on dumpsite, and Red - rectangles are bacteria sequences from pigs continuously scavenging on the dumpsite. The black - circles with GenBank accession numbers gi|210063436| and gi|444439579| are reference sequences of *Enterococcus faecium* and *Shigella sonnei*, respectively, both known to be pathogenic. The yellow - circle is *Methanosarcina* sp from Achaea (gi|37222667|) which was used as an out-group.

Table 5.1 Similarity of bacterial sequences from pigs scavenging on dumpsite to known pathogens

This work			From literature		
Accession #	# of clones	Description	Accession #	% ID	Ref
KM244771	6	*Shigella sonnei*	NR_074894.1	99	(Yang *et al*., 2005)
KM244773	5	*Escherichia furgosonii*	NR_074902.1	99	(Forgetta *et al.*, 2012)
KM244781	3	*Enterococcus faecium*	NR_102790.1	99	(Lam *et al.*, 2012)
KM244796	3	*Escherichia coli O157:H7*	NR_074891.1	99	(Hayashi *et al*., 2001)

5.3.2. Antimicrobial sensitivity test

Eighty pure bacteria isolates from different solid wastes were used for antimicrobial sensitivity test. The study revealed three susceptibility patterns shown in (Fig.5.2), where resistant, intermediate resistant and susceptible isolates were found after measuring the diameter of the zone of inhibition in (mm) and interpretation by the Clinical Laboratory Standard Institute guideline.

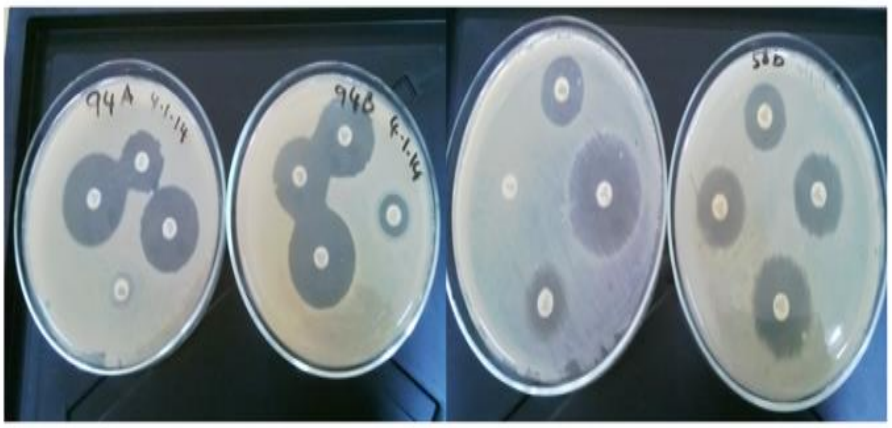
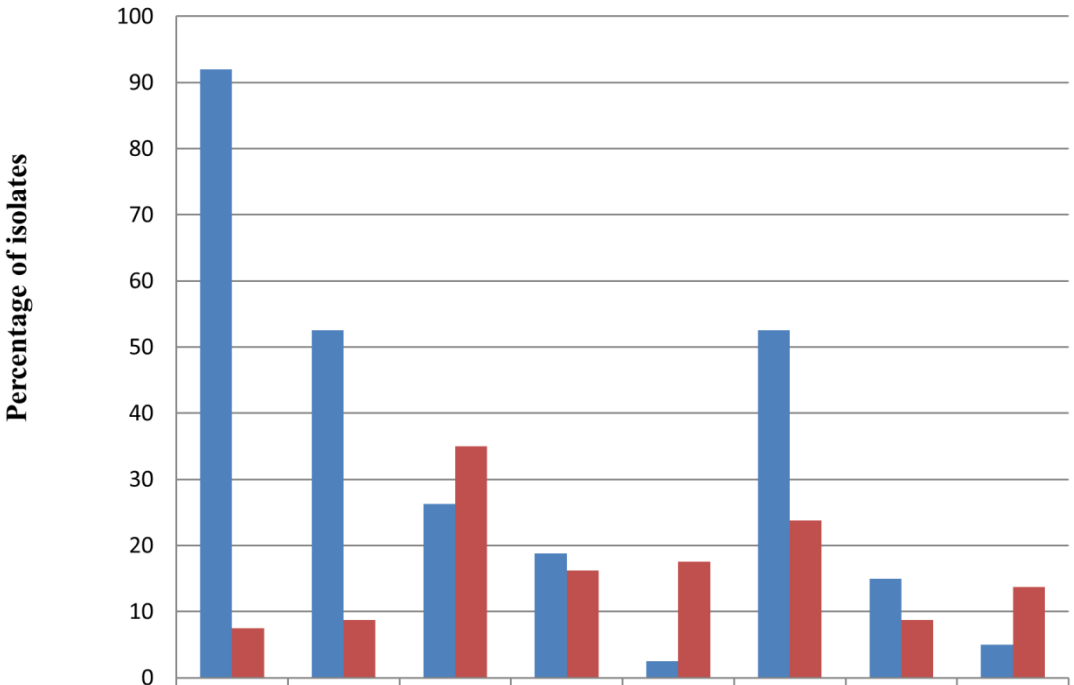


Figure 5.2. Phenotypic expression of enteric bacteria isolate to different antibiotics. White patches in the middle are the discs impregnated with antibiotics; clear circular zones around the discs are the inhibition zones of no bacteria growth due to antibiotics.

Phenotypic profile analysis revealed that, over 50% of all the isolates were resistant to Penicillin G, Ceftazidime and Nalidixic Acid antibiotics (Fig.5.3). While for penicillin G most bacteria

showed resistance to antibiotic (92% of all isolates); Ciprofloxacin and Gentamycin were the most effective antibiotics with 81% and 79%, respectively susceptible isolates. When the isolates exhibiting intermediate and total resistance are put together, it was found that, in the third generation cephalosporin β- lactam antibiotics CAZ and CTXM resistance was evident in over 60% of all isolates tested (61% for CTXM and 62% for CAZ).



	P	CAZ	CTXM	AMC	CN	NA	FOX	CIP
% Resistant isolate	92	53	26	19	3	53	15	5
% Intermediate resistant	8	9	35	16	18	24	9	14

Figure 5.3 Antimicrobial resistance phenotypic profile of bacteria isolates. Percentage of enteric bacterial isolates with different degrees of resistance; P - Penicillin G, CAZ - Ceftazidime, CTXM - Cefotaxime, AMC - Amoxycillin/Clavulanic, CN - Gentamicin, NA - Nalidixic Acid, FOX- Cefoxitin, CIP - Ciprofloxacin. Blue bars represent resistant isolates and red bars represent isolates with intermediate resistance.

Further, phenotypic profiling revealed prevalence of multidrug resistant bacteria on the dumpsite (Table 5.2). Of all the isolates, 56% (45/80) were resistant to at least two antibiotics. Some isolates were resistant to more than four antibiotics. For example, *Escherichia coli* from faecal material of pigs scavenging on dumpsites was resistant to Gentamycin, Amoxy/Clavulanic, Penicillin G, Ceftazidime, Cefoxitin and Nalidixic Acid; *Shigella flexneri* and *Pseudomonas luteola* both from faecal material of pigs were resistant to Amoxycillin/Clavulanic Acid,

Penicillin G, Ceftazidime, Cefotaxime, Cefoxitin and Nalidixic acid. *Pseudomonas luteola* from solid biomedical wastes and faecal material of pigs scavenging on dumpsite were multidrug resistant. Interestingly, multidrug resistant bacteria were also found in faecal material of pigs reared indoors. Overall summary of identified bacterial isolates and antibiotics inhibition zones and resulting sensitivity profile is shown in (Appendix 5.5 and Appendix 5.6).

Table 5.2 Multidrug resistance profile of bacteria isolates from the municipal dumpsite

Isolates expressing resistance to more than four antibiotics are shown with an asterisk; * *Escherichia coli* isolated from faecal material of indoor reared pigs; *[r]*Shigella* sp isolates from the river near the dumpsite; *[a] *Shigella flexneri* isolated from faecal material of pigs scavenging on dumpsite; ** *Pseudomonas luteola* from faecal material of pigs scavenging on dumpsite; *** *Pseudomonas luteola* from solid biomedical waste. *y *Serratia rubidae* isolated from solid biomedical waste, *[z] *Enterococcus casseliflavus* isolated from pigs scavenging on dumpsite; [x]* *Bacillus* sp isolated from biomedical waste

Isolate	Source	Genus/ Species	# Antibiotics	Resistance pattern	#Isolates
FecDX03	FecD	*Escherichia furgosonii*	2	P, NA	4
FecDX04	FecI	*Escherichia coli*	3	P,CAZ, NA	2
FecDX05	FecD	*Escherichia coli*	3	CIP, P, NA	1
FecDX07	FecD	*Escherichia coli*	4	CIP,CAZ, P,NA	1
FecDX11	FecD	*Escherichia coli*	4	P, CAZ, CTXM, NA	1*
FecIX13	FecI	*Escherichia coli*	5	AMC, P, CAZ, CTXM, FOX	1*
FecIX06	FecI	*Escherichia furgosonii*	6	CN, AMC, P, CAZ, FOX, NA	1*
FecDX15	FecD	*Shigella* spp	2	P, NA	1
FecDX17	FecD	*Shigella* spp	3	P, CAZ, NA	1
RivX03	Riv	*Shigella spp*	4	P, CAZ, CTXM, NA	1*[r]
FecDX08	FecD	*Shigella Flexneri*	5	AMC, P, CAZ, CTXM, NA	1*[a]
FecDX20	FecD	*Shigella flexeneri*	6	AMC, CAZ, P, CTXM, FOX ,NA	1*[b]
DomX33	Dom	*Pseudomonas* sp	2	P, NA	1
BiomX25	Biom	*Pseudomonas luteola*	3	P, CAZ, CTXM	1
BiomX22	Biom	*Pseudomonas luteola*	3	CIP, CAZ, NA	1
FecDX09	FecD	*Pseudomonas luteola*	4	CIP, P, CAZ, NA	2**
BiomX06	Biom	*Pseudomonas luteola*	5	AMC, P, CAZ, CTXM, NA	1 ***
BiomX11	Biom	*Pseudomonas luteola*	6	AMC, P, CAZ, CTXM, FOX, NA	1***
DomX09	Dom	*Serratia* spp	3	P, CAZ, NA	1
DomX21	Dom	*Serratia plymuthica*	3	AMC, P NA	1
BiomX14	Biom	*Serratia rubidae*	7	CIP, CN, AMC, P, CAZ, CTXM, NA	1*[y]
FecDX08	FecD	*Enterococcus* spp	2	CIP, NA	1
FecDX17	FecD	*Enterococcus casseliflavus*	3	AMC, P, CAZ, CTXM, FOX	1*[z]
BiomX23	Biom	*Bacillus* spp	2	P, CAZ	1
BiomX33	Biom	*Bacillus* spp	2	P, NA	2
DomX37	Dom	*Bacillus* spp	3	P, CAZ, CTXM	1
BiomX46	Biom	*Bacillus* spp	3	P, CAZ, NA	1
BiomX06	Biom	*Bacillus* spp	4	P, CAZ, CTXM, FOX	4*
BiomX13	Biom	*Bacillus* spp	5	AMC, P, CAZ, CTXM, FOX	1*
BiomX17	Biom	*Bacillus* spp	5	P, CAZ, CTXM, FOX, NA	1*
BiomX67	Biom	*Bacillus* spp	5	AMC, P, CAZ, CTXM, FOX	1*
FecDX42	FecD	*Bacillus* spp	6	AMC, P, CAZ,CTXM, FOX, NA	1[x*]

5.4. Discussion

This study determined the prevalence and antibiotic resistance profile of enteric bacteria from a municipal dumpsite in Arusha, Tanzania. High prevalence of bacteria resistant to most commonly used antibiotics was revealed on the dumpsite. Since the dumpsite was composed of solid waste from diverse sources such as hospitals, domestic and industrials, it is therefore expected that microbes found therein were brought to the dumpsite along with solid wastes from the respective sources. The fact that antimicrobial resistant genes are common in environments (Jensen *et al.,* 2001; Riesenfeld *et al.,* 2004; Sengeløv *et al.,* 2003; Wright, 2010) and play an important role for bacterial survival; the high prevalence of multidrug resistant bacteria on the dumpsite is probably due to a multitude of biological as well as ecological factors.

The complex interaction of microbes from different sources on the dumpsite creates a favourable environment for genetic material exchange between microbes, hence the possible prevalence of antibiotic resistant bacteria detected in this study. The fact that most of *Escherichia coli* and *Shigella* sp were multidrug resistant implies that there is possibility of these bacteria to harbour plasmids with several genes conferring resistance to a broad array of antibiotics. This finding is in agreement with previous studies where *Escherichia coli* from animals previously treated with antibiotics were found to harbour genes conferring resistance to β-lactam antibiotics (Horton, *et al*., 2011; Jørgensen *et al*., 2007; Pitout *et al.,* 2004). The presence of multidrug resistant bacteria on dumpsite may also be attributed to by the selection pressure from variety of drugs on dumpsite and the noted high interaction between microbes from different sources.

The study has shown that multidrug resistant *Escherichia coli* were also detected in faecal material of indoor reared pigs with no history of antimicrobial use. By sampling faecal material of pigs managed differently from those scavenging on the dumpsite we anticipated to confirm whether pig management has a significant impact on composition of faecal enteric bacteria. This finding is similar to previous reports (Allen, 2014; Horton *et al.,* 2011; Pallecchi *et al*., 2008), where resistant genes to given antibiotics were found in animal microbiota in the absence of treatment with particular antibiotics. This suggest that probably there is a broad spread of yet unknown resistant genes in both an environment and animal, hence further research is needed.

The prevalence of multidrug resistant *Pseudomonas* sp mostly from solid biomedical waste is also reported by (Odjadjare *et al*., 2012) in effluent of municipal waste water treatment plant.

Pseudomonas is associated with diseases in humans and animals, for example, Casalta *et al.*,(Casalta *et al.,* 2005) isolated *P. luteola* in patient with prosthetic valve endocarditis, Benoit reported chromosome encoding *β- lactamase* gene in *Pseudomonas luteola*; hence their resistance to β-lactam antibiotics. Other researchers reported the potential of *Pseudomonas luteola* in degrading natural and man-made chemicals with their extracellular enzymes *lipase* and *amylase* (Khannous *et al.,* 2014). The fact that these multidrug resistant bacteria were found on dumpsite, suggests that there is high chance of spreading these pathogens and the associated resistant genes to humans and animals. *Shigella* sp from the river near the dumpsite was among the multidrug resistant isolate. As documented in this study (Fig.5.1), the river near the dumpsite is used by local people around the dumpsite for domestic chores and their animals. People using the river have a possibility of contracting multidrug resistant bacteria. The study further speculates the risk of spreading resistant genes from the dumpsite to a larger population through the river.

Bacillus species was the second most abundant group after *Escherichia* sp. This group expressed high multidrug resistance to most of the antibiotics. Gentamycin was the most effective antibiotics to *Bacillus* sp with most isolates susceptible. Similarly, previous studies reported multidrug resistant *Bacillus* sp in municipal waste and tanneries, and they associated it with presence of mega plasmid with resistant genes (Naraian *et al.,* 2012; Samanta *et al.,* 2012). The fact that *Bacillus* sp is associated with several diseases of humans and animals, such as non-gastrointestinal tract infections (Bottone, 2010); eye infections (Kivanç *et al.,* 2014) and foodborne diseases (Logan, 2012; Scallan *et al.,* 2011). The prevalence of bacillus and the multidrug resistance shown in this study signifies presence of human and animal health risks on the dumpsite.

Many of the known antibiotic resistance genes are found on transposons and plasmids, which can be mobilized and transferred to other bacteria of the same or different species through horizontal gene transfer (Andam *et al.,* 2011; Kristiansson *et al.,* 2011; Palmer *et al.,* 2010). The fact that there is high diversity of antimicrobial resistant bacteria on dumpsite, and that animals and humans are commonly interacting on dumpsite; there is high chance of resistant genes from the dumpsite to be transferred to previously susceptible bacterial groups in human and animal

populations through horizontal gene transfer. This situation could further broaden the spectrum of resistant pathogenic bacteria in the environment.

The presence of high interaction between people working on dumpsite without any protective gear and domestic animals scavenging on dumpsite; presents a viable interface with high risks of contacting and spreading resistant genes from the dumpsite to the public. This could be through food animals scavenging on dumpsite, shedding of the infected faecal material on the environment and through people working on dumpsite.

In Tanzania, the prevalence of antibiotic resistant bacteria has been reported mostly in hospital settings. Reported cases in Tanzania includes, the prevalence of *β-lactamase* producing gram negative bacteria of nosocomial origin in hospital (Ndugulile *et al.,* 2005), antimicrobial resistance in urinary isolates (Moyo *et al.,* 2010b), and antibiotic resistant bacteria in diabetic women's (Lyamuya *et al.,* 2011), nasal carriage of methicillin resistant *Staphylococcus* to under 5 children (Moyo *et al.,* 2014) and antimicrobial resistant isolates from blood stream (Moyo *et al*., 2010b). Most of these studies reported *Escherichia coli* as the most prominent aetiological agent with high resistance to most of the drugs. As the case here, all studies were conducted in hospital settings; implying that little is known of the prevalence of the antimicrobial resistant bacteria and other pathogens in the environment and the possible association to growing antimicrobial resistance levels in Tanzania.

The study has also found high sequence similarity of bacteria from the dumpsite to known pathogens, including *Shigella sonnei*, *Enterococcus faecium*, *Enterococcus furgosonii* and *Escherichia coli*. Public health risks associated with these bacteria have been extensively reported and includes food borne diseases outbreaks caused by *Shigella sonnei* (Funke *et al*., 1993; Lam *et al.,* 2012; Thomas *et al.,* 2013; Yang *et al.,* 2005); nosocomial infections (Bernardini *et al.,* 1989) as well as various food-borne diseases (Escobar *et al*., 2013; Moore *et al.,* 1987; Robinson *et al.,* 1984). This finding suggest that probably these pathogens are present on the dumpsite, and the fact that there is high interaction between animals and human on the dumpsite they could easily be spread to human setting through food animals as well as people working on the dumpsite.

The prevalence of antibiotic resistant bacteria (with 56% multidrug resistant) on dumpsite, which represents an 'end-point' of biodegradable and unrecyclable garbage from diverse human

activities has demonstrated the microbial complexity on a municipal dumpsite and shows the role of such dumpsites as hotspots for emergence of new pathogens.

5.5. Conclusion

This study has shown high prevalence of antibiotic resistant enteric bacteria on the dumpsite. Some isolates have high similarity to known pathogens. This indicates a possible risk of spreading of these pathogens and resistant genes from the dumpsite to human or clinical setting. The finding calls for further research to study the shared resistome in bacteria from the environment, humans and animals using functional metagenomic approach to capture known and new resistant genes

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CHAPTER SIX

6.0. General discussion

This study has led to the following four major findings: (i) High abundance and diversity of bacteria has been demonstrated in the municipal dumpsite in Arusha, some of the bacteria representing pathogens of public health importance, (ii) Free range pigs scavenging in the municipal dumpsite were shown to be potential reservoirs of pathogenic bacteria, (iii) Some bacteria with metabolic processes related to known industrial applications were shown in this study and (iv) High prevalence of antibiotic resistant enteric bacteria was shown in the municipal dumpsite (Appendix 6.1). Considering the complex interface between microbes from diverse solid wastes, domestic and wild animals as well as humans working on the dumpsite without protective gear; it was of interest to investigate the abundance and molecular diversity of bacteria in the municipal dumpsite.

As reported in Chapter 2 of this dissertation, thirty-five bacteria phyla with 76,862 operational taxonomic units were found on dumpsite, presenting the highest diversity than any of the previous reports by (Oviasogie *et al*., 2010; Awisan, 2013) based on culture method, as well as (Cherif *et al*, 2008) based on molecular approach. However, it is important to mention here that there no report using a similar highthroghput sequencing techniques for study of bacteria community on the dumpsite. This finding implies that the urban dumpsite is a favourable environment for microbial proliferation whose diversity can be efficiently described using a culture independent approach such as the 16S rRNA amplicons of the total genomic DNA sampled directly from the environment, coupled with high through-put sequencing. Laurie Garrett reported that "cities and towns are microbial heavens" (Garrett, 1994) and John Cairns perceives them as "graveyards of mankind" (King and Henderson, 2013). The finding of high abundance and diverse bacteria on dumpsites, with some of them related to known pathogens supports the above phenomenon.

The dumpsite scenario puts humans and animals at risk of exposure to a range of pathogens from diverse garbage and also through intense animal interactions on the dumpsite. As reported in previous studies, 75% of the emerging pathogens are zoonotic, accounting for over 60% of all human diseases (Karesh *et al.,* 2012; Taylor *et al*., 2001). The human-animal interface has

elsewhere been reported to be associated with zoonotic diseases transmission (Godfroid *et al.*, 2011; Greger, 2007; Lloyd-Smith *et al.*, 2009; Michel *et al.*, 2010). From this study, the revealed complex interface between domestic and wild animals as well as humans on dumpsite with polymicrobials, ascertain that the dumpsite is a potential hotspot for emergence of new pathogens. This study has demonstrated the co-existence of bacterial communities in solid waste and animals scavenging on the dumpsite. This indicates that the dumpsite environment facilitates microbes to change hosts and this may potentially be accompanied with development of virulence and multi-host pathogens adaptation to new environment. Moreover, public health risks that may be associated with food products from domestic animals scavenging on dumpsite are another major concern.

