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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 common 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 a common dumpsite. A qualitative survey was done to identify types of solid biomedical waste on the 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 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 *Chloroflex* 1.7%. Catchall analysis predicted a mean of 9399 species per sample. Overall, 31402 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 spp* and *Acinetobacter spp* 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 unknown. **Conclusions** 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.

Keywords: *solid biomedical waste, bacteria, molecular diversity, 16S rRNA, dumpsite, illumina MiSeq, Tanzania*

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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 [1,2,3,4]; their disposal have remained a serious challenge in most cities of developing countries [5,6,7,8] even to-date. 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 Wallace *et al*., and DeRoos [4,9] 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 [6,10,11], where *Escherichia spp, Pseudomonas spp, Klebsiella spp, Salmonella spp, Staphylococcus spp, Serratia spp, Acinetobacter spp, Enterococcus spp* and *Streptococcus spp* 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 [12,13], 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 [6,11,14]. Unfortunately, none of the 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 environments 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 surprisingly dominated by Operational Taxonomic Units (OTUs) of industrial importance.

2. Materials and Methods

2.1. Study Site

Site for this study was the Arusha municipal dumpsite, where unsorted waste from different urban sources is thrown. 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.

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).

2.3. Extraction of Total DNA

About 250 mg of biomedical waste was used to extract total DNA using PowerSoil™ DNA extraction kit (MOBIO Laboratories, Carlsbad, CA) according to per manufacturer's protocol. Quality and quantity of total DNA was verified with NanoDrop ND-2000c spectrophotometer (Thermo Scientific) and gel electrophoresis run in 0.8% agarose and visualized by ultraviolet illumination after staining with gel red™. The DNA was stored at -20°C till further processing

2.4. 16S rRNA Amplification, Library Construction and Sequencing

Illumina sequencing preparation guide [15] was used to prepare a pooled amplicons of v4 region of 16S rRNA gene for sequencing. Primers (515F/806R) designed for v4 region of 16S rRNA and protocols were adapted from Caporaso [16]. Duplicate reactions were done in PCR master mix reaction in 20 µl AccuPower® Taq PCR PreMix composed of 0.5µl 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°C for 3 min, 35 cycles of 94°C for 45 s, 50°C for 60 s and 72°C for 90 s and a final extension at 72°C for 10 min. Amplicons quality was visualized using gel electrophoresis, 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 further quality control using Agilent DNA 1000 Chip in Agilent 2100 Bioanalyzer (Agilent Technologies, Waldbronn, Germany). Library denaturing, dilution and PhiX control preparation was done as described in 16S metagenomic sequencing library preparation guide [15]. Libraries were denatured and primers used according to the method described in Caporaso [16]. Sequencing of the library was done in Illumina MiSeq platform (San Diego, USA) using 2×250 paired- end chemistry at the BecA - ILRI Hub genomic platform, Nairobi, Kenya

2.5. Sequence Data Analysis and Statistics

The Mothur package algorithms (v1.34.1) was used for both quality control and sequence data analysis [17]. After paired end reads were assembled, sequences were aligned with the Silva 16S rRNA reference database (www.arb-silva.de) [18]. 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 [19] and eliminated. Taxonomy was assigned using the RDP taxonomy database (http://rdp.cme.msu.edu/index.jsp) [20]. Sequences were binned into operational taxonomic units (OTUs) at 97% sequence similarity level.

Species richness was assessed with Chao1 richness estimator [21], abundance based coverage estimator ACE [22], Shannon weaver [23] and inverse Simpson [24] indices as well as with CatchAll analysis [25]. 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 [26] 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 [27] 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 [28] where further taxonomic profile comparison were performed. A p-value of ≤ 0.05 was considered significant for all comparisons.

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].

3. Results

3.1. Qualitative Survey of the Dumpsite

A qualitative survey revealed presence of diverse solid biomedical waste on the municipal dumpsite. Expired drugs, used syringes and swabs, catheters and drugs containers were among the most prevalent biomedical wastes (Figure 1).

Figure 1. Types of solid biomedical waste on the dumpsite. (A) Expired drugs, (B) used syringes (C) drugs and drugs containers (D) drugs containers and residues

3.2. 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 70664 to 174456 (mean 121765, SD 35853). Catchall analysis of richness predicted a mean of 9399 species per sample (range 544 - 16621, SD 3678). Good's coverage ranged from 0.9625 – 0.9926 (mean 0.9835, SD 0.0074). Rarefaction curves showing sampling efficiency is displayed in Figure 2.

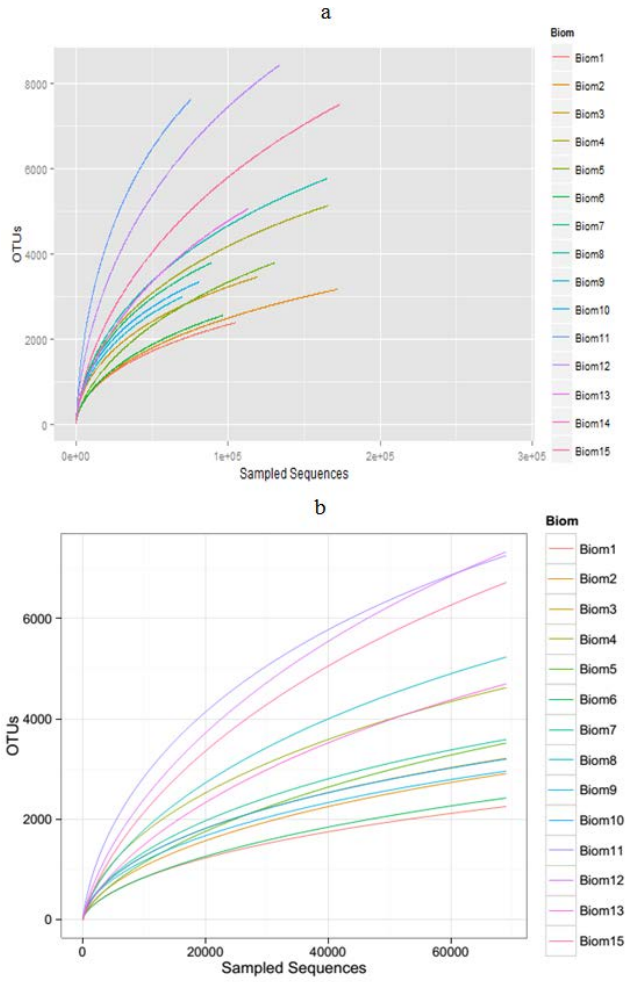

Figure 2. Rarefaction curves of v4 region of 16S rRNA gene sequences from 15 samples. Figure 2a show rarefaction curves as per sequences generated from each sample and Figure 2b is a rarefaction curve after subsampling of 69,000 sequences from each sample

The overall bacterial diversity was high with an average Chao1 richness of 6330 (range 3728 – 12287, SD 2760); ACE 7682 (range 4476 – 15167, SD 3383); 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%. Figure 3 summarizes predominant bacterial phyla.

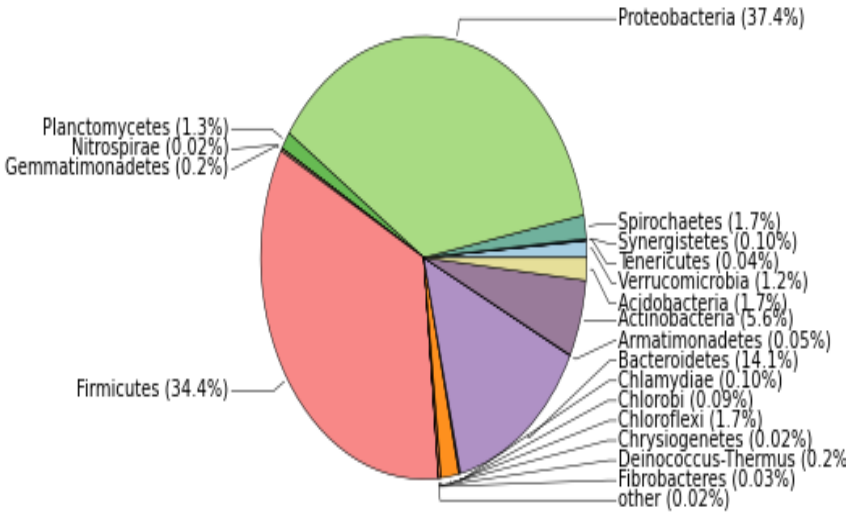

Figure 3. Predominant bacteria phyla in solid biomedical wastes. OTUs were assigned at 97% sequence similarity cut-off

Table 1. Summary of good quality sequence data and diversity indices of different samples at species - level (OTUs definition at > 97% identity cut-off)