In Chapter 3, this study has reported on the faecal microbiota of pigs, whereby pigs under different management regimen were compared. The significant difference in bacteria community between pigs scavenging on dumpsite and indoor reared pigs, and the presence of a clusters of bacteria exclusive to pigs scavenging on dumpsite; clearly demonstrates the influence of management on composition of faecal microbiota of pigs. For example, the finding of some pathogenic bacteria like *Brucella* (Cannella *et al*., 2012; Dean *et al*., 2012), *Rickettsia* (Johnston *et al*., 2013), *Listeria* (Cartwright *et al.*, 2013; Heiman *et al.*, 2015) and *Afipia* (Lo *et al.*, 2013) exclusively to pigs scavenging on the dumpsite suggests that the dumpsite is a suitable environment for such bacteria. Furthermore, presence of bacterial taxa related to pathogens exclusively in pigs scavenging on dumpsite points to the potential of these food animals to be reservoirs of pathogens of public health importance. Presence of bacteria with high sequence similarity to known pathogens was also found in pigs under free range. The potential of pigs as carreer of pathogens has been reported elsewhere (Cavaco *et al*., 2008; Hamill *et al*., 2013; Horton *et al.*, 2011; Jørgensen *et al.*, 2007; Ocaido *et al.*, 2013).The finding is an important alert on the risks of food animals scavenging on municipal dumpsite. With increasing demand for pork, hence a massive expansion in urban and peri urban agriculture, where small-holder farmers are practicing free range pig management system; appropriate policy guiding urban and peri-urban agriculture needs to be instituted to mitigate the possible associated risks. Since during study period scavenging pigs were clinically healthy, it could be that bacteria specific to these aninals confers some benefits; thus, further study on the roles of these bacteria to pigs, and their potential to be probiotics is warranted.

The fact that taxonomic-to-phenotypic mapping of bacteria from the dumpsite indicated that most of the bacteria from solid biomedical waste mapped to phenotype (metabolism) with industrial application, a detailed study specific to solid biomedical waste was conducted as reported in Chapter 4 of this dissertation. *Proteiniclasticum* was the most abundant in solid biomedical waste. Since this genus has been reported in ruminants (Zhang *et al*., 2010) and tannery waste water (Desta *et al.,* 2014), its overall predominance in solid biomedical waste could be attributed in part by ruminant animals scavenging on the dumpsite and also by solid waste from abbatoirs. The fact that *Proteiniclasticum* has been reported in uranium mines (Khan *et al.,* 2013) and in cold and alkaline environment from which novel enzymes with industrial potential were discovered (Vester *et al.,* 2014); the predominance of this genera in solid biomedical waste suggests that they might have acquired features to adapt in solid biomedical waste which could be of industrial potential. The second predominant genus of *Firmicutes* was *Planococcus* spp (1.3%). This group of bacteria has been associated with the reduction of Chromium VI under high salt conditions (Subramanian, 2012), bioremediation of petroleum refinery effluents (Vennila and Kannan, 2013) and in production of *chitinase* enzymes of potential use in biotechnology and agro-industry (Essghaier *et al*., 2010). These genera might have developed unique features to adapt extreme dumpsite environment.

From the *Proteobacteria* phylum, *Halomonas* sp accounted for 2% of all sequences in solid biomedical waste. This genus is reported to produce exopolysaccharides capable of emulsifying most of hydrocarbons (Bouchotroch *et al.,* 2000); industrially used in synthesis of ectoine which is used as a stabilizer for enzymes and cell protectant in skin and health care (Schwibbert *et al.,* 2011). Predominance of *Acinetobacter* sp (9.2% of all sequences) is in agreement with findings of (Saini *et al.,* 2004; Hossain *et al.,* 2013; Omer *et al.,* 2015) in solid biomedical waste. This group of bacteria resists and detoxify chromium VI thus having bioremediation potential in removal of heavy metal from industrial waste and contaminated sites (Bhattacharya and Gupta, 2013; Franzetti *et al*., 2014). The detection of *Pseudomonas* spp (1.7%) in solid biomedical waste is also reported by (Hossain *et al.,* 2013; Hossain *et al.,* 2011; Oviasogie *et al*., 2010; Rastogi *et al*., 2011) based on culture methods. Apart from this bacteria being associated with multidrug resistance (Jensen *et al*., 2001; Ndugulile *et al.,* 2005; Odjadjare *et al.,* 2012; Pfeifer *et al*., 2010) with impact to humans and animals health, it has been reported to be the first bacterium to degrade chloroaromatic compounds and also a novel *amylase* and *lipase* producer

of industrial application (Khannous *et al*., 2014; Miyazaki *et al.,* 2015). The presence of these bacteria in solid biomedical waste suggests further research based on both functional metagenomics and culture methods for thorough explore of their economic importance.

This study further investigated the prevalence and antimicrobial sensitivity of enteric bacteria from the dumpsite as reported in Chapter 5. Enteric bacteria were identified from variety of solid waste on the dumpsite as well as faecal material of pigs scavenging on the dumpsite. Later, isolates were tested for their antimicrobial sensitivity to commonly used antibiotics. Since the most predominant enteric bacteria, *Escherichia* / *Shigella* (12%) and *Bacillus* (11%) were from faecal material of pigs scavenging on dumpsite; further study of the phylogenetic relation of enteric bacteria from pigs under different management was conducted. The phylogenetic relationship showed uniqueness of microbiota from indoor reared pigs compared to that from pigs scavenging on the dumpsite. The clustering of some bacterial sequences from indoor reared pigs away from sequences of bacteria from free range pigs strongly suggests that microbiota composition is influenced by animal management system.

Despite reported presence of antimicrobial resistant genes in environments (Jensen *et al*., 2001; Riesenfeld *et al.,* 2004; Sengeløv *et al.,* 2003; Wright, 2010); high prevalence (56%) of multidrug resistant bacteria from the dumpsite could be attributed to several biological factors, such as interaction between microbes from different sources as well as ecological factors like exposure to variety of chemicals/drugs from industries, pharmaceuticals and hospitals. The fact that most of the *Escherichia* sp and *Shigella* sp were multidrug resistant suggests that these bacteria harbour plasmids with several genes conferring resistance to a broad array of antibiotics. Since many of the known antibiotic resistance genes are found on transposons and plasmids, which can be mobilized and transferred to other bacteria of the same or different species (Andam *et al.,* 2011; Kristiansson *et al.,* 2011; Palmer *et al.,* 2010), the finding of several multi-drug resistant bacteria in a complex polymicrobial environment of the dumpsite suggests presence of this process on the dumpsite.

The revealed multidrug resistant *Pseudomonas luteola* in biomedical waste is consistent to thefinding in effluent of municipal waste water (Odjadjare *et al*., 2012). Despite the fact that this bacterium has been implicated to cause diseases in both humans and animals (Altinok *et al.,* 2007; Casalta *et al.,* 2005); it has been reported to also degrade chemicals/drugs from the

environment with their extracellular enzymes, lipase and amylase (Khannous *et al.,* 2014). Thus, the frequency of *Pseudomonas luteola* on dumpsite is probably attributed to by their ability to degrade chemicals including drugs/ antimicrobial, thus rendering them resistant to most antibiotics; consequently, potential for this bacterium in spreading resistance genes to other non-pathogenic bacteria.

The fact that *Bacillus* sp is associated with several diseases of humans and animals (Bottone, 2010; Kivanç *et al.,* 2014; Logan, 2012; Scallan *et al.,* 2011); the detected high prevalence of multidrug resistant *Bacillus* sp on the dumpsite is a serious alarm to public health worthy further investigation. Previous works have reported multidrug resistant *Bacillus* sp in municipal waste and tanneries (Naraian *et al.,* 2012; Samanta *et al.,* 2012) and associated their resistance with presence of mega plasmid. Presence of high interaction between animals, humans and microbes on the municipal dumpsite expose humans and animals at risk of contracting multidrug resistant bacteria. This study reports also presence of multidrug resistant *Shigella* sp in the Burka river, located adjacent to the dumpsite. The fact that this river is used for both domestic purposes and as a source of water for animals, this finding further suggests there is high risk of spreading resistant genes from the dumpsite to a larger population.

Most reported cases of antimicrobial resistance in Tanzania focused mainly in hospital settings. Such cases are the lactamase producing gram negative bacteria of nosocomial origin in hospital (Ndugulile *et al*., 2005); antimicrobial resistance in urinary isolates (Moyo *et al*., 2010a); antibiotic resistant bacteria in diabetic women (Lyamuya *et al.,* 2011); nasal carriage of methicillin resistant Staphylococcus to under 5 children (Moyo *et al.,* 2014) and antimicrobial resistant isolates from blood stream (Moyo *et al*., 2010b). These studies reported *Escherichia coli* as the most prominent aetiological agent with high resistance to most commonly used drugs. Since none of these studies used environmental samples, the high prevalence of antibiotic resistant bacteria on the dumpsite, which represents an 'end-point' of biodegradable and unrecyclable garbage from diverse human activities, demonstrates the potential role of the dumpsite as a hotspot for emergence of antimicrobial resistant bacteria.

Despite the growing trend of emerging and re-emerging infectious diseases, none of the most pandemic pathogens have been predicted before infecting human beings (Morse *et al*., 2012). This study, therefore, advocates investigation of any possible public health risks in areas with

high interaction or interface of both macro and microorganisms, such as dumpsites so that research findings can inform preventive measures for intervening before pathogens reach the human population.

6.2. Conclusions

The following are the major conclusions from this study:

i. There is an abundant and diverse bacterial community in the Arusha municipal dumpsite some of which represent pathogens of public health importance ,
ii. Free range pigs scavenging in the dumpsite are reservoirs of pathogenic bacteria that could get into the human population through food chain or people handling these animals,
iii. There is high prevalence of antibiotic resistant enteric bacteria in the municipal dumpsite and,
iv. Some of the bacteria present in the municipal dumpsite have been shown to have potential industrial application.

6.3. Recommendations and future implications

This study recommends the following;

i. In-depth studies on the types, dynamics and the associated public health risks of microbes from the dumpsite. The study may involve screening of zoonotic and other public health diseases in human and animal population around the dumpsite.
ii. Instituting working policies that govern livestock farming in urban and peri-urban areas to mitigate possible risks associated with the free range system. This would entail communicating research findings to local, regional and national authorities.
iii. Study is recommended on the shared antibiotic resistant genes (resistome) in bacteria from the environment, humans and animals using both PCR and metagenomic approaches to capture both known and new resistant genes and unravel their relationship. The study would involve determination of antibiotic resistant genes in bacteria from the dumpsite, people working on dumpsite including family members, people living closer to the dumpsite, and animals scavenging on the dumpsite. The finding would then be used to assess presence of any shared antibiotic resistant genes between the environments and

humans. The output would assist in designing the strategy to manage spread of these antibiotic resistant genes

6.4. Novelity of the study

Using 16S metagenomics and high throughput sequencing technology, this study has established a database of bacteria community found on the dumpsite ecosystem. This data can be used to design tailor-made solid wate management system. Further more, this study found bacterial sequences with with high similarity to known pathogens. These seaquences can be used to design diagnostic tools for examining health status of people working on the dumpsite.

6.5. Limitation of the study

Sampling of solid wastes and faecal materials of pigs on the municipal dumpsite was done for four month only (March – June 2013). Therefore, bacterial diversity reported here is not representing the entire bacterial population of all seasons of the year. A study which covers all seasons of the year would give a better view of bacterial diversity and dynamics on the municipal dumpsite.

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APPENDICES

Appendix 1.1 Description of the Arusha municipal dumpsite

The Arusha municipal dumpsite is located in the Arusha peri-urban at about 12 km south east of the city at latitude 03°25'S, longitude 36°40'E, and at an elevation 1260m above sea level. The Arusha municipal with an area 93 km^2 and population of about 800,000 people (according to 2012 National census) is estimated to produce 400 tons/day of solid waste, and only 35 – 40% is collected and dumped. The dumpsite receives unsorted domestic, biomedical, industrial and abattoir solid wastes. The dumpsite is characterized by heaps of garbage varying from the time it was established to fresh ones. There are water-logged areas within the dumpsite due to the collection of water between garbage heaps following rains, thus making it a good breeding site for pathogens and vectors. Presently, the area is surrounded by residential houses and it is common to see domestic animals such as dogs, cattle, goats, pigs, chickens and birds scavenging on the dumpsite. The dumpsite borders with river Burka from the north (Fig.A1) and during rains, wastes from the dumpsite drain into the river. Residents surrounding the dumpsite use Burka River for domestic and animal drinking purposes.

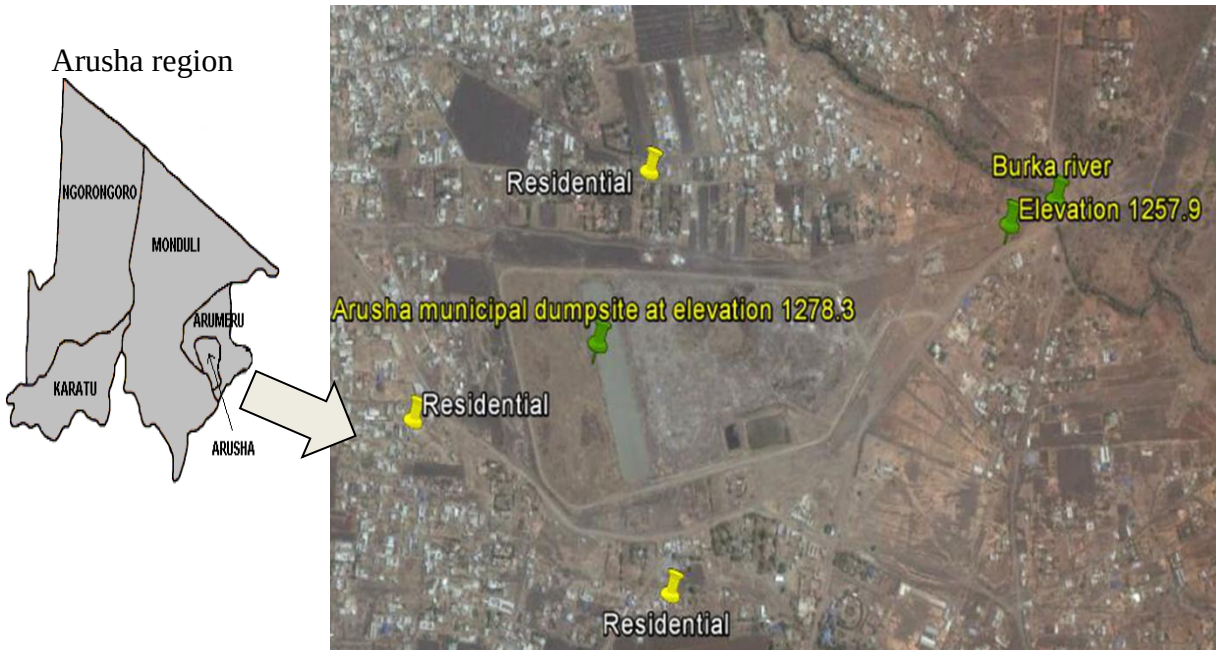


Figure A1. The location of the Arusha municipal dumpsite. The dumpsite is surrounded with residential houses at an elevation 1278.3m above sea level. Downhill there is Burka river at an elevation 1257.9m above sea level.

Interaction between animals and people at the dumpsite is shown in Fig.A2, and type of solid waste co-disposed in a municipal dumpsite is shown in Fig.A3. Human-animal interactions were common on the dumpsite. People, mostly children and women pick recyclable materials from the dumpsite daily. Domestic animals such as pigs, dogs, cattle, goats and sheep scavenge on the dumpsite. Wild animals such rodents are also found on the dumpsite, mostly during evenings. Birds, mostly crows, chickens and storks also scavenge on the dumpsite.

(a) (b)

(c) (d)

Figure A2. Animal and human interaction at the dumpsite (a) a truck offloading garbage and people scramble for recyclable materials, (b) pigs feed on dumped solid waste and a woman search for recyclable material, (c) goats and pigs feed on waste and (d) show people and cows using water from the Burka river which receives waste leaches from the dumpsite.

(a)

(b)

(c)

(d)

(e)

(f)

Figure A3. Types of solid wastes at the Arusha municipal dumpsite; (a) Diapers from domestic, (b) used syringes from hospitals /laboratories, (c) rotten onion and potatoes from market, (d) expired drugs from hospitals/ pharmaceuticals (e) skin-hides from abattoirs and (f) filter cakes and worn boxes from brewery factory.

Hypothesized microbial circulation in the dumpsite ecosystem

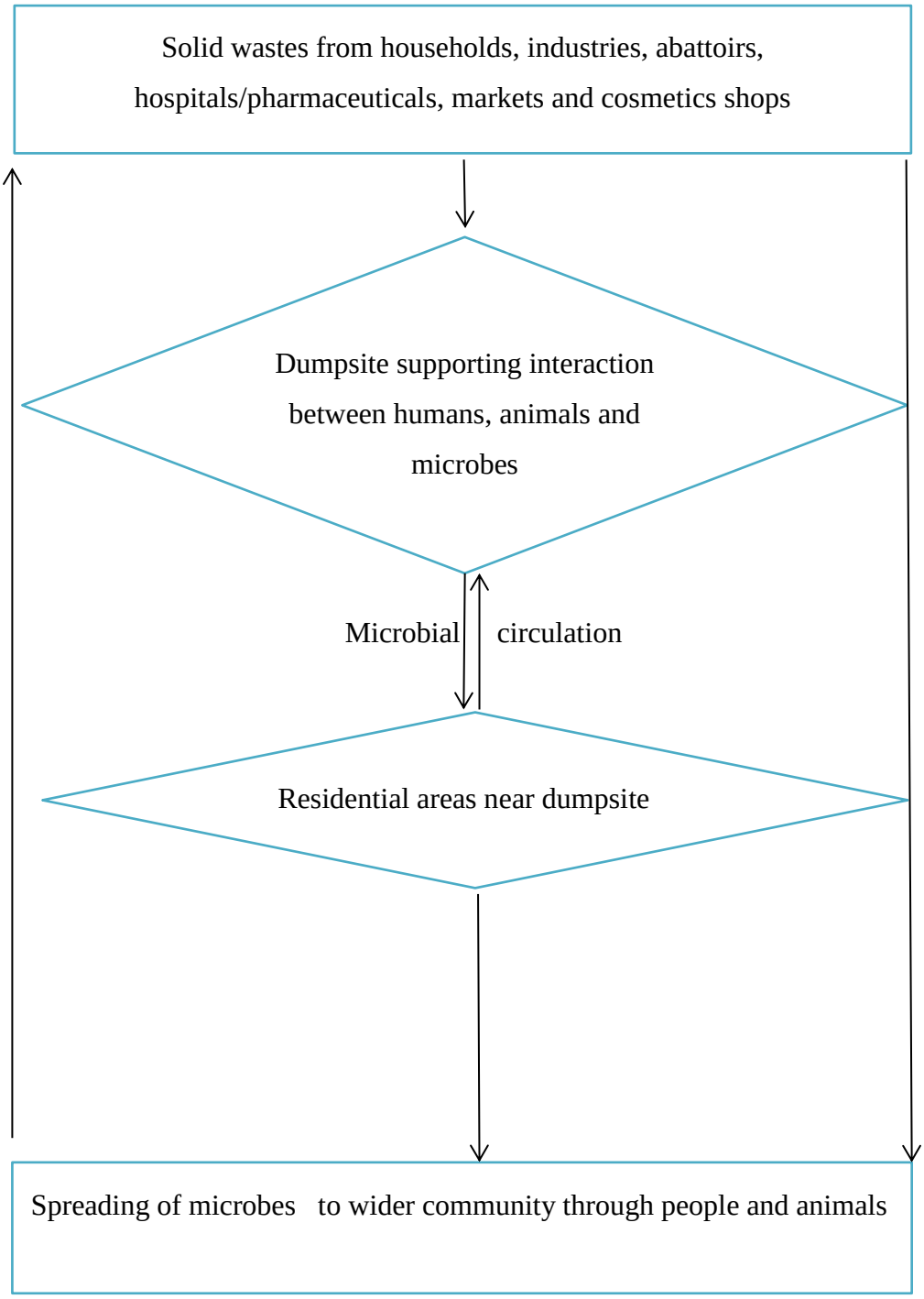


Figure A4. Hypothesized microbial circulation in the dumpsite ecosystem

Appendix 2.1 Genomic DNA extraction using PowerSoil® DNA isolation kit

Protocol for total genomic DNA extraction using PowerSoil® DNA isolation kit

i. To the PowerBead Tubes provided, add 0.25 grams of solid waste sample.
ii. Gently vortex to mix.
iii. Check Solution C1. If Solution C1 is precipitated, heat solution to 60^{o}C until dissolved before use.
iv. Add 60 ul of Solution C1 and invert several times or vortex briefly.
v. Secure PowerBead tubes in vortex adapter tube holder for the vortex, vortex at maximum speed for 10 minutes.
vi. Centrifuge tubes at 10,000 x g for 30 seconds at room temperature.
vii. Transfer the supernatant to a clean 2 ml Collection Tube (provided).
viii. Add 250 ul of Solution C2 and vortex for 5 seconds. Incubate at 4^{o}C for 5 minutes.
ix. Centrifuge the tubes at room temperature for 1 minute at 10,000 x g.
x. Avoiding the pellet, transfer up to, but no more than, 600 ul of supernatant to a clean 2 ml Collection Tube (provided).
xi. Add 200 ul of Solution C3 and vortex briefly. Incubate at 4^{o}C for 5 minutes.
xii. Centrifuge the tubes at room temperature for 1 minute at 10,000 x g.
xiii. Avoiding the pellet, transfer up to, but no more than, 750 ul of supernatant into a clean 2 ml Collection Tube (provided).
xiv. Shake to mix Solution C4 before use. Add 1200 ul of Solution C4 to the supernatant and vortex for 5 seconds.
xv. Load approximately 675 ul onto a Spin Filter and centrifuge at 10,000 x g for 1 minute at room temperature.
xvi. Discard the flow through and add an additional 675 ul of supernatant to the Spin Filter and centrifuge at 10,000 x g for 1 minute at room temperature.
xvii. Load the remaining supernatant onto the Spin Filter and centrifuge at 10,000 x g for 1 minute at room temperature.
xviii. Add 500 ul of Solution C5 and centrifuge at room temperature for 30 seconds at 10,000 x g. Discard the flow through.
xix. Centrifuge again at room temperature for 1 minute at 10,000 x g.

xx. Carefully place spin filter in a clean 2 ml Collection Tube (provided). Avoid splashing any Solution C5 onto the Spin Filter.

xxi. Add 100 ul of Solution C6 to the center of the white filter membrane. Alternatively, sterile DNA-Free PCR Grade Water may be used for elution from the silica Spin

xxii. Centrifuge at room temperature for 30 seconds at 10,000 x g.

xxiii. Discard the Spin Filter. The DNA in the tube is now ready for any downstream application. No further steps are required.

xxiv. Store DNA frozen at (-20 ^{o}C to -80^{o}C).

Appendix 2.2 Caporaso primers for V4 region of 16S rRNA

Primer ID	Golay barcode	Primer ID	Golay barcode	Primer ID	Golay barcode
806rcbc0	TCCCTTGTCTCC	806rcbc22	CAAACAACAGCT	806rcbc44	TAATACGGATCG
806rcbc1	ACGAGACTGATT	806rcbc23	GCAACACCATCC	806rcbc45	TCGGAATTAGAC
806rcbc2	GCTGTACGGATT	806rcbc24	GCGATATATCGC	806rcbc46	TGTGAATTCGGA
806rcbc3	ATCACCAGGTGT	806rcbc25	CGAGCAATCCTA	806rcbc47	CATTCGTGGCGT
806rcbc4	TGGTCAACGATA	806rcbc26	AGTCGTGCACAT	806rcbc48	TACTACGTGGCC
806rcbc5	ATCGCACAGTAA	806rcbc27	GTATCTGCGCGT	806rcbc49	GGCCAGTTCCTA
806rcbc6	GTCGTGTAGCCT	806rcbc28	CGAGGGAAAGTC	806rcbc50	GATGTTCGCTAG
806rcbc7	AGCGGAGGTTAG	806rcbc29	CAAATTCGGGAT	806rcbc51	CTATCTCCTGTC
806rcbc8	ATCCTTTGGTTC	806rcbc30	AGATTGACCAAC	806rcbc52	ACTCACAGGAAT
806rcbc9	TACAGCGCATAC	806rcbc31	AGTTACGAGCTA	806rcbc53	ATGATGAGCCTC
806rcbc10	ACCGGTATGTAC	806rcbc32	GCATATGCACTG	806rcbc54	GTCGACAGAGGA
806rcbc11	AATTGTGTCGGA	806rcbc33	CAACTCCCGTGA	806rcbc55	TGTCGCAAATAG
806rcbc12	TGCATACACTGG	806rcbc34	TTGCGTTAGCAG	806rcbc56	CATCCCTCTACT
806rcbc13	AGTCGAACGAGG	806rcbc35	TACGAGCCCTAA	806rcbc57	TATACCGCTGCG
806rcbc14	ACCAGTGACTCA	806rcbc36	CACTACGCTAGA	806rcbc58	AGTTGAGGCATT
806rcbc15	GAATACCAAGTC	806rcbc37	TGCAGTCCTCGA	806rcbc59	ACAATAGACACC
806rcbc16	GTAGATCGTGTA	806rcbc38	ACCATAGCTCCG	806rcbc60	CGGTCAATTGAC
806rcbc17	TAACGTGTGTGC	806rcbc39	TCGACATCTCTT	806rcbc61	GTGGAGTCTCAT
806rcbc18	CATTATGGCGTG	806rcbc40	GAACACTTTGGA	806rcbc62	GCTCGAAGATTC
806rcbc19	CCAATACGCCTG	806rcbc41	GAGCCATCTGTA	806rcbc63	AGGCTTACGTGT
806rcbc20	GATCTGCGATCC	806rcbc42	TTGGGTACACGT	806rcbc64	TCTCTACCACTC
806rcbc21	CAGCTCATCAGC	806rcbc43	AAGGCGCTCCTT	806rcbc65	ACTTCCAACTTC

Primer ID	Golay barcode	Primer ID	Golay barcode	Primer ID	Golay barcode
806rcbc66	CTCACCTAGGAA	806rcbc82	ATGGCTGTCAGT	806rcbc92	AGCAGAACATCT
806rcbc67	GTGTTGTCGTGC	806rcbc83	GTTCTCTTCTCG	806rcbc93	TGGAGTAGGTGG
806rcbc68	CCACAGATCGAT	806rcbc82	ATGGCTGTCAGT	806rcbc94	TTGGCTCTATTC
806rcbc69	TATCGACACAAG	806rcbc83	GTTCTCTTCTCG	806rcbc95	GATCCCACGTAC
806rcbc70	GATTCCGGCTCA	806rcbc84	CGTAAGATGCCT	806rcbc96	TACCGCTTCTTC
806rcbc71	CGTAATTGCCGC	806rcbc85	GCGTTCTAGCTG	806rcbc97	TGTGCGATAACA
806rcbc72	GGTGACTAGTTC	806rcbc86	GTTGTTCTGGGA	806rcbc98	GATTATCGACGA
806rcbc73	ATGGGTTCCGTC	806rcbc84	CGTAAGATGCCT	806rcbc99	GCCTAGCCCAAT
806rcbc74	TAGGCATGCTTG	806rcbc85	GCGTTCTAGCTG	806rcbc100	GATGTATGTGGT
806rcbc75	AACTAGTTCAGG	806rcbc86	GTTGTTCTGGGA	806rcbc96	TACCGCTTCTTC
806rcbc76	ATTCTGCCGAAG	806rcbc87	GGACTTCCAGCT	806rcbc97	TGTGCGATAACA
806rcbc77	AGCATGTCCCGT	806rcbc88	CTCACAACCGTG	806rcbc98	GATTATCGACGA
806rcbc78	GTACGATATGAC	806rcbc89	CTGCTATTCCTC	806rcbc99	GCCTAGCCCAAT
806rcbc80	TAGTATGCGCAA	806rcbc90	ATGTCACCGCTG	806rcbc100	GATGTATGTGGT
806rcbc81	TGCGCTGAATGT	806rcbc91	TGTAACGCCGAT		

Reverse complement of 3' Illumina adapter	Reverse primer pad	Reverse primer linker
CAAGCAGAAGACGGCATACGAGAT	AGTCAGTCAG	CC
5' Illumina adapter	**Forward primer pad**	**Forward primer linker**
AATGATACGGCGACCACCGAGATCTACAC	TATGGTAATT	GT

Appendix 2.3 Detailed process of quality control of sequences and statistical analysis

The following commands were used in Mothur package for the quality control and statistical analysis of paired end sequence reads of v4 – 16S rRNA sequences

The customized silva-based bacterial reference sequences for v4 region of 16S rRNA gene (**silva.v4.fasta**) and mothur-formatted version of the RDP training set (**trainset14_032015.rdp.fasta** and **trainset14_032015.rdp.tax**) were prepared. Paired-end sequences and the metadata (stability.files) (e.g. for FecD1, FecD1_R1.fastq and FecD1-R2.fastq) were uploaded into work environment

Quality control of sequences: Reducing sequencing and PCR errors

1. Assembling of paired-end reads:

Paired end reads for each sample were combined to form contiguous sequence using the command [**make.contigs]**

> *make.contigs(file=stability.files, processors=12)*

This command extract sequence and quality score data from fastq files and then create the reverse complement of the reverse read and then join the reads into contigs. The command produces a file [*stability.trim.contigs.fasta* and *stability.contigs.groups*]. These files will contain the sequence data and group identity for each sequence. Pairs of sequences are aligned and then, positions where two reads disagree are identified. If one sequence has a base and the other has a gap, the quality score for a base must be over 25 to be considered real. If both sequences have a base at that position, then one of the bases must have a quality score of 6 or more points better than the other. If is less than 6 points better, then the consensus base is set to be N (ambiguous)

The command (**summary.seqs**) was used to generated a percentile summary for the number of sequences formed, the size (N bases), the start and end positions, the number of ambiguous bases and homo polymer in each group, the

> *summary.seqs(fasta=stability.trim.contigs.fasta)*

Sequences with an ambiguous bases more than 2 and anything less than 239bp and longer than 260bp were removed using the command (**screen.seqs**). The target region was about 251bp

> *screen.seqs(fasta=stability.trim.contigs.fasta, group=stability.contigs.groups, maxambig=2,*

minlength=239, maxlength=260)

2. Removing duplicate sequences and preparation for alignment

The command (**unique.seqs**) merges identical sequences. The output will serve computational power during alignment. Note: In the output file there will be a column for a number of sequences characterized and a column for a number of unique sequences remaining

> *unique.seqs(fasta=stability.trim.contigs.good.fasta)*

Simplification of the names and group file was done using the command (**count.seqs**). This command generates a table where rows are the names of the unique sequences and columns are the names of different groups of solid wastes. The table is then filled with the number of times each unique sequence shows up in each group. The output will be a file called *stability.trim.contigs.good.count_table*

> *count.seqs(name=stability.trim.contigs.good.names, group=stability.contigs.good.groups)*

3. Aligning sequences to the reference

The command (**align.seqs**) aligns sequences to the customised v4-16S rRNA Silva-based reference sequences.

> *align.seqs(fasta=stability.trim.contigs.good.unique.fasta, reference=silva.v4.fasta)*

To asses for proper alignment (most sequences to have the same start and end position i.e. 1968 – 11550), the same number of Nbases (~ 250bp), no or minimum ambiguous base calls, and the number of homopolymers; the (**summary.seqs**) command was used to generates a summary table.

>***summary.seqs(fasta=stability.trim.contigs.good.unique.align, count=stability.trim.contigs.good.count_table)***

To screen sequences and remain with only those with required start and end position and a limited number of homopolymer, a command (**screen.seqs**) was used

>***screen.seqs(fasta=stability.trim.contigs.good.unique.align,count=stability.trim.contigs.good.count_table, summary=stability.trim.contigs.good.unique.summary, start=1968, end=11550, maxhomop=8)***

Since many columns in the alignments will contain gap characters (i.e. "-"); these columns, together with overhangs at both ends are removed using the command (**filter.seqs**)

>filter.seqs(fasta=stability.trim.contigs.good.unique.good.align, vertical=T, trump=.)

Further de-noising of sequences was done using the pre-cluster command, where up to 2 nucleotide differences between sequences was allowed. This command split sequences by group and then sorts them by abundance and goes from most abundant to least and identify sequences that are within 2 nucleotides of each other. If they are then they get merged.

>pre.cluster(fasta=stability.trim.contigs.good.unique.good.filter.unique.fasta, count=stability.trim.contigs.good.unique.good.filter.count_table, diffs=2)

4. Removing chimeras (PCR errors)

Having removed as much sequencing error as possible, at this stage chimeras are removed using UCHIME algorithm that is called within mothur using the command (***chimera.uchime***). This command will split the data by sample and check for chimeras.

>chimera.uchime(fasta=stability.trim.contigs.good.unique.good.filter.unique.precluster.fasta, count=stability.trim.contigs.good.unique.good.filter.unique.precluster.count_table, dereplicate=t)

Running chimera.uchime with the count file removes the chimeric sequences from the count file. At this stage chimeric sequences were removed from the fasta file using the command (***remove.seqs***)

>remove.seqs(fasta=stability.trim.contigs.good.unique.good.filter.unique.precluster.fasta, accnos=stability.trim.contigs.good.unique.good.filter.unique.precluster.uchime.accnos)

5. Removing non bacteria sequences

A final step of quality control is to remove any undesirables in a data set such as Archaea, Chloroplast, Eukaryotes and Mitochondria that might have been amplified. To achieve this, all sequences are classified based on the reference set using the command (**classify.seq).** The cut-off was set to be 51%

>classify.seqs(fasta=stability.trim.contigs.good.unique.good.filter.unique.precluster.pick.fasta, count=stability.trim.contigs.good.unique.good.filter.unique.precluster.uchime.pick.count_table, reference=trainset14_032015.rdp.fasta, taxonomy=trainset14_032015.rdp.tax, cutoff=51, relabund=T, processors=12)

All undesirables (Archaea, Chloroplast, Mitochondria and Eukaryota) were removed using the command (**remove.lineage)**

>*remove.lineage(fasta=stability.trim.contigs.good.unique.good.filter.unique.precluster.pick.fasta, count=stability.trim.contigs.good.unique.good.filter.unique.precluster.uchime.pick.count_table, taxonomy=stability.trim.contigs.good.unique.good.filter.unique.precluster.pick.rdp.wang.taxonomy, taxon=Chloroplast-Mitochondria-Archaea-Eukaryota)*

The command (**summary.seqs**) was used to generate a summary table of sequences remaining after the quality control processes. The command (**count.groups)** generated a table showing a number sequences that passed quality control in each group.

>summary.seqs (fasta=current, count=current)

>count.groups(count=stability.trim.contigs.good.unique.good.filter.unique.precluster.uchime.pick.count_table)

6. Assigning sequences to operational taxonomic units (OTUs)

Sequences were clustered into OTUs using the (**cluster.split**) command. Taxonomic informations were used to split sequences into bins and then cluster within each bin. Sequences were first split at ‘‘family level’’ and then clustered to a 0.03 cut-off level.

>cluster.split(fasta=stability.trim.contigs.good.unique.good.filter.unique.precluster.pick.pick.fasta, count=stability.trim.contigs.good.unique.good.filter.unique.precluster.uchime.pick.pick.count_table, taxonomy=stability.trim.contigs.good.unique.good.filter.unique.precluster.pick.rdp.wang.pick.taxonomy, splitmethod=classify, taxlevel=5, cutoff=0.08, processors=12)

The (**make.shared)** command was used to show many sequences are in each OTU from each group. The cut-off level was set to 0.03

>make.shared(list=stability.trim.contigs.good.unique.good.filter.unique.precluster.pick.pick.an.unique_list.list,count=stability.trim.contigs.good.unique.good.filter.unique.precluster.uchime.pick.pick.count_table, label=0.03)

The consensus taxonomy for each OTU was generated using the command (**classify.otu**)

>classify.otu(list=stability.trim.contigs.good.unique.good.filter.unique.precluster.pick.pick.an.unique_list.list, count=stability.trim.contigs.good.unique.good.filter.unique.precluster.uchime.pick.pick.count_table, taxonomy=stability.trim.contigs.good.unique.good.filter.unique.precluster.pick.rdp.wang.taxonomy, label=0.03)

For simplicity, the shared and consensus taxonomy files were renamed using the command **system (mv)** as shown.

>System(mv_stability.trim.contigs.good.unique.good.filter.unique.precluster.pick.pick.pick.an.unique_list.shared stability.an.shared)

>System(mv_stability.trim.contigs.good.unique.good.filter.unique.precluster.pick.pick.pick.an.unique_list .0.03.cons.taxonomy stability.an.cons.taxonomy)

7. Statistical analyses

Alpha (α) diversity estimation: The measure of the bacterial diversity within the dumpsite ecosystem was done by generating the collector's curve of the Chao1 richness estimators and the inverse Simpson diversity index using the command **(collect.single)**

>collect.single(shared=stability.an.shared, calc=chao-invsimpson, freq=100)

Rarefaction analyses: To determine the number of OTUs observed as a function of sampling effort, a rarefaction curve was established using a command **(rarefaction.single**) and the data generated were ploted using gplot package in R

>rarefaction.single(shared=stability.an.shared, calc=sobs, freq=100)

To calculate the number of sequences, sample coverage, number of observed OTUs and the Inverse Simpson measure of diversity, and Shannon measure of evenness, the command **(summary.single)** was used. Subsampling of sequences (size = 10,000) thousand times from each sample was done.

>summary.single(shared=stability.an.shared, calc=nseqs-coverage-sobs-invsimpson, subsample=10,000)

Beta (β) diversity estimation

To be able to compare bacterial community membership and structure between different solid wastes, the command (**dist.shared**) was used to generate a phylip-formatted distance matrix that describes the dissimilarity (1-similarity) among multiple groups. The command calculates the Yue and Clayton (thetayc) measure of dissimilarities between the structures of two communities, and the jaccard index (jclass) which describes the dissimilarity of bacterial membership between two communities.

>dist.shared(shared=stability.an.shared, calc=thetayc-jclass, subsample=10000)

The command generates two distance matrix files (*stability.an.thetayc.0.03.lt.ave.dist* and *stability.an.jclass.0.03.lt.ave.dist*). To describe the similarity of samples to each other, dendrograms were generated for both jclass (community membership) and thetayc (community structure) distance matrixes using (**tree.shared**) command.

>tree.shared(phylip=stability.an.jclass.0.03.lt.ave.dist)

>tree.shared(phylip=stability.an.thetayc.0.03.lt.ave.dist)

This command generates a newick-formatted tree file (*stability.an.thetayc.0.03.lt.ave.tre*) and (*stability.an.thetayc.0.03.lt.ave.tre*) which was visualized using FigTree software.

To determine whether the clustering within the tree was statistically significant or not, the commands (**parsimony)** and a distance metric (**unifrac)** were used.

>parsimony(tree=stability.an.thetayc.0.03.lt.ave.tre, group=waste.design, groups=all)

Note: [***waste.design***] is a metadata file which describes samples. The command (**unifrac.unweighted**) was used to test for bacterial community membership, while community structure was assessed using a command (**unifract.weighted**)

>unifrac.weighted(tree=stability.an.thetayc.0.03.lt.ave.tre, group=waste.design, random=T, groups=all)

>unifrac.unweighted(tree=stability.an.jclass.0.03.lt.ave.tre, group=waste.design, random=T, groups=all)

The significance of the difference in genetic diversity of bacterial community within each waste type from the average genetic diversity of both communities pooled together was assessed using the (**amova)** command (analysis of molecular variance**)**

>amova(phylip=stability.an.thetayc.0.03.lt.ave.dist, design=waste.design)

To test whether there's significant difference in separation between the centers of clouds of each solid waste type was assessed using homogeneity of molecular variance (**homova)** command

>homova(phylip=stability.an.thetayc.0.03.lt.ave.dist, design=waste.design)

The two distance matrices (*stability.an.jclass.0.03.lt.ave.dist)* for community membership and (*stability.an.thetayc.0.03.lt.ave.dist*) for community structures were also visualized using principal coordinate analysis command (**pcoa)**

>pcoa(phylip=stability.an.thetayc.0.03.lt.ave.dist)

Population level diversity

To determine whether there are any OTUs that are differentially represented between solid wastes, the command (**metastats)** was used. This is a non-parametric T-test that determines whether there are any OTUs that are differentially represented between the samples.

>metastats(shared=stability.an.0.03.subsample.shared, design=waste.design)

To determine bacterial taxa that are responsible for the difference between groups of solid wastes was done by performing indicator analysis using the command **(indicator)**

>indicator(shared=stability.an.0.03.subsample.shared, design=waste.design)

To ensure that further analysis of data is possible in different metagenomics packages, the shared file generated in MOTHUR (***stability.an.shared***) was converted to BIOM format file using (**make.biom**) command. This format is compatible to several metagenomics analysis packages.

>Make.biom(shared=stability.an.shared, constaxonomy=stability.an.cons.taxonomy)

The BIOM file was imported into MEtaGenomics ANalyser (MEGAN5) where further analyses for relative abundance, principal coordinate analysis and rarebiome was done.

The taxonomy file, shared file and metadata generated in MOTHUR were also imported into METAGENassist, a web-based analysis pipeline, where further analysis for taxonomy to metabolic processes mapping; taxonomy to pathogen mapping, principal coordinate analysis and heatmaps were done.