Sample	Quality reads	OTUs	Chao1	Inv Simpson	Shannon
Biom1	106046	2369	3728	19	4.3
Biom2	172105	3101	4627	7	3.8
Biom3	119512	3426	4970	22	4.9
Biom4	167643	5075	7284	83	5.8
Biom5	130218	3670	5613	12	4.0
Biom6	96878	2533	3847	9	4.0
Biom7	90308	3788	5402	25	5.2
Biom8	167215	5781	8568	45	5.5
Biom9	70664	2973	4336	54	5.4
Biom10	81145	3352	4752	54	5.5
Biom11	77074	7615	10590	496	7.4
Biom12	137828	8353	12287	37	6.2
Biom13	113620	4952	7464	5	4.1
Biom14	1730	285	420	30	4.5
Biom15	174456	7357	11110	16	4.9

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

Table 2. Abundance of predominant OTUs in solid biomedical waste

OTU	Reads	%	Phylum	Genus
1	175640	10.4	*Firmicutes*	*Proteiniclasticum*
2	116657	6.9	*Proteobacteria*	*Acinetobacter*
3	65854	3.9	*Proteobacteria*	*Halomonas*
4	38308	2.3	*Proteobacteria*	*Acinetobacter*
5	28125	1.7	*Proteobacteria*	*Pseudomonas*
6	26140	1.5	*Proteobacteria*	*Escherichia/Shigella*
7	22324	1.3	*Firmicutes*	*Planococcus*
8	20506	1.2	*Proteobacteria*	*Oligella*
9	17020	1.0	*Proteobacteria*	*Paracoccus*

A total of 31402 OTUs were found, however only 6,201 (19.8%) were identified more than 10 times. 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 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 OUT was found across all samples tested although. Only *Proteiniclasticum* and *Acinetobacter* were detected in 67% (10/15) of samples at relative abundance of 1%.

Despite the fact that all samples were collected from the same dumpsite, the phylogenetic tree established using UPGMA(Unweighted Pair Group Method Arithmetic mean) clearly grouped sequences into two clusters (Figure 4). Cluster "Biom A" comprised sequences from 10 samples while cluster "Biom B" comprised sequences from 5 samples. Further, we revealed predominance of *Firmicutes*, *Proteobacteria* and *Bacteroidetes* phyla in cluster A and *Proteobacteria*, *Bacteroidetes* and *Firmicutes* in cluster "Biom B" in that order.

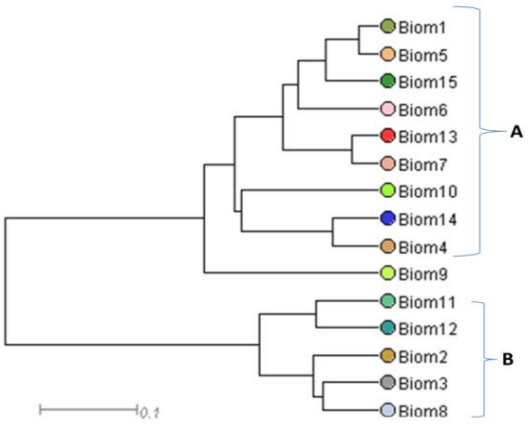

Figure 4. Population structure of bacteria from 15 solid biomedical waste samples (cluster A, n = 10 cluster B, n = 5). Phylogenetic tree was established using UPGMA (Unweighted Pair Group Method with Arithmetic Mean) based on Bray Curtis distances of dissimilarity. 16S rRNA sequence similarity was established 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 Deinoccocu-*Thermus*) were significantly different between clusters A and B (Table 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 1092 bacterial genera, 88 genera and 26 unclassified genera were significantly different between the two clusters (Additional file 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* (Figure 5).

Table 3. Differential abundance of bacterial phyla in two clusters of solid 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