Appendix 2.4 Significantly different bacteria at genus level between solid wastes

S/N	Genera	Mean_ Biom	Mean Dom	P-value
1	*5_genus_incertae_sedis*	0.002426508	0.003941834	0.046
2	*Afipia*	6.52E-05	0	0.004
3	*Aidingimonas*	3.97E-05	0	0.036
4	*Akkermansia*	0.00015699	0.000409762	0.03
5	*Alkalimonas*	4.84E-05	0	0.036
6	*Allofustis*	6.53E-05	0	0.001
7	*Anaeroplasma*	4.43E-05	0.000299431	0.004
8	*Anaerotruncus*	0.000106643	0.000296284	0.019
9	*Anoxynatronum*	7.04E-05	3.26E-06	0.044
10	*Atopobium*	6.54E-05	7.20E-06	0.044
11	*Duganella*	0.000149495	2.94E-05	0.02
12	*Gordonibacter*	0.000159673	3.78E-05	0.05
13	*Haematobacter*	7.53E-05	0	0.012
14	*Haliangium*	0.001044774	0.000721728	0.035
15	*Haliscomenobacter*	0.000258662	0.000457528	0.014
16	*Halothiobacillus*	0.000173944	4.41E-05	0.002
17	*Janthinobacterium*	0	7.30E-05	0.02
18	*Lewinella*	0.000465988	0.000825868	0.03
19	*Massilia*	7.95E-06	7.07E-05	0.045
20	*Microcella*	5.57E-05	8.22E-06	0.017
21	*Millisia*	0.000109893	2.18E-05	0.007
22	*Oligella*	0.000325157	0.000694592	0.045
23	*Peptoniphilus*	0.000104073	0	0.004
24	*Perlucidibaca*	0.000183572	5.72E-05	0.015
25	*Propionivibrio*	8.55E-05	9.66E-06	0.001
26	*Pseudorhodoferax*	0.000150644	0	0
27	*Schlesneria*	9.14E-05	3.26E-06	0.001
28	*Solimonas*	0.00015094	5.24E-05	0.028
29	*Sphingobium*	0.001195233	0.000629521	0.048
30	*Sporobacter*	0.003330822	0.005496117	0.048
31	*Sporosalibacterium*	4.40E-05	0	0.036
32	*Sulfuritalea*	3.25E-05	0	0.036
33	*Waddlia*	0.000135668	4.76E-05	0.021
34	*Xiphinematobacter*	0.000177622	6.94E-05	0.015

Apppendix 2.4 continues

S/N	Feature	mean-Biom	Mean_FecD	p-val
1	*5_genus_incertae_sedis*	0.002426508	0.005089808	0.001
2	*Acetanaerobacterium*	0.000583545	0.001421545	0.013
3	*Acetitomaculum*	5.52E-05	0.000391802	0.001
4	*Acetobacterium*	0.000290781	0.000123016	0.049
5	*Achromobacter*	8.13E-05	0	0.031
6	*Acidaminobacter*	8.84E-05	0.000199251	0.04
7	*Aciditerrimonas*	0.002123494	0.001172092	0.027
8	*Aerococcus*	0.00031643	0.000799141	0.011
9	*Aggregatibacter*	2.63E-05	0.000161169	0.035
10	*Akkermansia*	0.00015699	0.000739684	0.002
11	*Algoriphagus*	0.000799868	0.000368239	0.03
12	*Altererythrobacter*	0.001082274	0.000605289	0.039
13	*Alterococcus*	0.000182825	4.21E-05	0.009
14	*Anaerobranca*	7.94E-05	0	0.031
15	*Anaeromyxobacter*	0.001439154	0.000599278	0.004
16	*Anaeroplasma*	4.43E-05	0.000384874	0.001
17	*Anaerostipes*	0.00019243	0.000793852	0.009
18	*Anaerovibrio*	0.000205033	0.000634306	0.01
19	*Aquiflexum*	0.000309648	0.000140051	0.036
20	*Azospirillum*	0.000503889	0.000188962	0.023
21	*Azotobacter*	0.000324228	0.000135911	0.032
22	*Bacteriovorax*	0.000407831	0.000133989	0.044
23	*Bacteroides*	0.002604564	0.005023329	0.019
24	*Barnesiella*	0.001193624	0.003575533	0.001
25	*Bdellovibrio*	0.001165384	0.000378753	0.01
26	*Bifidobacterium*	0.000606521	0.001655834	0.006
27	*Blastopirellula*	0.006694716	0.002605901	0.009
28	*Blautia*	0.002548571	0.006993896	0.001
29	*Brachymonas*	0.000259213	0.000106128	0.016
30	*BRC1_genera_incertae_sedis*	0.001521976	0.000607712	0.016
31	*Brevibacillus*	0.00039301	0.000112637	0.025
32	*Bulleidia*	0.000447597	0.001575639	0.001
33	*Butyrivibrio*	6.89E-05	0.000242104	0.017

Appendix 2.4 continues

S/N	Feature	mean-Biom	Mean_FecD	p-val
34	*Caldilinea*	0.002985043	0.001367198	0.017
35	*Campylobacter*	0.000194295	0.000478345	0.005
36	*Caryophanon*	0	0.000156577	0.028
37	*Cellulomonas*	0.000282331	0.000621455	0.041
38	*Cellulosilyticum*	0.001234297	0.003434429	0.003
39	*Chlamydia*	3.53E-05	0.000280499	0.011
40	*Chondromyces*	0.001410409	0.000693577	0.015
41	*Clostridium_IV*	0.003907005	0.010845164	0.002
42	*Clostridium_sensu_stricto*	0.008737839	0.019360076	0.008
43	*Clostridium_XI*	0.001556673	0.004209043	0.001
44	*Clostridium_XlVa*	0.00776595	0.015202681	0.003
45	*Clostridium_XlVb*	0.000940319	0.00179939	0.044
46	*Conexibacter*	0.002506939	0.001257355	0.007
47	*Coprobacillus*	4.07E-05	0.00027902	0.007
48	*Coprococcus*	0.001146161	0.002609547	0.006
49	*Coxiella*	0.000385615	0.000131023	0.041
50	*Dasania*	0.000114035	1.04E-05	0.039
51	*Desulfocapsa*	0.000280995	9.77E-05	0.019
52	*Desulfomicrobium*	0.00037522	0.000142273	0.01
53	*Desulfonatronum*	9.61E-05	0	0.016
54	*Desulfonispora*	1.76E-05	8.22E-05	0.035
55	*Dialister*	8.59E-05	0.000204914	0.045
56	*Dorea*	0.001238467	0.002564325	0.004
57	*Dysgonomonas*	0.00273743	0.000530496	0.015
58	*Elusimicrobium*	8.84E-05	0.000362769	0.032
59	*Ensifer*	0.000992385	0.000361396	0.038
60	*Enterorhabdus*	0.000138352	0.000341413	0.04
61	*Erysipelotrichaceae_incertae_sedis*	0.000450977	0.001462786	0.001
62	*Ethanoligenens*	0.000398183	0.000896362	0.012
63	*Eubacterium*	0.001034209	0.001716617	0.045
64	*Euzebya*	0.000595835	0.000183618	0.011
65	*Faecalibacterium*	0.001034857	0.002859885	0.004
66	*Ferruginibacter*	0.00100485	0.000243701	0.03

Appendix 2.4 continues

S/N	Feature	mean-Biom	Mean_FecD	p-val
67	*Filomicrobium*	0.00023388	7.81E-05	0.029
68	*Flavihumibacter*	0.000841979	0.000289566	0.022
69	*Flavonifractor*	0.000460392	0.001273833	0.005
70	*Geminicoccus*	0.002718103	0.001407646	0.01
71	*Gp4*	0.007847593	0.00476597	0.036
72	*Haliangium*	0.001044774	0.000350226	0.001
74	*Haloferula*	0.000472279	8.21E-05	0.001
75	*Helicobacter*	0.000171411	0.000440455	0.022
76	*Heliothrix*	0.00078312	0.000179573	0.007
77	*Hespellia*	0.000183968	0.000340507	0.033
78	*Holophaga*	1.59E-05	7.06E-05	0.02
79	*Howardella*	5.02E-05	0.000428798	0
80	*Ilumatobacter*	0.000971178	0.000444624	0.017
81	*Indibacter*	0.000307953	7.43E-05	0.025
82	*Inquilinus*	0.000217073	6.29E-05	0.01
83	*Isobaculum*	1.32E-05	0.000159372	0.035
84	*Klebsiella*	0	7.66E-05	0.014
85	*Kofleria*	0.000383076	0.00010108	0.004
86	*Lachnospiracea_incertae_sedis*	0.002968346	0.008519407	0.001
87	*Lactobacillus*	0.003576022	0.006459193	0.02
88	*Levilinea*	0.000512346	0.000153702	0.012
89	*Mitsuokella*	0.000232957	0.000702384	0.012
90	*Mogibacterium*	0.000718045	0.001508814	0.04
91	*Nannocystis*	0.000375784	0.000204572	0.013
92	*Natronincola*	0.00018337	2.08E-05	0.013
93	*Nitriliruptor*	0.001240434	0.000450545	0.008
94	*Nitrosomonas*	0.00051806	7.05E-05	0.003
95	*Nitrospira*	0.000939735	0.000416037	0.016
96	*Oligella*	0.000325157	0.000847594	0.004
97	*Ornithinibacillus*	8.37E-05	0	0.031
98	*Oscillibacter*	0.002316169	0.006724864	0.002
99	*Papillibacter*	0.001451009	0.002683703	0.032
100	*Paraeggerthella*	0.000127749	0.000446858	0.028

Appendix 2.4 continues

S/N	Feature	mean-Biom	Mean_FecD	p-val
101	*Parapedobacter*	0.001050208	0.00015628	0.006
102	*Paraprevotella*	0.000629754	0.001798605	0.013
103	*Pasteuria*	0.004959617	0.002365603	0.041
104	*Pediococcus*	0.000171146	0.000484569	0.021
105	*Peptococcus*	0.000443701	0.000865515	0.035
106	*Planctomyces*	0.008608864	0.003534475	0.002
107	*Plesiocystis*	0.000365227	6.44E-05	0.002
108	*Porphyrobacter*	0.001104133	0.000459605	0.041
109	*Porticoccus*	0.000321678	8.08E-05	0.007
110	*Prevotella*	0.007005839	0.01596701	0.007
111	*Proteiniclasticum*	0.010281141	0.001920867	0.009
112	*Proteiniphilum*	0.000800115	0.000223395	0.002
113	*Proteocatella*	0.000350909	7.47E-05	0.03
114	*Pseudobutyrivibrio*	0.000231659	0.000675493	0.011
115	*Pseudoflavonifractor*	0.000419625	0.000875676	0.012
116	*Pseudofulvimonas*	0.000658656	0.000331255	0.024
117	*Pseudoramibacter*	0.00016755	0.00052823	0.008
118	*Pseudorhodoferax*	0.000150644	1.13E-05	0.003
119	*Pusillimonas*	0.001502818	0.000612179	0.008
120	*Pyramidobacter*	1.98E-05	0.000163291	0.019
121	*Rhodopirellula*	0.002079498	0.001094807	0.015
122	*Robinsoniella*	0.000150932	0.000509029	0.001
123	*Roseburia*	0.000778761	0.0025137	0.005
124	*Ruminococcus*	0.003709739	0.009873302	0.001
125	*Salegentibacter*	0.00011536	0.000204082	0.012
126	*Salinibacter*	0.000752282	0.000318732	0.012
127	*Salisaeta*	0.001472241	0.000595253	0.001
128	*Sandarakinorhabdus*	0.000281942	0.00013003	0.042
129	*Sarcina*	5.77E-05	0.000441	0.006
130	*Selenomonas*	3.13E-05	0.000143218	0.035
131	*Sharpea*	0.000119644	0.000363381	0.033
132	*Sinomonas*	1.76E-05	0.00031118	0.005
133	*Slackia*	7.31E-05	0.000382641	0.001

Appendix 2.4 continues

S/N	Feature	mean-Biom	Mean_FecD	p-val
134	*Solitalea*	0.000172218	5.15E-05	0.035
135	*Spartobacteria_genera_incertae_sedis*	0.005345601	0.002713114	0.004
136	*Sphaerotilus*	4.20E-05	0.000122209	0.035
137	*Sporobacter*	0.003330822	0.009297008	0.001
138	*Subdoligranulum*	0.000328919	0.000915867	0.01
139	*Thiohalophilus*	0.000235943	3.41E-05	0.001
140	*Treponema*	0.004574724	0.01002677	0.001
141	*Truepera*	0.002573534	0.001312322	0.003
142	*Turicibacter*	0.000944458	0.002883276	0.003
S/N	**Bacterial genera**	**Mean_Biom**	**Mean_Riv**	**P_val**
1	*Acetoanaerobium*	0.00061967	0.000222318	0.022
2	*Acetobacterium*	0.000290781	8.86E-05	0.02
3	*Acholeplasma*	0.001430354	0.000320976	0.006
4	*Achromobacter*	8.13E-05	0	0.04
5	*Alicyclobacillus*	0.000102063	0	0.012
6	*Alishewanella*	0.000338792	0.000116253	0.003
7	*Alkaliphilus*	0.000489367	0.000146316	0.006
8	*Allofustis*	6.53E-05	0	0.04
9	*Aminomonas*	0.000142857	2.07E-05	0.017
10	*Ancylobacter*	8.86E-05	0	0.04
11	*Aquimonas*	0.000339816	0.000556013	0.05
12	*Armatimonadetes_gp5*	0.001028273	0.002514725	0.016
13	*Azotobacter*	0.000324228	0.000161228	0.025
14	*Bacillus*	0.004259462	0.002546007	0.02
15	*Balneola*	0.000579132	0.000138129	0.05
16	*Bellilinea*	0.003279469	0.006183781	0.033
17	*Bordetella*	0.00025439	0.000150645	0.026
18	*Brevibacillus*	0.00039301	6.22E-05	0.003
19	*Brevibacterium*	0.000476087	0.000157321	0.001
20	*Brevundimonas*	0.001913801	0.000870046	0.02
21	*Caenispirillum*	0.000168443	0	0.003
22	*Caldalkalibacillus*	7.22E-05	0	0.022
23	*Castellaniella*	0.000574975	0.000141446	0.003

Appendix 2.4 continues

S/N	Bacterial genera	Mean_Biom	Mean_Riv	P_val
24	*Cerasicoccus*	0.000599666	0.00013752	0.012
25	*Clostridium_XII*	0.000225394	1.80E-05	0.029
26	*Cohnella*	0.000138181	1.06E-05	0.029
27	*Corynebacterium*	0.003438129	0.000735171	0.003
28	*Defluviicoccus*	9.31E-05	0.000335578	0.025
29	*Demequina*	0.000575873	0.00034179	0.033
30	*Desmospora*	0.000380469	9.64E-05	0.003
31	*Desulfitibacter*	0.000127262	1.89E-05	0.029
32	*Desulfitispora*	9.27E-05	0	0.04
33	*Desulfitobacterium*	0.000254379	7.70E-05	0.031
34	*Dethiosulfatibacter*	0.000120091	0	0.007
35	*Devosia*	0.002834287	0.001332308	0.003
36	*Dietzia*	0.000615376	0.000306872	0.031
37	*Dysgonomonas*	0.00273743	0.000561384	0.04
38	*Ectothiorhodospira*	0.000134833	1.06E-05	0.017
39	*Ensifer*	0.000992385	0.000341668	0.002
40	*Erysipelothrix*	0.000721174	4.31E-05	0.002
41	*Euzebya*	0.000595835	0.000222228	0.048
42	*Exiguobacterium*	0.000844862	0.000280124	0.002
43	*Fervidicella*	8.32E-05	0.000370454	0.034
44	*Fodinicurvata*	0.000278575	0.000107854	0.045
45	*Garciella*	0.000411299	4.23E-05	0.019
46	*Geobacillus*	0.000600794	9.65E-05	0.001
47	*Gp17*	0.001109287	0.002473336	0.037
48	*Halothiobacillus*	0.000173944	1.63E-05	0.006
49	*Holophaga*	1.59E-05	0.000318101	0.001
50	*Inquilinus*	0.000217073	9.07E-05	0.024
51	*Janthinobacterium*	0	0.000112223	0.007
52	*Jonesia*	0.000364863	0.000177101	0.022
53	*Lacibacter*	3.62E-05	0.000278803	0.003
54	*Leifsonia*	0.000599249	0.000140062	0.004
55	*Lentibacillus*	0.000160385	0	0.003
56	*Leptolinea*	0.000133519	0.000588909	0.033

Appendix 2.4 continues

S/N	Bacterial genera	Mean_Biom	Mean_Riv	P_val
57	*Leucobacter*	0.001590789	0.000197388	0.001
58	*Litoribacter*	0.000415029	6.03E-05	0.007
59	*Longilinea*	0.001572846	0.00347066	0.021
60	*Lutibacter*	7.95E-06	0.000109254	0.048
61	*Lutispora*	0.000263074	8.19E-05	0.015
62	*Marinilactibacillus*	0.000138407	0	0.007
63	*Marinobacter*	0.001201697	0.000166413	0.022
64	*Massilia*	7.95E-06	0.00016198	0.003
65	*Methylocaldum*	0.000248913	9.43E-05	0.034
66	*Methylophilus*	5.93E-05	0.000222089	0.03
67	*Micrococcus*	0.000595883	0.000231622	0.04
68	*Millisia*	0.000109893	0	0.04
69	*Nitriliruptor*	0.001240434	0.00038727	0.011
70	*Ochrobactrum*	0.000754082	0.000245794	0.002
71	*Ornithinibacillus*	8.37E-05	0	0.04
72	*Ornithinimicrobium*	0.000433374	0.00013654	0.003
73	*Paenibacillus*	0.002018443	0.000589064	0.041
74	*Paenochrobactrum*	0.000348968	0	0.022
75	*Parapedobacter*	0.001050208	0.000157601	0.007
76	*Parvibaculum*	0.000263808	6.91E-05	0.01
77	*Phaeobacter*	0.000464737	0.000150645	0.02
78	*Pigmentiphaga*	0.00030466	7.14E-05	0.013
79	*Planococcaceae_incertae_sedis*	0.000625782	0.000144608	0.011
80	*Planococcus*	0.000744091	6.78E-05	0.021
81	*Pontibacter*	0.004151875	0.001678583	0.023
82	*Porphyrobacter*	0.001104133	0.000515242	0.044
83	*Prosthecobacter*	0.000422317	0.001124921	0.027
84	*Proteiniclasticum*	0.010281141	0.00235731	0.023
85	*Proteiniphilum*	0.000800115	0.000262651	0.015
86	*Pseudorhodoferax*	0.000150644	1.63E-05	0.01
87	*Pusillimonas*	0.001502818	0.000397256	0.001
88	*Runella*	7.95E-06	0.00013042	0.013
89	*Saccharofermentans*	0.00369516	0.001739781	0.008

Appendix 2.4 continues

S/N	Bacterial genera	Mean_Biom	Mean_Riv	P_val
90	*Saccharomonospora*	0.000197867	1.89E-05	0.006
91	*Salegentibacter*	0.00011536	2.07E-05	0.029
92	*Sandaracinobacter*	4.71E-05	0.000258946	0.016
93	*Sedimentibacter*	0.001078412	0.000504036	0.011
94	*Serpens*	0.000583352	0.00013042	0.002
95	*Solimonas*	0.00015094	1.06E-05	0.017
96	*Sphaerotilus*	4.20E-05	0.000155411	0.013
97	*Sphingobacterium*	0.003736698	0.000786535	0.003
98	*Sphingopyxis*	0.001059063	0.000316557	0.024
99	*Spirosoma*	0	0.000154412	0.007
100	*Sporichthya*	4.22E-05	0.000150812	0.038
101	*Sporolactobacillaceae_incertae_sedis*	0.001004021	0.00020946	0.023
102	*Sulfurovum*	0.000216121	7.70E-05	0.04
103	*Symbiobacterium*	0.000265756	2.78E-05	0.002
104	*Tepidimicrobium*	7.54E-05	0	0.022
105	*Thalassobacillus*	0.000316269	0.000111777	0.033
106	*Thermomonas*	3.71E-05	0.000173965	0.024
107	*Thiohalophilus*	0.000235943	4.01E-05	0.004
108	*Tissierella*	0.003579311	0.000481825	0.001
109	*Trichococcus*	0.000597136	0.00022386	0.035
110	*Truepera*	0.002573534	0.001330641	0.004
111	*Uliginosibacterium*	7.95E-06	0.000125557	0.025
112	*Vagococcus*	0.000639466	0.000155284	0.008
113	*Vasilyevaea*	0.000372893	0.000149769	0.016
114	*Victivallis*	0.000132577	3.61E-05	0.031
115	*Virgibacillus*	0.000561843	0.000184003	0.043
116	*Weissella*	0.00063445	0.000207813	0.004
117	*Wohlfahrtiimonas*	0.00013007	0	0.022
118	*Zavarzinella*	0.003027712	0.006536424	0.049
S/N	**Features**	**Mean_Dom**	**Mean-FecD**	**P-value**
1	*Acetanaerobacterium*	0.000746603	0.001422	0.012
2	*Acetitomaculum*	0.000120935	0.000392	0.008
3	*Acidaminobacter*	0.000104934	0.000199	0.041

Appendix 2.4 continues

S/N	Features	Mean_Dom	Mean-FecD	P-value
4	*Aciditerrimonas*	0.002512702	0.001172	0.002
5	*Acidocella*	0	6.43E-05	0.033
6	*Afifella*	0.000134467	1.23E-05	0.021
7	*Aggregatibacter*	4.90E-05	0.000161	0.018
8	*Alkanibacter*	0	5.04E-05	0.033
9	*Altererythrobacter*	0.001015035	0.000605	0.028
10	*Alterococcus*	0.000293837	4.21E-05	0.003
11	*Amaricoccus*	0.000958262	0.000523	0.011
12	*Anaerobacter*	1.39E-05	0.000103	0.039
13	*Anaerofustis*	0.000199679	0.000559	0.003
14	*Anaeromyxobacter*	0.001668016	0.000599	0.001
15	*Anaerovibrio*	0.000217448	0.000634	0.016
16	*Aquiflexum*	0.000388661	0.00014	0.013
17	*Arenimonas*	0.000691665	0.000304	0.005
18	*Armatimonadetes_gp5*	0.001310721	0.000496	0.002
19	*Atopobium*	7.20E-06	0.00017	0.001
20	*Azospirillum*	0.000502205	0.000189	0.002
21	*Azotobacter*	0.000286	0.000136	0.017
22	*Bacteriovorax*	0.000416966	0.000134	0.011
23	*Bauldia*	0.000581718	0.00028	0.023
24	*Bdellovibrio*	0.001338809	0.000379	0.001
25	*Bifidobacterium*	0.000844653	0.001656	0.021
26	*Blastopirellula*	0.00479964	0.002606	0.02
27	*Blautia*	0.003389678	0.006994	0.007
28	*Brachymonas*	0.000363351	0.000106	0.002
29	*BRC1_genera_incertae_sedis*	0.001433748	0.000608	0.003
30	*Bulleidia*	0.000611672	0.001576	0.001
31	*Butyricicoccus*	0.000997864	0.001763	0.016
32	*Byssovorax*	0.00078541	0.000327	0.001
33	*Caldilinea*	0.004152518	0.001367	0.001
34	*Campylobacter*	0.000264356	0.000478	0.033
35	*Cellulosilyticum*	0.001626327	0.003434	0.013
36	*Cesiribacter*	0.000829334	0.00042	0.009

Appendix 2.4 continues

S/N	Features	Mean_Dom	Mean-FecD	P-value
37	*Chondromyces*	0.001381393	0.000694	0.003
38	*Cloacibacterium*	0.000235321	0.000464	0.006
39	*Clostridium_IV*	0.005511419	0.010845	0.002
40	*Clostridium_sensu_stricto*	0.007506046	0.01936	0.002
41	*Clostridium_XI*	0.001954737	0.004209	0.007
42	*Clostridium_XlVa*	0.008145327	0.015203	0.001
43	*Conexibacter*	0.002212573	0.001257	0.008
44	*Coprobacillus*	0.000106587	0.000279	0.008
45	*Coprococcus*	0.001228479	0.00261	0.004
46	*Cucumibacter*	7.66E-05	0	0.012
47	*Dasania*	0.000126226	1.04E-05	0.013
48	*Defluviicoccus*	5.09E-05	9.65E-05	0.006
49	*Desulfocapsa*	0.000391447	9.77E-05	0.001
50	*Desulfosarcina*	0.000155236	3.22E-05	0.044
51	*Dethiobacter*	0.000373165	0.00015	0.019
52	*Dorea*	0.001250003	0.002564	0.003
53	*Duganella*	2.94E-05	0.00015	0.034
54	*Dyella*	6.19E-05	0.000178	0.027
55	*Dysgonomonas*	0.001469088	0.00053	0.011
56	*Ensifer*	0.000549336	0.000361	0.033
57	*Enterorhabdus*	0.000170542	0.000341	0.046
58	*Erysipelotrichaceae_incertae_sedis*	0.000686744	0.001463	0.002
59	*Erythrobacter*	0.000515678	0.00025	0.006
60	*Ethanoligenens*	0.000416154	0.000896	0.002
61	*Eubacterium*	0.001005086	0.001717	0.022
62	*Euzebya*	0.000722786	0.000184	0.002
63	*Faecalibacterium*	0.001553658	0.00286	0.011
64	*Filomicrobium*	0.000247885	7.81E-05	0.022
65	*Flavihumibacter*	0.000512247	0.00029	0.045
66	*Flavobacterium*	0.004622564	0.002613	0.003
67	*Flavonifractor*	0.000656434	0.001274	0.022
68	*Fulvivirga*	0.000381059	0.000154	0.008
69	*Fusobacterium*	0.000153858	0.000447	0.041

Appendix 2.4 continues

S/N	Features	Mean_Dom	Mean-FecD	P-value
70	*Geminicoccus*	0.002238506	0.001408	0.028
71	*Gemmatimonas*	0.009127539	0.005655	0.04
72	*Geobacter*	0.000442748	0.000187	0.021
73	*Gordonibacter*	3.78E-05	0.000168	0.014
74	*Gp10*	0.001544591	0.000876	0.045
75	*Gp16*	0.003334035	0.002232	0.046
76	*Gp4*	0.008213745	0.004766	0.004
77	*Gp7*	0.00241369	0.001254	0.005
78	*Guggenheimella*	0.000497485	0.001003	0.031
79	*Haliangium*	0.000721728	0.00035	0.013
80	*Haloferula*	0.000381233	8.21E-05	0.001
81	*Heliothrix*	0.000793215	0.00018	0.002
82	*Holdemania*	0.00053518	0.00088	0.037
83	*Holophaga*	1.63E-05	7.06E-05	0.001
84	*Howardella*	0.00013478	0.000429	0.004
85	*Ilumatobacter*	0.000833083	0.000445	0.014
86	*Isobaculum*	3.11E-05	0.000159	0.046
87	*Kineococcus*	3.26E-06	7.49E-05	0.04
88	*Klebsiella*	1.60E-05	7.66E-05	0.036
89	*Kofleria*	0.000362884	0.000101	0.005
90	*Lachnospiracea_incertae_sedis*	0.004356635	0.008519	0.003
91	*Lacibacter*	1.74E-05	4.92E-05	0.016
92	*Lentibacillus*	0.000108757	0.000263	0.037
93	*Levilinea*	0.000652562	0.000154	0.001
94	*Lewinella*	0.000825868	0.000372	0.004
95	*Marmoricola*	0.00017075	2.27E-05	0.004
96	*Meniscus*	0.000584182	0.000236	0.04
97	*Micromonospora*	0.000275405	9.87E-05	0.002
98	*Mitsuokella*	0.000308978	0.000702	0.023
99	*Mogibacterium*	0.000718639	0.001509	0.013
100	*Mucispirillum*	5.72E-05	9.28E-05	0.036
101	*Nannocystis*	0.000352557	0.000205	0.042
102	*Nitriliruptor*	0.001411924	0.000451	0.003

Appendix 2.4 continues

S/N	Features	Mean_Dom	Mean-FecD	P-value
103	*Nitrosomonas*	0.000331386	7.05E-05	0.002
104	*Nitrospira*	0.001108145	0.000416	0.005
105	*Ochrobactrum*	0.000405741	0.000698	0.008
106	*Olivibacter*	7.38E-05	0.000194	0.043
107	*Orientia*	2.71E-05	0.000239	0.003
108	*Oscillibacter*	0.003855594	0.006725	0.021
109	*Oxalicibacterium*	0.000348488	0.00061	0.045
110	*Papillibacter*	0.001719014	0.002684	0.045
111	*Parapedobacter*	0.000438052	0.000156	0.008
112	*Pasteuria*	0.00383163	0.002366	0.049
113	*Pelotomaculum*	0.000524176	0.0002	0.033
114	*Peptoniphilus*	0	0.000107	0
115	*Peredibacter*	0.001210559	0.000626	0.026
116	*Perlucidibaca*	5.72E-05	0.00035	0.037
117	*Phaselicystis*	0.000738131	0.000255	0.002
118	*Phycisphaera*	0.000813696	0.000234	0.002
119	*Planctomyces*	0.007419911	0.003534	0.003
120	*Planobispora*	0.000136178	1.65E-05	0.009
121	*Plesiocystis*	0.000281507	6.44E-05	0.003
122	*Pontibacter*	0.007202116	0.001755	0.011
123	*Porticoccus*	0.000234785	8.08E-05	0.006
124	*Propionivibrio*	9.66E-06	5.03E-05	0.003
125	*Proteiniclasticum*	0.003163074	0.001921	0.025
126	*Proteiniphilum*	0.000639268	0.000223	0.002
127	*Pseudoflavonifractor*	0.000498561	0.000876	0.042
128	*Pseudofulvimonas*	0.000614602	0.000331	0.03
129	*Pseudoramibacter*	0.000179478	0.000528	0.003
130	*Pusillimonas*	0.001228017	0.000612	0.026
131	*Rhizobium*	0.000861047	0.001309	0.04
132	*Rhodococcus*	0.000381501	0.000803	0.038
133	*Robinsoniella*	0.000234044	0.000509	0.018
134	*Roseburia*	0.000964684	0.002514	0.012
135	*Rubrobacter*	0.001167148	0.000649	0.029

Appendix 2.4 continues

S/N	Features	Mean_Dom	Mean-Riv	P-value
136	*Ruminococcus*	0.005368898	0.009873	0.006
137	*Rummeliibacillus*	0.000157895	0.000496	0.01
138	*Salegentibacter*	0.000107375	0.000204	0.047
139	*Salinibacter*	0.000945083	0.000319	0.001
140	*Salinimicrobium*	0.000506092	0.000245	0.02
141	*Salisaeta*	0.001746535	0.000595	0.001
142	*Sarcina*	4.69E-05	0.000441	0.005
143	*Sediminibacterium*	9.20E-05	0.000292	0.01
144	*Selenomonas*	5.95E-05	0.000143	0.027
145	*Sharpea*	0.000163336	0.000363	0.021
146	*Sinomonas*	1.74E-05	0.000311	0.001
147	*Skermanella*	0.001206684	0.000659	0.012
148	*Slackia*	0.000136585	0.000383	0.002
149	*Solitalea*	0.00026293	5.15E-05	0.001
150	*Sorangium*	0.000309402	0.000144	0.047
151	*Spartobacteria_genera_incertae_sedis*	0.004300779	0.002713	0.022
152	*Sphaerobacter*	0.004549194	0.003089	0.021
153	*Sphingosinicella*	0.000970546	0.000519	0.006
154	*Sporobacter*	0.005496117	0.009297	0.007
155	*Staphylococcus*	0.000354923	0.000965	0.008
156	*Steroidobacter*	0.001434563	0.000919	0.008
157	*Streptococcus*	0.00049342	0.00144	0.002
158	*Syntrophobacter*	0.000456531	0.000157	0.007
159	*Thermoleophilum*	0.000923491	0.000504	0.026
160	*Thermomicrobium*	8.56E-05	6.21E-06	0.047
161	*Thioalkalispira*	0.000107837	1.04E-05	0.049
162	*Thiohalobacter*	0.000331364	0.000118	0.013
163	*Thiohalomonas*	0.000214997	7.73E-05	0.009
164	*Thiohalophilus*	0.000266669	3.41E-05	0.002
165	*Thiomonas*	0	7.44E-05	0.011
166	*Truepera*	0.002877661	0.001312	0.002
167	*Tumebacillus*	0.000487761	0.000188	0.005
168	*Turicibacter*	0.000908706	0.002883	0.002

Appendix 2.4 continues

S/N	Features	Mean_Dom	Mean-Riv	P-value
1	*Acetoanaerobium*	0.00048	0.000222	0.013
2	*Acholeplasma*	0.001022	0.000321	0.037
3	*Aequorivita*	9.79E-05	0	0.042
4	*Alishewanella*	0.000265	0.000116	0.004
5	*Alkaliphilus*	0.000543	0.000146	0.015
6	*Aminomonas*	0.000116	2.07E-05	0.035
7	*Anaerofilum*	0.000156	4.01E-05	0.038
8	*Anaeroplasma*	0.000299	5.24E-05	0.027
9	*Anderseniella*	8.17E-05	0.000295	0.007
10	*Armatimonadetes_gp5*	0.001311	0.002515	0.033
11	*Asanoa*	2.38E-05	9.02E-05	0.016
12	*Atopostipes*	0.000525	0.000149	0.025
13	*Azomonas*	5.14E-05	0	0.045
14	*Azotobacter*	0.000286	0.000161	0.027
15	*Bellilinea*	0.003647	0.006184	0.045
16	*Blastococcus*	0.000864	0.000502	0.048
17	*Brachybacterium*	0.000297	0.000137	0.016
18	*Brevibacillus*	0.000222	6.22E-05	0.005
19	*Brevibacterium*	0.000451	0.000157	0.007
20	*Brevundimonas*	0.001523	0.00087	0.002
21	*Caenispirillum*	0.000132	0	0.006
22	*Caldalkalibacillus*	5.02E-05	0	0.042
23	*Caldicoprobacter*	0.000103	1.89E-05	0.023
24	*Castellaniella*	0.000469	0.000141	0.011
25	*Caulobacter*	9.49E-05	0.00032	0.042
26	*Cerasicoccus*	0.000386	0.000138	0.033
27	*Cetobacterium*	5.39E-06	5.63E-05	0.022
28	*Clostridium_XII*	9.12E-05	1.80E-05	0.035
29	*Cohnella*	0.000104	1.06E-05	0.035
30	*Corynebacterium*	0.001991	0.000735	0.002
31	*Defluviicoccus*	5.09E-05	0.000336	0.007
32	*Desmospora*	0.000323	9.64E-05	0.001
33	*Desulfatirhabdium*	1.45E-05	9.38E-05	0.04

Appendix 2.4 continues

S/N	Features	Mean_Dom	Mean-Riv	P-value
34	*Dethiobacter*	0.000373	0.000105	0.023
35	*Devosia*	0.00214	0.001332	0.028
36	*Dietzia*	0.000651	0.000307	0.021
37	*Duganella*	2.94E-05	0.000123	0.024
38	*Ensifer*	0.000549	0.000342	0.037
39	*Enterococcus*	0.00075	0.000253	0.007
40	*Erysipelothrix*	0.000479	4.31E-05	0.002
41	*Euzebya*	0.000723	0.000222	0.01
42	*Exiguobacterium*	0.000603	0.00028	0.015
43	*Ferruginibacter*	0.000453	0.001499	0.012
44	*Fervidicella*	0.000119	0.00037	0.048
45	*Fulvimonas*	0	3.57E-05	0.021
46	*Fulvivirga*	0.000381	0.000135	0.012
47	*Garciella*	0.000447	4.23E-05	0.002
48	*Gemmata*	0.002323	0.004526	0.033
49	*Geobacillus*	0.000315	9.65E-05	0.01
50	*Georgenia*	0.000311	0.000175	0.049
51	*Geothrix*	3.01E-05	0.000168	0.04
52	*Gp1*	0.000306	0.00074	0.034
53	*Gp17*	0.000766	0.002473	0.011
54	*Gp20*	0	7.74E-05	0.006
55	*Gp25*	0.000222	0.001257	0.007
56	*Hallella*	1.56E-05	0.000239	0.04
57	*Halochromatium*	0	8.54E-05	0.002
58	*Holdemania*	0.000535	0.00027	0.034
59	*Holophaga*	1.63E-05	0.000318	0.001
60	*Hyphomicrobium*	0.001	0.002226	0.022
61	*Ignatzschineria*	0.000329	0.000118	0.036
62	*Jonesia*	0.000363	0.000177	0.018
63	*Kerstersia*	8.69E-05	0	0.042
64	*Kocuria*	0.000471	0.000218	0.035
65	*Ktedonobacter*	1.08E-05	7.28E-05	0.007
66	*Labrys*	2.24E-05	0.000151	0.002

Appendix 2.4 continues

S/N	Features	Mean_Dom	Mean-Riv	P-value
67	*Lacibacter*	1.74E-05	0.000279	0.001
68	*Lentibacillus*	0.000109	0	0.009
69	*Leucobacter*	0.000718	0.000197	0.002
70	*Leuconostoc*	0.000818	0.000264	0.018
71	*Litoribacter*	0.000318	6.03E-05	0.033
72	*Longilinea*	0.001674	0.003471	0.018
73	*Lutibacter*	2.15E-05	0.000109	0.031
74	*Lutispora*	0.000255	8.19E-05	0.047
75	*Marichromatium*	0	6.65E-05	0.006
76	*Marinilactibacillus*	8.62E-05	0	0.042
77	*Marinobacter*	0.000579	0.000166	0.023
78	*Meiothermus*	5.01E-06	6.03E-05	0.022
79	*Methylomonas*	3.77E-05	0.000131	0.038
80	*Methylophilus*	3.39E-05	0.000222	0.012
81	*Methylopila*	0	4.14E-05	0.021
82	*Micromonospora*	2.75E-04	0.00011	0.012
83	*Nakamurella*	2.59E-05	8.54E-05	0.04
84	*Nesterenkonia*	0.000322	5.81E-05	0.04
85	*Nitriliruptor*	0.001412	0.000387	0.006
86	*Ochrobactrum*	0.000406	0.000246	0.03
87	*Ohtaekwangia*	0.002061	0.003737	0.006
88	*Ornithinimicrobium*	0.000311	0.000137	0.006
89	*Paenibacillus*	0.001424	0.000589	0.019
90	*Paenisporosarcina*	0.000195	0	0.001
91	*Paenochrobactrum*	0.000161	0	0.003
92	*Parapedobacter*	0.000438	0.000158	0.025
93	*Pasteuria*	0.003832	0.007981	0.04
94	*Phaeobacter*	0.000384	0.000151	0.004
95	*Planococcus*	0.000428	6.78E-05	0.025
96	*Planomicrobium*	0.000121	0	0.026
97	*Pontibacter*	0.007202	0.001679	0.012
98	*Propionivibrio*	9.66E-06	0.00028	0.002
99	*Prosthecobacter*	0.00055	0.001125	0.034

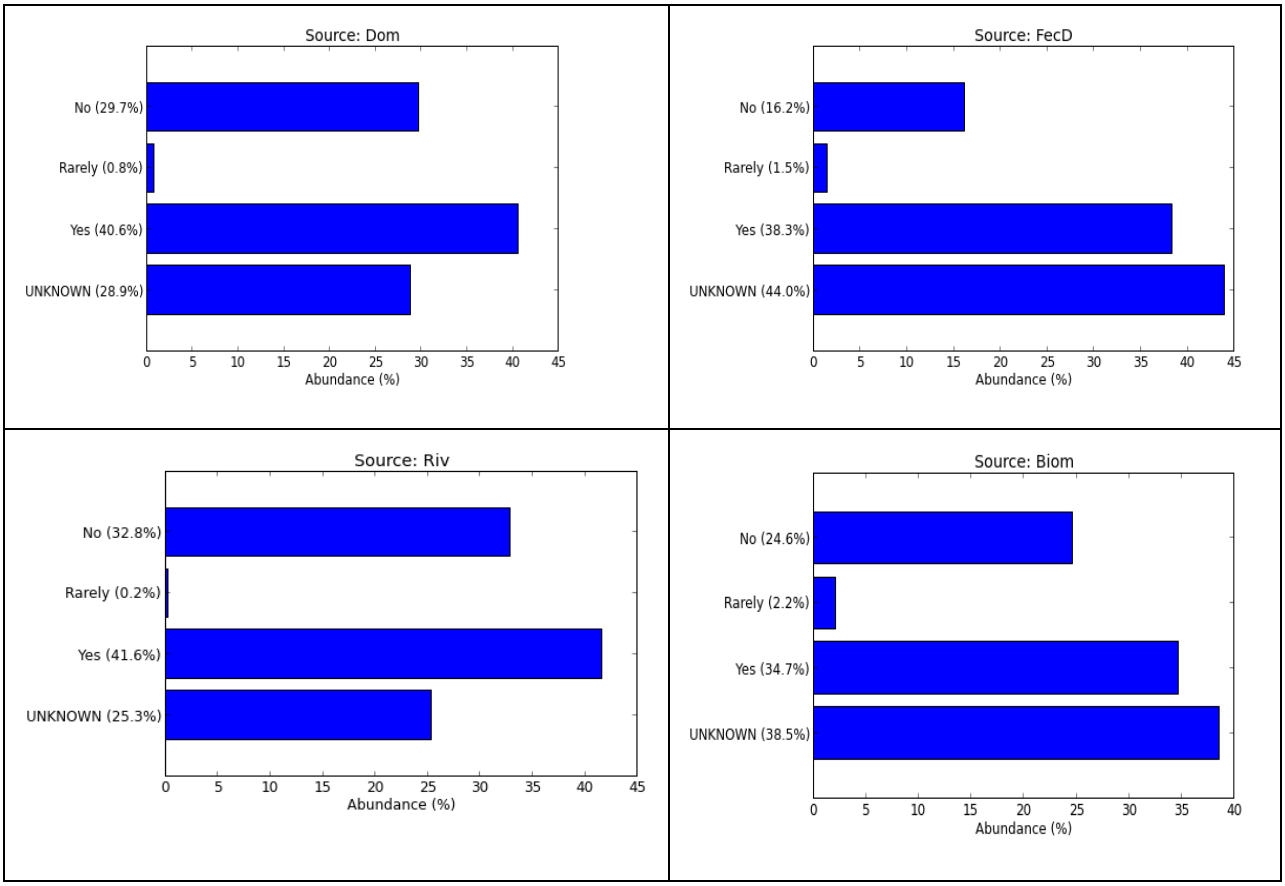
Appendix 2.4 continues

S/N	Features	Mean_Dom	Mean-Riv	P-value
100	*Proteiniborus*	7.57E-05	0	0.042
101	*Proteiniphilum*	0.000639	0.000263	0.032
102	*Pusillimonas*	0.001228	0.000397	0.001
103	*Riemerella*	0.000646	0.000319	0.017
104	*Rivibacter*	0	4.75E-05	0.021
105	*Runella*	1.66E-05	0.00013	0.001
106	*Saccharomonospora*	0.000171	1.89E-05	0.009
107	*Salisaeta*	1.75E-03	0.000994	0.03
108	*Sandaracinobacter*	0.000114	0.000259	0.025
109	*Schlesneria*	3.26E-06	5.07E-05	0.022
110	*Sediminibacterium*	9.20E-05	0.000443	0.012
111	*Serpens*	0.000313	0.00013	0.006
112	*Shinella*	0.000325	0.000197	0.045
113	*Silanimonas*	3.32E-06	8.54E-05	0.007
114	*Skermanella*	0.001207	0.000654	0.033
115	*Soehngenia*	0.000169	3.96E-05	0.038
116	*Sphaerobacter*	0.004549	0.002999	0.034
117	*Sphingobacterium*	0.002833	0.000787	0.002
118	*Spirosoma*	3.26E-06	0.000154	0.002
119	*Sporichthya*	2.87E-05	0.000151	0.002
120	*Sporolactobacillaceae_incertae_sedis*	0.000467	0.000209	0.021
121	*Subdoligranulum*	0.000586	0.000231	0.042
122	*Sulfuritalea*	0	4.81E-05	0.006
123	*Sulfurovum*	0.000232	7.70E-05	0.032
124	*Symbiobacterium*	0.000195	2.78E-05	0.003
125	*Syntrophaceticus*	0.000128	1.89E-05	0.006
126	*Tepidanaerobacter*	0.00036	1.73E-05	0.001
127	*Tepidibacter*	1.05E-05	8.50E-05	0.019
128	*Tepidimicrobium*	0.000108	0	0
129	*Thalassobacillus*	0.000239	0.000112	0.025
130	*Thermoactinomyces*	0.000483	0.000191	0.045
131	*Thiobacter*	9.35E-06	9.60E-05	0.016
132	*Thiohalomonas*	0.000215	8.09E-05	0.039

Appendix 2.4 continues

S/N	Features	Mean_Dom	Mean-Riv	P-value
133	*Thiohalophilus*	0.000267	4.01E-05	0.005
134	*Tissierella*	0.001648	0.000482	0.003
135	*Truepera*	0.002878	0.001331	0.004
136	*Uliginosibacterium*	1.24E-05	0.000126	0.002
137	*Ureibacillus*	0.000247	2.95E-05	0.017
138	*Vasilyevaea*	0.000265	0.00015	0.033
139	*Veillonellaceae_genus_incertae_sedis*	0	4.51E-05	0.021
140	*Victivallis*	0.000151	3.61E-05	0.038
141	*Weissella*	0.001025	0.000208	0.004
142	*Wohlfahrtiimonas*	0.000154	0	0.006
143	*Zavarzinella*	0.002096	0.006536	0.013

Appendix 2.5 Taxonomy to pathogen mapping of bacterial OTUs from the dumpsite



Appendix 3.1. Rarefaction curves of bacteria from different pigs' management for subsampled 9016 sequences

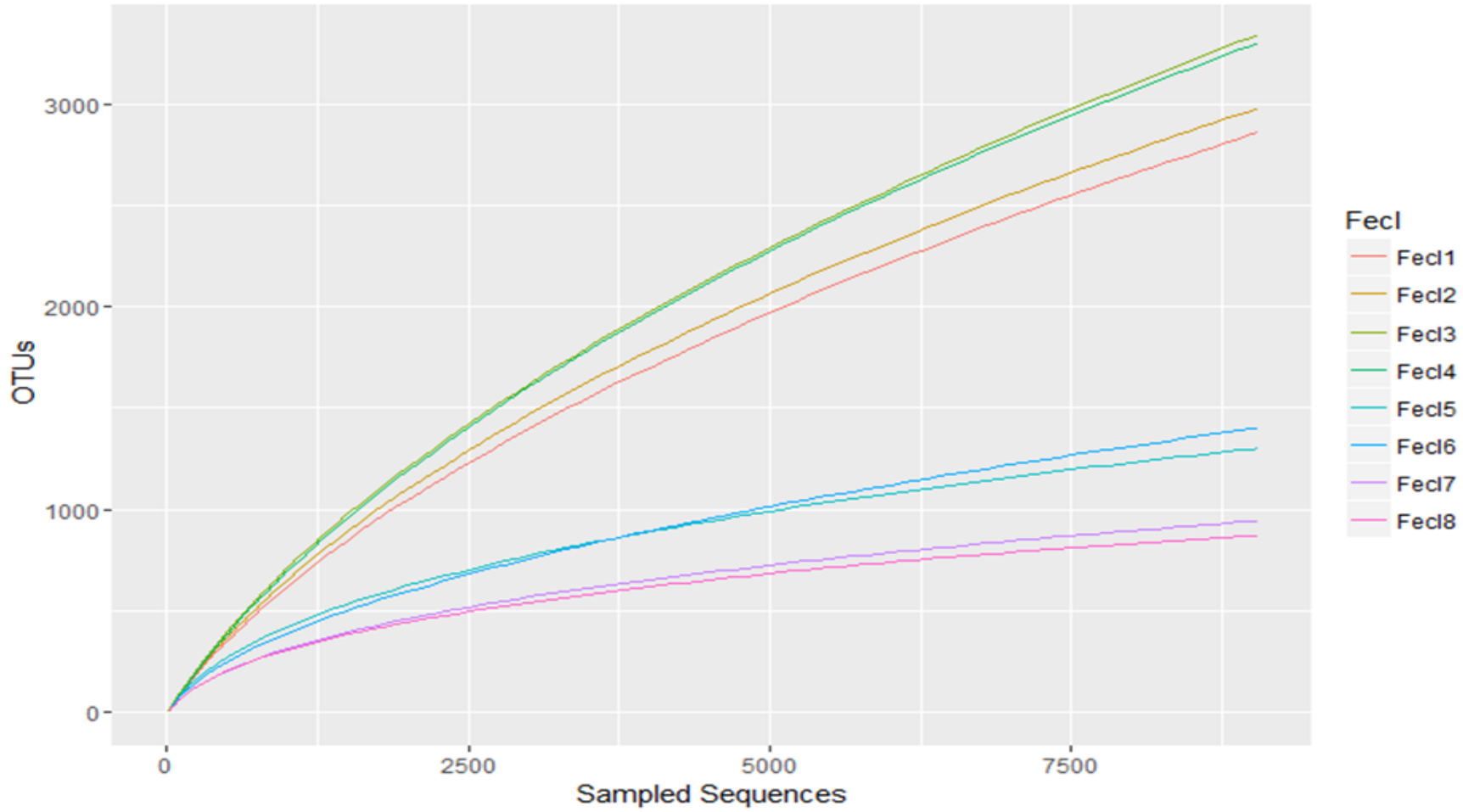


Fig 3.1 (a) Rarefaction curves of bacteria OTUs from faecal samples of pigs reared indoors

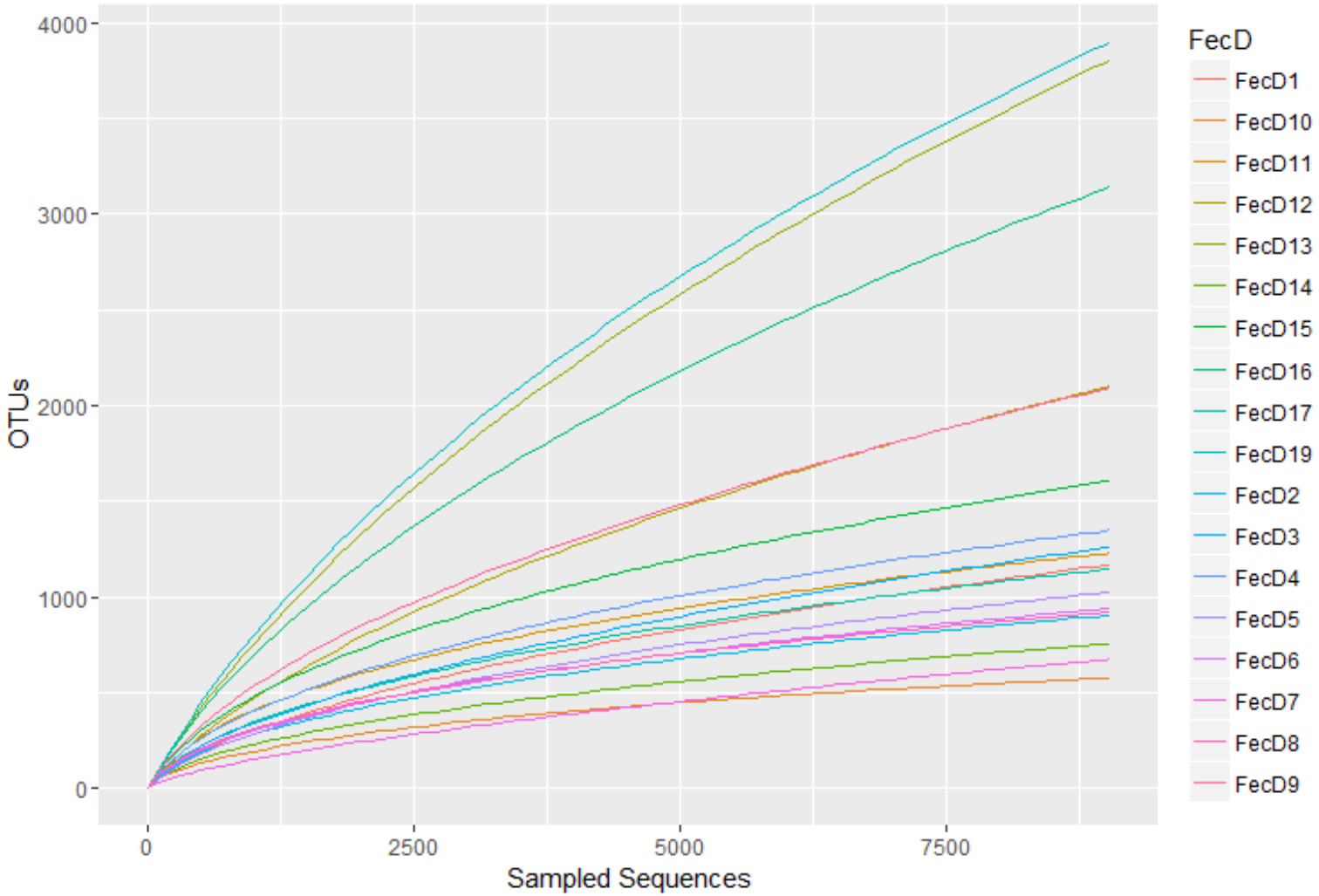


Fig 3.1 (b). Rarefaction curves of bacteria OTUs from faecal sample of pigs permanently scavenging on the dumpsite

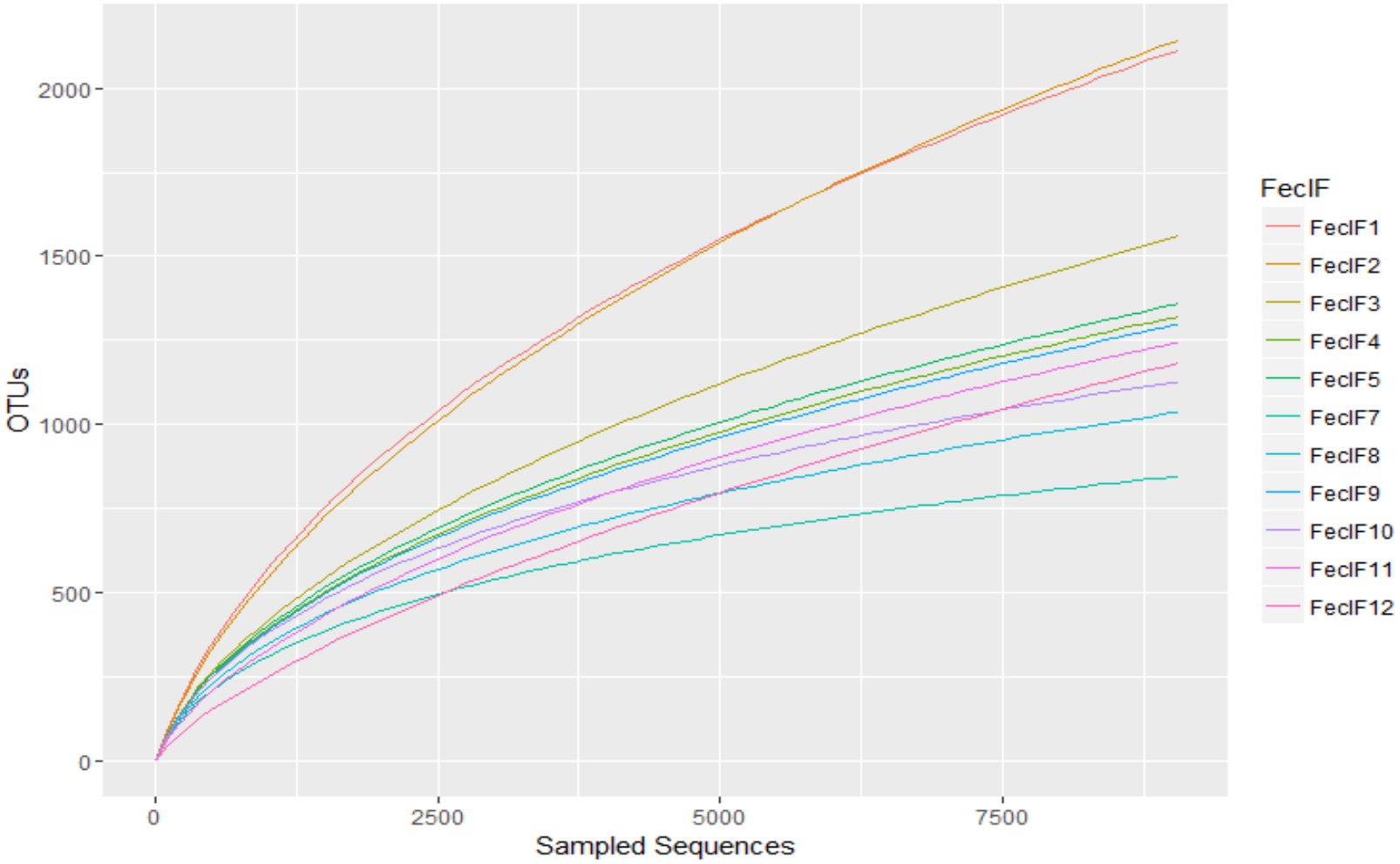


Fig 3.1 (b) Rarefaction curves of bacteria OTUs from faecal sample of pigs shifted from indoor to free range on the dumpsite

Appendix 3.2 Summary of quality sequence and diversity indices of faecal microbiota

Sample ID	Quality reads	OTUs	Chao1	ACE	Shannon	InvSimpson
FecD1	133018	2246	3256	4120	2.93	4
FecD10	9155	576	823	843	4.31	28
FecD11	91176	2226	3390	4124	5.09	36
FecD12	116135	4434	6546	8330	4.49	10
FecD13	180143	8202	12120	15382	5.39	31
FecD14	36314	1356	1812	1907	3.90	7
FecD15	145587	3242	4753	5787	5.40	70
FecD16	169992	7025	10377	12894	5.85	66
FecD17	130993	2231	3410	4370	4.40	23
FecD18	551	246	466	518	4.79	43
FecD19	192897	9718	13667	14349	6.74	99
FecD2	113513	1661	2436	3056	3.67	12
FecD3	101017	2705	4548	6187	4.50	27
FecD4	62726	2499	3378	3522	5.66	100
FecD5	116233	2055	3032	3761	3.07	7
FecD6	122921	1692	2596	3371	3.54	8
FecD7	136362	1484	2582	3787	1.97	3
FecD8	99238	1573	2452	2913	4.25	18
FecD9	291467	4411	6776	8552	4.48	19
FecI1	95792	6126	8875	9356	6.08	73
FecI2	127603	6205	8958	9377	5.64	28
FecI3	152935	7132	10486	13260	5.69	47
FecI4	128478	7004	10126	12674	5.59	27
FecI5	150164	2313	3347	4075	4.94	44
FecI6	104502	2751	4150	5125	5.10	56
FecI7	159828	1664	2477	2801	3.47	6
FecI8	16927	1107	1518	1541	5.34	74
FecIF1	195573	4207	6023	7423	4.83	17
FecIF10	106021	2008	2979	3569	4.88	37
FecIF11	136995	2454	3780	4777	4.17	21
FecIF12	152463	2654	4395	6158	2.08	3
FecIF2	136491	4102	5989	7458	4.98	33
FecIF3	71527	3046	4735	6190	5.48	76
FecIF4	76324	2461	3529	4396	5.39	73
FecIF5	110690	2450	3539	4304	5.03	50
FecIF6	147	73	161	182	3.75	22
FecIF7	22146	1078	1423	1454	5.27	73
FecIF8	70454	1784	2710	3385	5.21	71
FecIF9	100009	2530	3877	4861	4.66	19

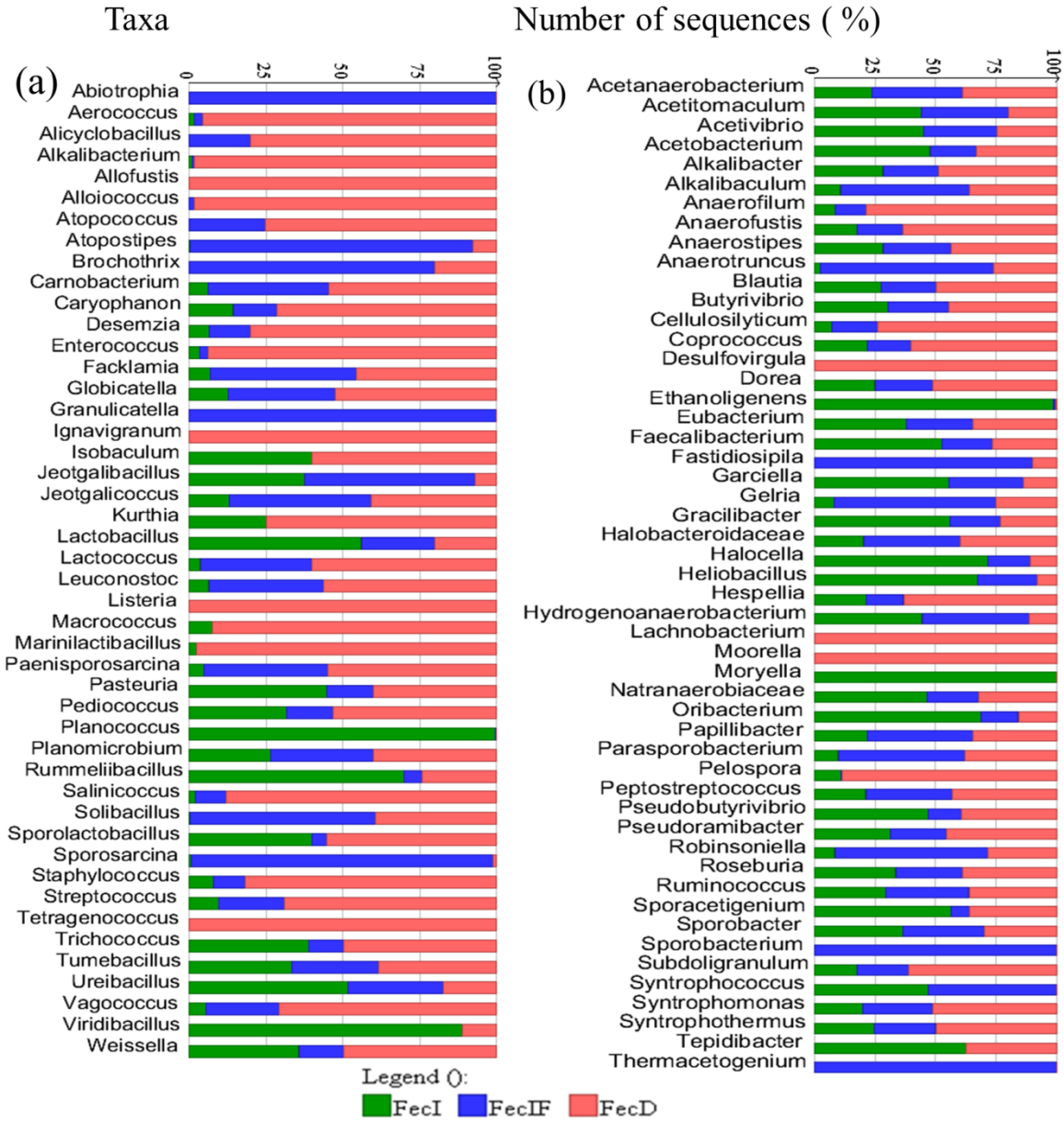
Appendix 3.3 Predominant bacterial genera in different pig management system

S/N	Predominant genera in FecI pigs	Number of sequences	%
1	*Lactobacillus*	90297	10
2	*Acinetobacter*	65213	7
3	*Comamonas*	61879	7
4	*Prevotella*	31894	4
5	*Clostridium_sensu_stricto*	25625	3
6	*Unclassified Ruminococcaceae*	23000	3
7	*Treponema*	17516	2
8	*Proteiniclasticum*	15048	2
9	*Kurthia*	12621	1
10	*Lachnospiraceae_incertae_sedis*	10822	1
11	*Unclassified Clostridiales*	10061	1
12	*Unclassified Sphingobacteriales*	9787	1
13	*Escherichia_Shigella*	6414	1
14	*Planococcus*	6327	1
15	*Unclassified Porphyromonadaceae*	6179	1
16	*Clostridium_XIa*	5859	1
S/N	**Predominant genera in FecIF**	**Number of sequences**	**%**
1	*Acinetobacter*	239413	19
2	*Unclassified Planococcaceae*	79081	6
3	*Clostridium_sensu_stricto*	51657	4
4	*Treponema*	48341	4
5	*Lactobacillus*	43798	3
6	*Escherichia_Shigella*	33244	3
7	*Unclassified Ruminococcus*	28399	2
8	*Prevotella*	21092	2
9	*Proteiniclasticum*	13952	2
10	*Pseudomonas*	18657	2
11	*Unclassified Enterobacteriaceae*	10138	1
12	*Unclassified Sphingobacteriales*	10138	1
13	*Turicibacter*	10138	1
14	*Unclassified Pseudomonadaceae*	10007	1
15	*Solibacillus*	8823	1

Appendix 3.3 continues

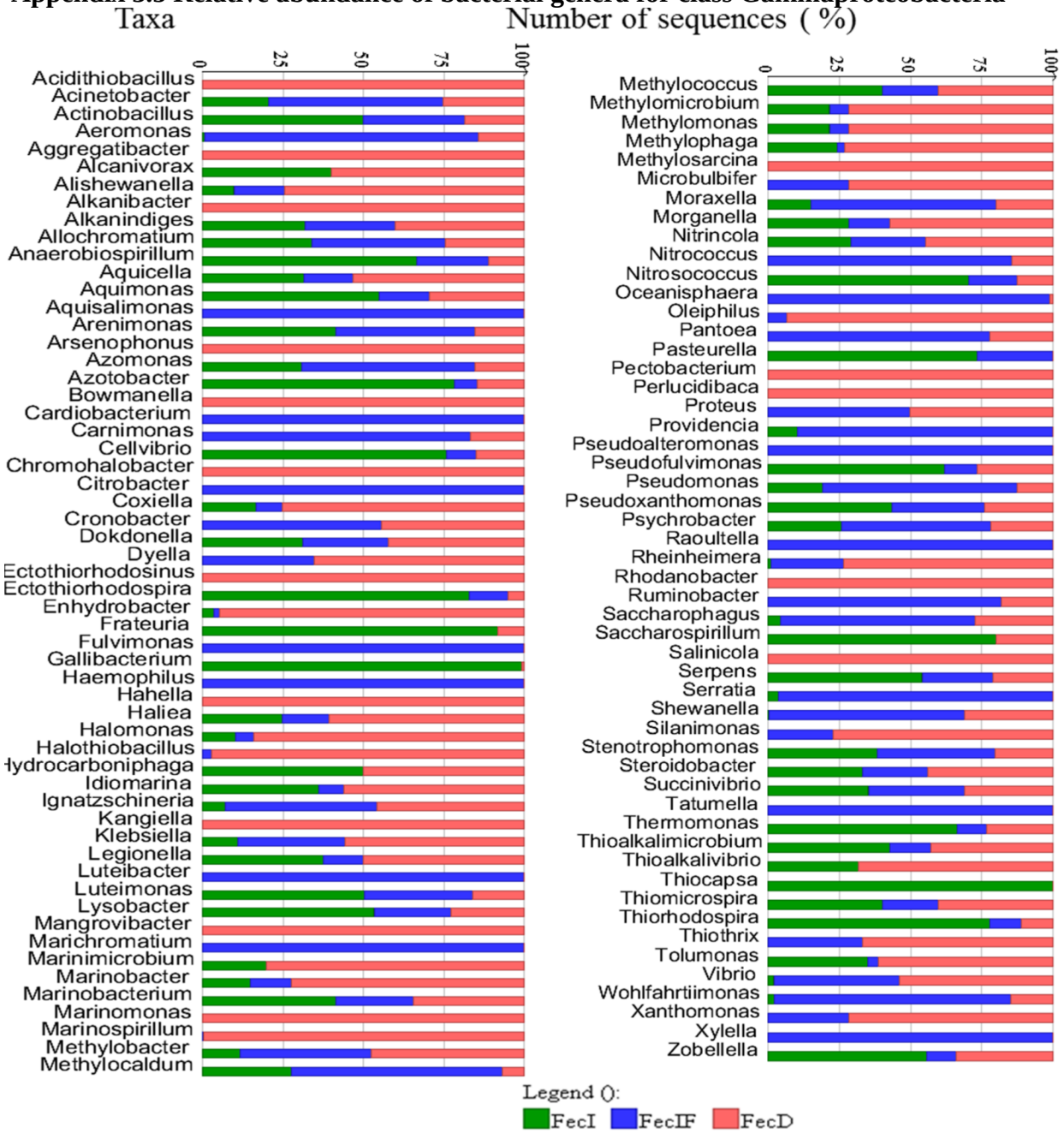
S/N	Predominant genera in FecD	Number of sequences	%
1	*Clostridium_sensu_stricto*	224116	11
2	*Acinetobacter*	197978	9
3	*Turicibacter*	64872	3
4	*Clostridium_XII*	43310	2
5	*Alkalibacterium*	56621	2
6	*Unclassified Enterobacteriaceae*	12158	2
7	*Unclassified Ruminococcaceae*	33516	2
8	*Oligella*	36609	2
9	*Corynebacterium*	93469	1
10	*Lachnospiraceae*	24595	1
11	*Aerococcus*	22370	1
12	*Leuconostoc*	11916	1
13	*Proteiniclasticum*	12158	1
14	*Solibacillus*	16440	1
15	*Treponema*	12960	1
16	*Solibacillus*	64872	1
18	*Unclassified Planococcaceae*	31688	1
19	*Prevotella*	19764	1

Appendix 3.4 Relative abundance bacterial genera for class Bacilli



Percentage of sequences assigned to each genus is presented out of 100. Different colours represent management systems. Green for indoor management "FecI", Blue for pigs shifted from indoor to free range on the dump "FecIF" and red for pigs permanently scavenging on dump "FecD". When only one colour appears to a given genera it means that a specific genera is found only to that type of management system.

Appendix 3.5 Relative abundance of bacterial genera for class Gammaproteobacteria



Percentage of sequences assigned to each genus is presented out of 100. Different colours represent management systems. Green for indoor management "FecI", Blue for pigs shifted from indoor to free range on dump "FecIF" and Red for pigs permanently scavenging on dump "FecD". When only one colour appears to a given genera it means that a specific genera is found only to that type of management system

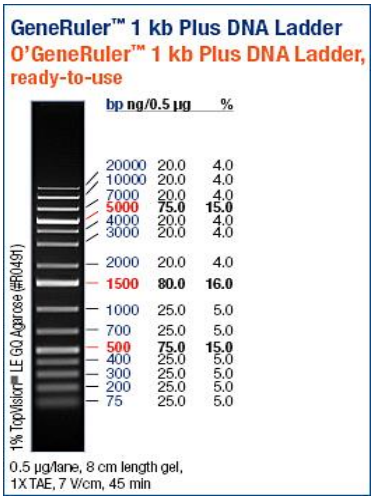
Appendix 4.1 Bacterial genera significantly different between cluster A and cluster B of solid biomedical waste

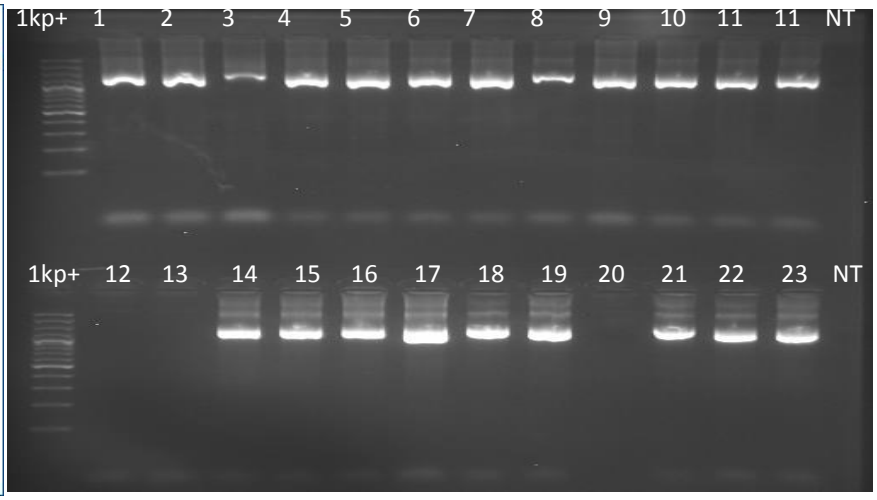
Genera	Mean_gp A	variance-gp A	Mean-gp B	variancegpB	p-val
Gp9	7.21E-06	5.20E-10	0.00017965	9.85E-09	0
Bdellovibrio	3.54E-05	1.31E-09	0.00079133	4.91E-08	0
Haliscomenobacter	2.04E-05	3.24E-10	0.00023838	2.54E-08	0.001
Pseudofulvimonas	0.0002	7.24E-08	0.00107392	3.12E-07	0.001
Arenimonas	0.00018	4.44E-08	0.00244939	2.75E-06	0.001
Shinella	4.45E-05	4.32E-09	0.00078426	3.27E-07	0.002
Eubacterium	0.000703	3.73E-07	0.00011987	2.03E-08	0.002
Enhygromyxa	5.64E-06	6.00E-11	3.77E-05	6.82E-10	0.004
Devosia	0.001329	4.06E-06	0.00622102	1.47E-05	0.004
Planctomyces	0.001088	1.21E-06	0.00841846	3.47E-05	0.004
Enterococcus	0.007947	6.35E-05	0.00104861	9.30E-07	0.004
Sunxiuqinia	2.00E-05	1.25E-09	0.00024672	3.59E-08	0.005
Flavobacterium	0.002024	5.25E-06	0.01329367	8.93E-05	0.005
Cohnella	8.83E-06	2.18E-10	0.00011235	8.03E-09	0.006
Butyricicoccus	0.000592	4.18E-07	6.18E-05	4.36E-09	0.006
Mesorhizobium	0.000373	2.57E-07	0.00182737	1.46E-06	0.006
Proteiniclasticum	0.16294	0.026745	0.02406387	0.000939	0.006
Clostridium sensu stricto	0.036991	0.001007	0.00942526	5.84E-05	0.006
Nonomuraea	6.97E-06	8.12E-11	3.57E-05	6.00E-10	0.007
Pantoea	3.74E-05	2.90E-09	0.00018436	1.68E-08	0.009
Gp4	0.002995	5.68E-06	0.00722334	1.20E-05	0.009
Filomicrobium	1.31E-05	1.95E-10	4.94E-05	1.05E-09	0.011
Gp5	3.25E-05	7.23E-09	0.00035684	8.77E-08	0.011
Jeotgalicoccus	0.000222	7.51E-08	1.58E-05	3.10E-10	0.011
Peptostreptococcus	0.000747	9.45E-07	1.31E-05	3.85E-10	0.011
WPS-1_genera_incertae_sedis	0.000123	9.26E-09	0.00080541	4.05E-07	0.011
WPS-2_genera_incertae_sedis	0.000411	7.76E-08	0.00167147	1.37E-06	0.011
Alkalibacterium	0.005326	4.49E-05	0.00027569	1.39E-07	0.011
Paraeggerthella	9.60E-05	1.10E-08	1.26E-05	7.90E-10	0.012
Fastidiosipila	5.29E-05	4.57E-09	2.97E-06	1.72E-11	0.013
Maricurvus	0	0	1.41E-05	7.91E-10	0.014
Opitutus	0.000163	3.26E-08	0.0008884	4.85E-07	0.014

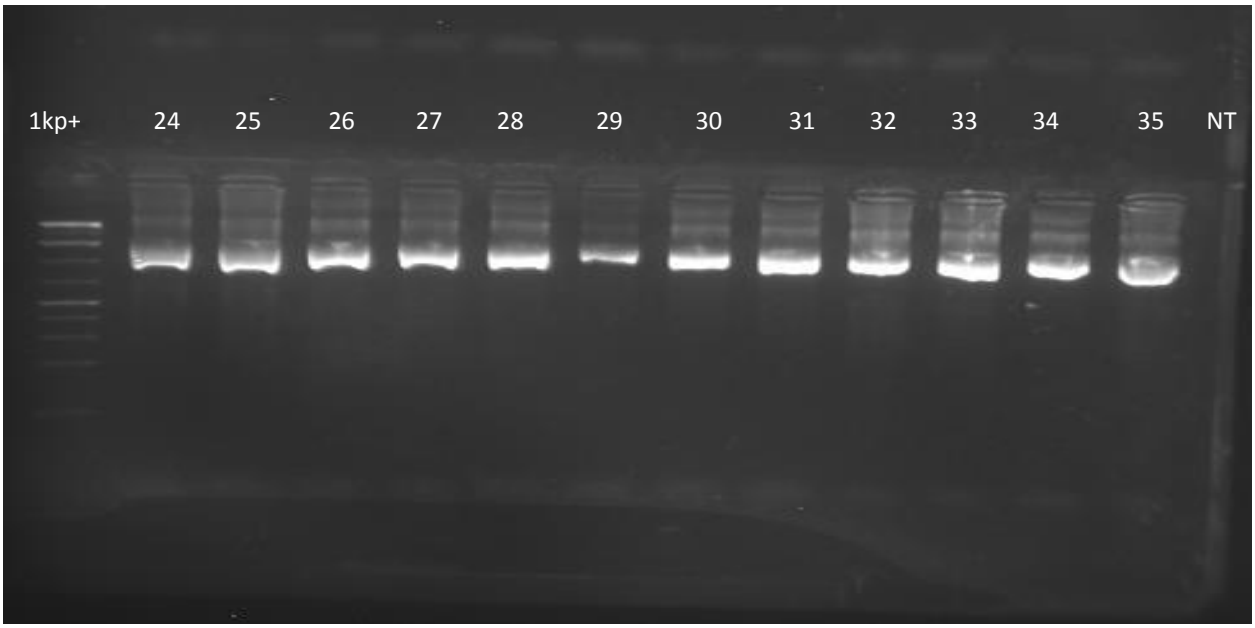
Appendix 4.1 continues

Genera	Mean_gp A	variance-gp A	Mean-gp B	variancegpB	p-val
Lactobacillus	0.014892	0.000242	0.00340136	1.01E-05	0.016
Thiohalobacter	6.91E-06	2.72E-10	6.13E-05	2.85E-09	0.017
Blastopirellula	0.000493	1.81E-07	0.00252911	4.16E-06	0.018
Coxiella	1.02E-05	4.75E-10	5.41E-05	1.80E-09	0.02
Bacteriovorax	1.59E-05	2.84E-10	0.00027969	7.48E-08	0.021
Gp25	4.98E-05	1.15E-08	0.00040539	1.33E-07	0.022
Nesterenkonia	0.000672	9.13E-07	2.73E-05	1.19E-09	0.023
Kofleria	2.32E-05	1.13E-09	0.00011957	9.94E-09	0.024
Gracilibacter	0.000111	3.91E-08	0.00077533	4.75E-07	0.024
Simplicispira	3.31E-05	2.76E-09	0.00044715	1.97E-07	0.026
Guggenheimella	0.007018	9.57E-05	0.00050843	1.13E-06	0.026
Macrococcus	6.66E-05	9.87E-09	1.72E-06	1.47E-11	0.027
Peredibacter	0.000184	6.89E-08	0.00396312	1.67E-05	0.027
Jonesia	0.000306	1.22E-07	6.73E-05	6.59E-09	0.028
Rhizobium	0.001189	1.17E-06	0.00599973	2.67E-05	0.028
Weissella	0.000506	1.79E-07	0.00020497	1.78E-08	0.028
Algoriphagus	0.000165	4.39E-08	0.00476102	2.54E-05	0.029
Saccharofermentans	0.010111	0.000178	0.00139203	2.28E-06	0.029
Sporomusa	4.64E-06	3.25E-11	4.46E-05	1.93E-09	0.03
Pontibacter	0.005415	3.67E-05	0.001499	3.35E-07	0.03
Pseudaminobacter	8.99E-06	1.10E-10	9.00E-05	8.03E-09	0.031
Pediococcus	5.16E-05	2.64E-09	1.63E-05	2.17E-10	0.031
Anaerosporobacter	0.00095	1.98E-06	5.30E-05	1.48E-09	0.031
Planococcus	0.011562	0.000206	0.00206823	7.57E-06	0.031
Comamonas	0.0021	1.08E-05	0.01111907	9.48E-05	0.031
Fluviicola	1.27E-05	1.08E-09	0.00024544	6.85E-08	0.034
Giesbergeria	1.74E-05	8.00E-10	0.00010334	9.06E-09	0.034
Paenibacillus	0.000365	3.18E-07	0.00123563	8.18E-07	0.035
Cesiribacter	0.000579	5.62E-07	0.00010833	4.65E-09	0.035
Ruminococcus	0.005269	4.29E-05	0.00104125	1.49E-06	0.035
Fulvivirga	2.31E-05	1.38E-09	0	0	0.036
Vagococcus	0.000963	2.04E-06	7.74E-05	6.10E-09	0.036
Erysipelotrichaceae_incertae_sedis	0.001653	4.52E-06	0.00030489	1.53E-07	0.038
Rickettsia	1.31E-06	1.72E-11	3.07E-05	1.16E-09	0.04

Appendix 5.1 Gel images of 16S rRNA amplicons







Appendix 5.2 Library preparation and cloning protocol for Sanger sequencing

i. Equal molar concentration of samples from the same type of solid waste were pooled together

ii. Ligation reaction composition was as follows:

Component	Volume
Vector pTZ57R/T (0.17pmo ends)	3µl
5X Ligation buffer	6µl
PCR product (0.52pm ends)	8µl
Nuclease free water	12µl
T4 DNA Ligase	1µl
Total volume	30µl

iii. Ligation mixture was incubated at room temperature for 3hours, then over night at 4^{o}C

Transformation step was done as follows:

iv. Ligation mixture was combined with competent cells (*E.coli* DH5α) in a ratio of 1:5 (ligation mixture: competent cells).

v. Incubation of ligation mixture and competent cells on ice for 20 min

vi. The mixture was heat shock for 30 s at 42^{o}C, then allowed to chill on ice for 2 min

vii. The mixture was then added in LB broth (250 µl) and then incubated in shaker for 45 min at 37^{o}C to allow multiplication of transformed cells

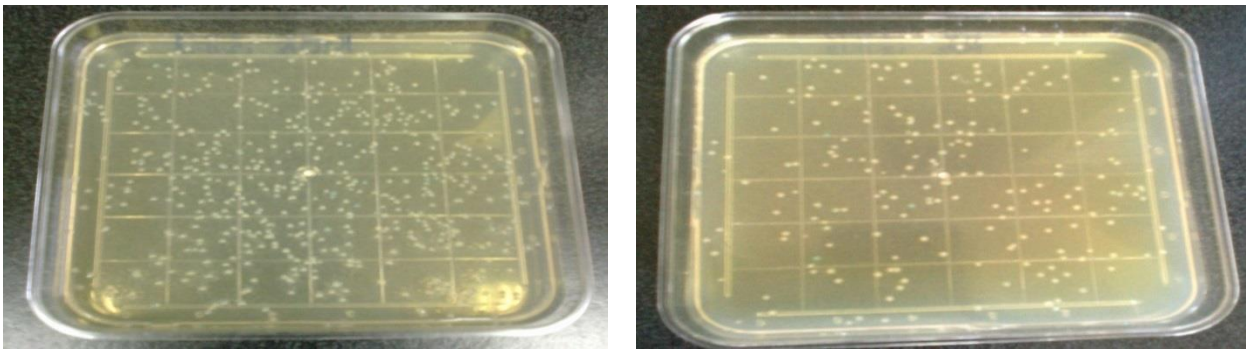
viii. 150µl of culture was inoculated into LB agar plates (supplemented with Ampicillin, X-gal and IPTG).

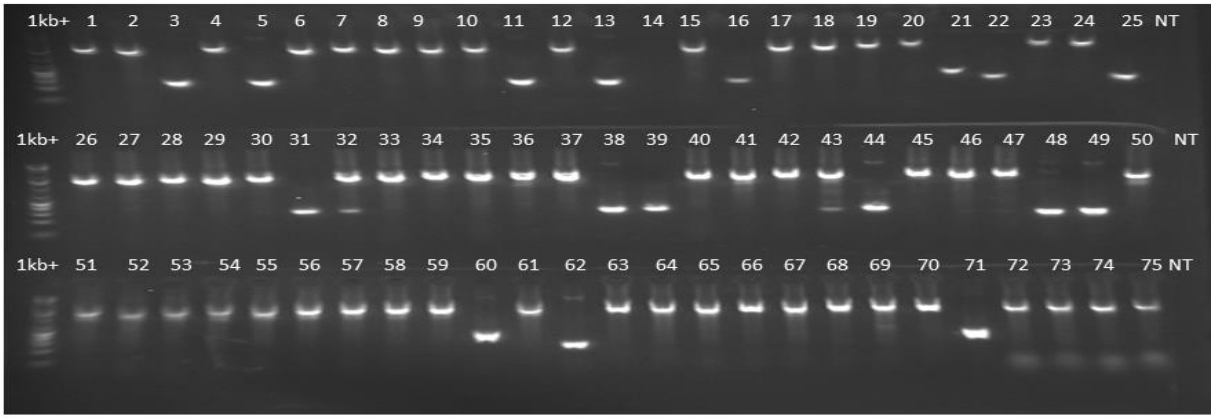
ix. The plate was incubated at 37 ^{o}C over for 18 hours.

x. Blue / white clone screening was then conducted to confirm clones with insert. (white clones – with insert, Blue clones – without insert)

The protocol was modified from InsTAclone PCR cloning kit (Fermentas, Lithuania).

Appendix 5.3 Multiplication of transformed E.coli DH5α and colony PCR





Appendix 5.4 Biochemical Identification of enteric bacteria isolates using API 20E kits

Appendix 5.5 Enteric bacteria isolates from different solid waste on the dumpsite

S/N	Sample/Clone	Genera	Sequence similarity (%)
Ref	K12	*Escherichia/Shigella*	100
1	Biom28	*Alkalitalea*	99
2	Biom60	*Alkalitalea*	97
3	Biom81	*Aquisphaera*	84
4	Biom123	*Bacillus*	100
5	Biom125	*Bacillus*	100
6	Biom127	*Bacillus*	100
7	Biom131	*Bacillus*	100
8	Biom135	*Bacillus*	76
9	Biom139	*Bacillus*	100
10	Biom145	*Bacillus*	100
11	Biom17	*Bacillus*	100
12	Biom70	*Cellvibrio*	100
13	Biom6	*Derxia*	56
14	Biom2	*Enterococcus*	100
15	Biom24	*Flavisolibacter*	98
16	Biom19	*Flavobacterium*	88
17	Biom56	*Luteimonas*	100
18	Biom142	*Lysinibacillus*	97
19	Biom143	*Lysinibacillus*	97
20	Biom146	*Lysinibacillus*	100
21	Biom149	*Lysinibacillus*	71
22	Biom39	*Massilia*	100
23	Biom83	*Micrococcineae*	96
24	Biom22	*Oligella*	100
25	Biom78	*Peptoniphilus*	66
26	Biom3	*Planomicrobium*	83
27	Biom15	*Proteiniclasticum*	100
28	Biom35	*Proteiniclasticum*	99
29	Biom37	*Proteiniclasticum*	100
30	Biom38	*Proteiniclasticum*	100
31	Biom61	*Proteiniclasticum*	100
32	Biom66	*Proteiniclasticum*	100
33	Biom69	*Proteiniclasticum*	100
34	Biom74	*Proteiniclasticum*	100
35	Biom80	*Proteiniclasticum*	99
36	Biom12	*Pseudomonas*	99
37	Biom77	*Rhodoplanes*	55
38	Biom86	*Stenotrophomonas*	100
39	Biom14	*Thauera*	100
40	Biom30	*Tissierella*	61

Appendix 5.5 continues

S/N	Sample	Genera	Sequence similarity (%)
41	Dom16	*Acinetobacter*	100
42	Dom23	*Acinetobacter*	100
43	Dom44	*Acinetobacter*	100
44	Dom7	*Allochromatium*	99
45	Dom52	*Atopostipes*	100
46	Dom113	*Bacillus*	100
47	Dom114	*Bacillus*	100
48	Dom132	*Bacillus*	100
49	Dom37	*CandidatusHydrogenedens*	100
50	Dom47	*Clostridium XI*	99
51	Dom11	*Fusibacter*	100
52	Dom28	*Kurthia*	58
53	Dom40	*Leuconostoc*	100
54	Dom5	*Mesorhizobium*	100
55	Dom54	*Mesorhizobium*	58
56	Dom48	*Oceanibaculum*	58
57	Dom30	*Phascolarctobacterium*	94
58	Dom36	*Pontibacter*	100
59	Dom8	*Pontibacter*	100
60	Dom35	*Proteiniclasticum*	100
61	Dom111	*Pseudomonas*	100
62	Dom12	*Saccharofermentans*	75
63	Dom19	*Sphingomonas*	100
64	Dom26	*Sporacetigenium*	100
65	Dom129	*Staphylococcus*	100
66	Dom34	*Thalassolituus*	100
67	Dom4	*Tindallia*	74
68	Dom39	*Treponema*	100
69	FecD12	*Bacillus*	100
70	FecD128	*Bacillus*	100
71	FecD26	*Bacillus*	84
72	FecD50	*Bacillus*	100
73	FecD60	*Bacillus*	100
74	FecD61	*Bacillus*	100
75	FecD84	*Bacillus*	100
76	FecD85	*Bacillus*	100
77	FecD87	*Bacillus*	86
78	FecD91	*Bacillus*	100
79	FecD99	*Bacillus*	81

Appendix 5.5 continues

S/N	Sample	Genera	Sequence similarity (%)
80	FecD83	*Clostridium sensu stricto*	100
81	FecD17	*Clostridium XI*	99
82	FecD19	*Clostridium XI*	75
83	FecD7	*Clostridium XI*	100
84	FecD120	*Enterococcus*	100
85	FecD144	*Enterococcus*	100
86	FecD35	*Enterococcus*	100
87	FecD77	*Enterococcus*	100
88	FecD86	*Enterococcus*	100
89	FecD1	*Escherichia/Shigella*	100
90	FecD21	*Escherichia/Shigella*	100
91	FecD3	*Escherichia/Shigella*	100
92	FecD3	*Escherichia/Shigella*	99
93	FecD34	*Escherichia/Shigella*	100
94	FecD44	*Escherichia/Shigella*	100
95	FecD48	*Escherichia/Shigella*	100
96	FecD50	*Escherichia/Shigella*	100
97	FecD51	*Escherichia/Shigella*	100
98	FecD61	*Escherichia/Shigella*	100
99	FecD63	*Escherichia/Shigella*	99
100	FecD81	*Escherichia/Shigella*	100
101	FecD82	*Escherichia/Shigella*	100
102	FecD83	*Escherichia/Shigella*	100
103	FecD87	*Escherichia/Shigella*	99
104	FecD93	*Escherichia/Shigella*	100
105	FecD97	*Escherichia/Shigella*	100
106	FecD75	*Fusobacterium*	66
107	FecD37	*Kandleria*	98
108	FecD13	*Lachnospiracea_incertae_sedis*	71
109	FecD10	*Lactobacillus*	100
110	FecD88	*Mitsuokella*	100
111	FecD58	*Oscillibacter*	48
112	FecD33	*Paenibacillus*	99
113	FecD40	*Planococcaceae_incertae_sedis*	96
114	FecD43	*Planococcaceae_incertae_sedis*	94
115	FecD14	*Sporacetigenium*	63
116	FecI11	*Acetivibrio*	68
117	FecI17	*Bacillus*	100
118	FecI19	*Bacillus*	100

Appendix 5.5 continues

S/N	Sample	Genera	Sequence similarity (%)
119	FecI2	*Clostridium IV*	80
120	FecI20	*Clostridium IV*	84
121	FecI21	*Clostridium sensu stricto*	100
122	FecI23	*Clostridium sensu stricto*	100
123	FecI27	*Clostridium sensu stricto*	98
124	FecI27	*Clostridium sensu stricto*	98
125	FecI29	*Clostridium sensu stricto*	100
126	FecI30	*Escherichia/Shigella*	100
127	FecI32	*Escherichia/Shigella*	100
128	FecI34	*Escherichia/Shigella*	100
129	FecI38	*Escherichia/Shigella*	100
130	FecI39	*Escherichia/Shigella*	100
131	FecI41	*Escherichia/Shigella*	100
132	FecI41	*Escherichia/Shigella*	100
133	FecI43	*Escherichia/Shigella*	100
134	FecI43	*Escherichia/Shigella*	100
135	FecI44	*Escherichia/Shigella*	100
136	FecI46	*Gemmiger*	57
137	FecI47	*Lachnospiracea_incertae_sedis*	79
138	FecI48	*Lachnospiracea_incertae_sedis*	97
139	FecI51	*Lachnospiracea_incertae_sedis*	74
140	FecI54	*Lactobacillus*	100
141	FecI58	*Lactobacillus*	100
142	FecI59	*Lactobacillus*	100
143	FecI6	*Lactobacillus*	100
144	FecI61	*Lactobacillus*	100
145	FecI64	*Lactobacillus*	100
146	FecI67	*Lactobacillus*	100
147	FecI68	*Megasphaera*	100
148	FecI7	*Megasphaera*	100
149	FecI79	*Oscillibacter*	76
150	FecI84	*Oscillibacter*	100
151	FecI86	*Prevotella*	99
152	FecI93	*Roseburia*	100
153	FecI97	*Tannerella*	63
154	FecI98	Tannerella	63
155	FecIF75	Anaerovorax	95
156	FecIF76	Anaerovorax	90
157	FecIF101	Bacillus	100

Appendix 5.5 continues

S/N	Sample	Genera	Sequence similarity (%)
158	FecIF53	*Bacillus*	100
159	FecIF71	*Clostridium IV*	90
160	FecIF19	*Clostridium sensu stricto*	100
161	FecIF56	*Clostridium sensu stricto*	100
162	FecIF2	*Clostridium XI*	100
163	FecIF35	*Clostridium XI*	64
164	FecIF41	*Clostridium XI*	100
165	FecIF42	*Clostridium XI*	100
166	FecIF46	*Clostridium XI*	100
167	FecIF60	*Coriobacterineae*	72
168	FecIF43	*Escherichia/Shigella*	100
169	FecIF55	*Escherichia/Shigella*	100
170	FecIF58	*Escherichia/Shigella*	100
171	FecIF62	*Escherichia/Shigella*	100
172	FecIF80	*Escherichia/Shigella*	100
173	FecIF92	*Escherichia/Shigella*	100
174	FecIF95	*Oscillibacter*	93
175	FecIF86	*Prevotella*	95
176	FecIF96	*Prevotella*	99
176	FecIF96	*Prevotella*	99
177	FecIF64	*Rikenella*	72
178	FecIF1	*Ruminococcus*	79
179	FecIF82	*Ruminococcus*	100
180	FecIF83	*Subdivision5_genera_incertae_sedis*	70
181	FecIF12	*Tannerella*	63
182	FecIF14	*Tannerella*	68
183	FecIF47	*Treponema*	94
184	Riv137	*Bacillus*	99
185	Riv138	*Bacillus*	100
186	Riv1	*Bacillus*	100
187	Riv2	*Bacillus*	100
188	Riv105	*Escherichia/Shigella*	99
189	Riv4	*Escherichia/Shigella*	97
190	Riv5	*Lysinibacillus*	59
191	Riv6	*Lysinibacillus*	100

Appendix 5.6 Antimicrobial resistance profiles of enteric bacteria from the dumpsite

Isolate	Source	Species / Genus	Resistance profile of isolates to different antibiotics (zone of inhibition, diameter in mm)							
			CIP	CN	AMC	P	CAZ	CTXM	FOX	NA
FecDX03	FecD	*Escherichia furgosonii*	28 (S)	26 (S)	26 (S)	20 (R)	16 (I)	30 (S)	26 (S)	0 (R)
FecXD05	FecD	*Escherichia coli*	11 (R)	13 (I)	28 (S)	24 (R)	12 (R)	19 (I)	18 (S)	0 (R)
FecDX07	FecD	*Escherichia coli*	23 (S)	16 (S)	18 (I)	7 (R)	20 (S)	24 (S)	22 (S)	20 (S)
FecDX10	FecD	*Escherichia coli*	21 (S)	14 (I)	18 (I)	10 (R)	21 (S)	26 (S)	24 (S)	21 (S)
FecDX11	FecD	*Escherichia coli*	30 (S)	20 (S)	19 (S)	12 (R)	12 (R)	14 (R)	27 (S)	13 (R)
FecDX12	FecD	*Escherichia coli*	12 (R)	15 (I)	18 (I)	0 (R)	20 (S)	23 (S)	22 (S)	0 (R)
FecDX23	FecD	*Escherichia coli*	25 (S)	13 (I)	20 (S)	10 (R)	22 (S)	25 (S)	23 (S)	21 (S)
FecDX25	FecD	*Escherichia coli*	26 (S)	22 (S)	31 (S)	21 (R)	22 (S)	32 (S)	34 (S)	20 (S)
FecDX26	FecD	*Escherichia coli*	24 (S)	16 (S)	18 (I)	8 (R)	21 (S)	25 (S)	19 (S)	21 (S)
FecDX28	FecD	*Escherichia coli*	28 (S)	16 (S)	21 (S)	10 (R)	25 (S)	27 (S)	25 (S)	22 (S)
FecDX29	FecD	*Escherichia*	24 (S)	21 (S)	37 (S)	34 (S)	23 (S)	29 (S)	27 (S)	0 (R)
FecDX30	FecD	*Escherichia coli*	24 (S)	16 (S)	18 (I)	0 (R)	18 (S)	22 (I)	21 (S)	18 (I)
FecDX32	FecD	*Escherichia coli*	31 (S)	14 (R)	18 (I)	0 (R)	21 (S)	23 (S)	21 (S)	19 (I)
FecDX33	FecD	*Escherichia coli*	21 (S)	15 (I)	20 (S)	9 (R)	22 (S)	24 (S)	23 (S)	20 (S)
FecDX15	FecD	*Shigella*	20(I)	16 (S)	10 (R	0 (R)	11 (R)	10 (R)	24 (S)	12 (R)
FecDX16	FecD	*shigella sp*	20 (I)	15 (I)	22 (S)	9 (R)	22(S)	25 (S)	21 (S)	7 (R)
FecDX17	FecD	*Shigella sp*	27 (S)	15 (I)	18 (I)	18 (R)	14 (R)	25 (S)	22 (S)	0 (R)
FecDX20	FecD	*Shigella flexneri*	24 (S)	21 (S)	7 (R)	0 (R)	10 (R)	6 (R)	9 (R)	0 (R)
FecDX08	FecD	*Shigella flexneri*	31 (S)	22 (S)	27 (S)	8 (R)	0 (R)	8 (R)	21 (S)	0 (R)
FecDX19	FecD	*Enterococcus casseliflavus*	21 (S)	13 (I)	14 (R)	9 (R)	6 (R)	14 (R)	14 (R)	14 (I)
FecDX34	FecD	*Enterobacter amnigenus*	18 (I)	16 (S)	23 (S)	23 (R)	0 (R)	19 (I)	15 (I)	0 (R)
FecDX35	FecD	*Enterococcus faecium*	18 (I)	14 (I)	30 (S)	30 (S)	18 (S)	21 (S)	20 (S)	0 (R)
FecDX36	FecD	*Enterococcus faecium*	25 (S)	18 (S)	36 (S)	29 (S)	18 (S)	25 (S)	27 (S)	0 (R)
FecDX37	FecD	*Bacillus sp*	32 (S)	25 (S)	25 (S)	21 (R)	0 (R)	9 (R)	23 (S)	0 (R)

Appendix 5.6 continues

Isolate	Source	Species / Genus	Resistance profile of isolates to different antibiotics (zone of inhibition diameter in mm)							
			CIP	CN	AMC	P	CAZ	CTXM	FOX	NA
FecDX38	FecD	*Bacillus sp*	19 (I)	16 (S)	8 (R)	0 (R)	0 (R)	0 (R)	8 (R)	8 (R)
FecDX40	FecD	*Bacillus* sp	32 (S)	19 (S)	21 (S)	16 (R	12 (R)	18 (I)	31 (S)	14 (I)
FecDX39	FecD	*Bacillus* sp	21 (S)	20 (S)	26 (S)	26 (S)	10 (R)	22 (I)	18 (S)	0 (R)
FecDX42	FecD	*Bacillus* sp	19 (I)	15 (I)	0 (R)	0 (R)	0 (R)	0 (R)	11 (R)	12 (R)
FecDX67	FecD	*Bacillus* sp	23 (S)	20 (S)	0 (R)	0 (R)	0 (R)	0 (R	9 (R)	18 (I)
FecDX68	FecD	*Bacillus* sp	30(S)	16 (S)	20 (S)	7 (R)	24 (S)	24 (S)	22 (S)	19 (I)
FecDX69	FecD	*Bacillus* sp	27 (S)	17 (S)	25 (S)	16 (R)	23 (S)	30 (S)	20 (S)	17 (I)
FecDX70	FecD	*Bacillus* sp	25 (S)	17 (S)	21 (S)	11 (R)	25 (S)	26 (S)	17 (I)	7 (R)
FecDX71	FecD	*Bacillus* sp	33 (S)	22 (S)	20 (S)	20 (R)	13 (R)	17 (S)	31 (S)	0 (R)
FecDX72	FecD	*Aeromonas hydrophila*	21 (S)	19 (S)	25 (S)	20 (R)	18 (S)	18 (I)	25 (S)	17 (I)
FecDX09	FecD	*Pseudomonas luteola*	15(R)	13 (I)	25 (S)	20 (R)	11 (R)	15 (S)	15 (I)	0 (R)
FecDX12	FecD	*Pseudomonas luteola*	25 (S)	19 (S)	11 (R)	0 (R)	0 (R)	7 (R)	21 (S)	8 (R)
FecIX03	FecI	*Escherichia vulneris*	21 (S)	17 (S)	17 (I)	7 (R)	16 (I)	21 (I)	21 (S)	17 (I)
FecIX04	FecI	*Escherichia coli*	25 (S)	17 (S)	19 (S)	11 (R)	10 (R)	17 (I)	20 (S)	12 (R)
FecIX05	FecI	*Escherichia coli*	25 (S)	14 (I)	20 (S)	9 (R)	23 (S)	25 (S)	21 (S)	19 (I)
FecIX13	FecI	*Escherichia coli*	21 (S)	7 (R)	9 (R)	0 (R)	13 (R)	16 (I)	12 (R)	12 (R)
FecIX14	FecI	*Escherichia coli*	22 (S)	14 (I)	18 (I)	0 (R)	21 (S)	24 (S)	21 (S)	0 (R)
FecIX15	FecI	*Escherichia coli*	16 (I)	17 (S)	17 (I)	0 (R)	19 (S)	20 (I)	21 (S)	14 (I)
FecIX16	FecI	*Escherichia coli*	17 (I)	17 (S)	20 (S)	10 (R)	22 (S)	25 (S)	23 (S)	0 (R)
FecIX17	FecI	*Escherichia coli*	26 (S)	21 (S)	7 (R)	0 (R)	7 (R)	12 (S)	18 (S)	9 (R)
FecIX18	FecI	*Escherichia coli*	25 (S)	20 (S)	0 (R)	0 (R)	0 (R)	6 (R)	8 (R)	19 (S)
FecIX19	FecI	*Escherichia coli*	32 (S)	22 (S)	28 (S)	21 (R)	21 (S)	32 (S)	17 (I)	6 (R)
FecIX20	FecI	*Escherichia coli*	22 (S)	16 (S)	19 (S)	0 (R)	14 (R)	19 (I)	19 (S)	0 (R)
FecIX21	FecI	*Escherichia coli*	30 (S)	14 (I)	19 (R)	0 (R)	19 (S)	22 (I)	20 (S)	18 (I)
FecIX22	FecI	*Escherichia coli*	26 (S)	17 (S)	18 (I)	0 (R)	18 (S)	20 (I)	21 (S)	12 (R)
FecIX23	FecI	*Escherichia coli*	26 (S)	14 (I)	16 (I)	6 (R)	14 (R)	15 (I)	21 (S)	14 (I)

Appendix5.6 continues

Isolate	Source	Species / Genus	Resistance profile of isolates to different antibiotics (zone of inhibition diameter in mm							
			CIP	CN	AMC	P	CAZ	CTXM	FOX	NA
FecIX24	FecI	*Escherichia coli*	23 (S)	16 (S)	20 (S)	8 (R)	24 (S)	27 (S)	24 (S)	21 (S)
BiomX01	Biom	*Bacillus* sp	19 (I)	16 (S)	14 (I)	7 (R)	0 (R)	10 (R)	12 (R)	18 (I)
BiomX06	Biom	*Bacillus sp*	29 (S)	19 (S)	16 (I)	9 (R)	7 (R)	17 (S)	24 (S)	19 (I)
BiomX02	Biom	*Bacillus* sp	36 (S)	24 (S)	29 (S)	21 (R)	15 (I)	24 (S)	32 (S)	14 (I)
BiomX07	Biom	*Bacillus licheniformis*	22 (S)	23 (S)	35 (S)	33 (S)	9 (R)	8 (R)	21 (S)	0 (R)
BiomX13	Biom	*Bacillus sp*	24 (S)	19 (S)	0 (R)	0 (R)	0 (R)	0 (R)	10 (R)	16 (I)
BiomX14	Biom	*Serratia rubidae*	0 (R)	11(R)	12 (R)	0 (R)	0 (R)	12 (R)	15 (I)	0 (R)
BiomX23	Biom	*Bacillus sp*	27 (S)	15 (I)	12 (R)	0(R)	7 (R)	13 (R)	19 (S)	21 (S)
BiomX46	Biom	*Bacillus sp*	35 (S)	27 (S)	17 (I)	14 (R)	11 (R)	19 (S)	25 (S)	12 (R)
BiomX11	Biom	*Pseudomonas luteola*	24 (S)	20 (S)	10 (R)	0 (R)	0 (R)	0 (R)	12 (R)	0 (R)
BiomX22	Biom	*Pseudomonas luteola*	12 (R)	23 (S)	31 (S)	39 (S)	12 (R)	29 (S)	23 (S)	0 (R)
BiomX25	Biom	*Pseudomonas luteola*	29 (S)	22 (S)	28 (S)	26 (R)	0 (R)	7 (R)	22 (S)	14 (I)
BiomX12	Biom	*Staphylococcus sciuri*	28 (S)	22 (S)	20 (S)	17 (R)	11 (R)	16 (S)	28 (S)	15 (I)
BiomX03	Biom	*Lysinibacillus* sp	23 (S)	21 (S)	32 (S)	22 (R)	17 (I)	27 (S)	25 (S)	0 (R)
BiomX04	Biom	*Lysinibacillus* sp	19 (I)	21 (S)	19 (S)	27 (S)	7 (R)	21 (S)	18 (S)	0 (R)
DomX05	Dom	*Bacillus* sp	18 (I)	21 (S)	23 (S)	24 (R)	24 (S)	30 (S)	29 (S)	16 (I)
DomX06	Dom	*Bacillus* sp	24 (S)	18 (S)	9 (R)	0 (R)	6 (R)	8 (R)	11 (R)	0 (R)
DomX 08	Dom	*Bacillus cereus*	29 (S)	18 (S)	13 (R)	0 (R)	0 (R)	0 (R)	10 (R)	24 (S)
DomX37	Dom	*Bacillus*sp	27 (S)	16 (S)	24 (S)	21 (R)	0 (R)	7 (R)	19 (S)	15 (I)
DomX09	Dom	*Serratia plymuthica*	21(S)	16 (S)	29 (S)	20 (R)	13 (R)	21 (S)	23 (S)	0 (R)
DomX10	Dom	*Pseudomonas luteola*	15 (R)	13 (I)	25 (S)	20 (R)	11 (R)	15 (S)	15 (I)	0 (R)
DomX30	Dom	*Pseudomonas sp*	27 (S)	23 (S)	23 (S)	6 (R)	17 (I)	30 (S)	25 (S)	0 (R)
RivX01	Riv	*Ewingella americana*	29 (S)	25 (S)	30 (S)	18 (R)	25 (S)	19 (S)	28 (S)	24 (S)
RivX02	RiV	*Shigella* sp.	28 (S)	21 (S)	24 (S)	20 (R)	12 (R)	24 (S)	30 (S)	20 (S)
RivX03	Riv	*Shigella* sp	21 (S)	19 (S)	24 (S)	22 (R)	0 (R)	7 (R)	15 (I)	0 (R)
RivX04	Riv	*Escherichia coli*	29 (S)	15 (I)	14 (I)	8 (R)	17 (I)	22 (I)	14 (R)	20 (S)

Appendix 6.1 Manuscripts and Publications

1. Mwaikono, K. S.; Maina, S.; Sebastian, A.; Schilling, M.; Kapur, V.; Gwakisa, P**., High-throughput sequencing of 16S rRNA Gene Reveals Substantial Bacterial Diversity on the Municipal Dumpsite:** *The manuscript was submitted to the Journal BMC-Microbiology: Status - Under review*

2. Mwaikono, K. S.; Maina, S.; Sebastian, A.; Kapur, V.; Gwakisa, P., **The faecal microbiota of free-range pigs (*Sus scrofa domesticus*) scavenging on a municipal dumpsite**: *The manuscript was submitted to the Journal PLOS one: Status - Under review*

3. Mwaikono, K. S.; Maina, S.; Sebastian, A.; Kapur, V.; Gwakisa, P., **16S rRNA Amplicons Survey Revealed Unprecedented Bacterial Community in Solid Biomedical Wastes**. *American Journal of Microbiological Research* **2015,** 3, (4), 135-143. http://pubs.sciepub.com/ajmr/3/4/3/ doi: 10.12691/ajmr-3-4-3

4. Mwaikono, K. S.; Maina, S.; Gwakisa, P., **Prevalence and Antimicrobial Resistance Phenotype of Enteric Bacteria from a Municipal Dumpsite**. *Journal of Applied & Environmental Microbiology* **2015,** 3, (3), 82-94. http://pubs.sciepub.com/jaem/3/3/4/. doi: 10.12691/jaem-3-3-4