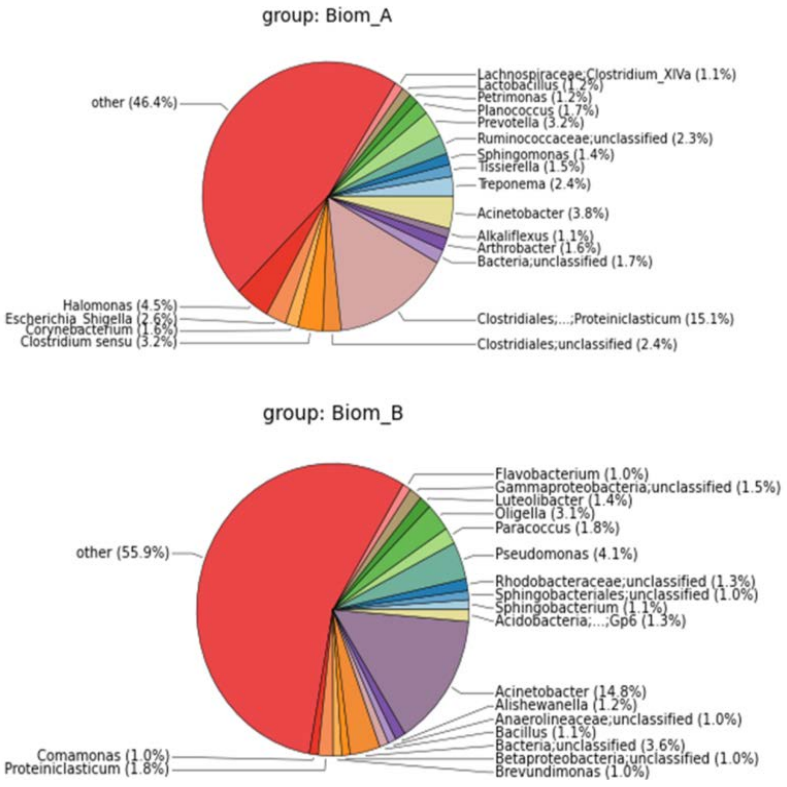

Figure 5. Abundance of predominant bacterial genera in clusters A and cluster B of solid biomedical wastes from the same dumpsite. Normalized sequence counts from each cluster were used in the comparison

3.3. Taxonomy to Phenotype Mapping

Taxonomy to metabolism mapping of the OTUs revealed presence of bacteria capable of degrading environmental pollutants. Bacteria involved with dehalogenation were 36.2%, degraders aromatic hydrocarbons 11.6%, chitin degradation 14.8%, chlorophenol degradation 8.5% and Atrazine metabolism 8.3% (Figure 6). Surprisingly, 46.5% of OTUs had unknown metabolism.

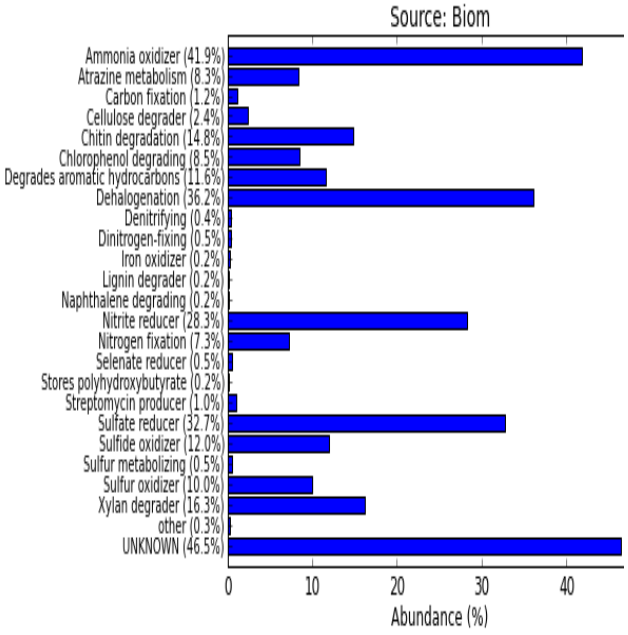

Figure 6. Taxonomy to metabolism mapping of bacteria OTUs of the solid biomedical waste

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 (Figure 7). Interestingly, 39.4% of the bacteria community were not known whether they are pathogens of not.

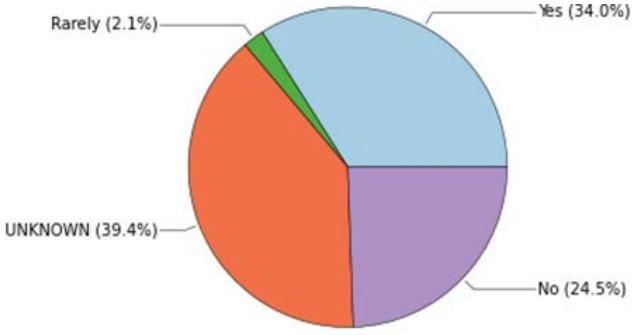

Figure 7. Taxonomy to human pathogens mapping. OTUs were assigned at 97% sequence similarity cut-off

4. Discussion

This study has identified a remarkable abundance and diversity of bacteria in solid biomedical waste in a municipal dumpsite in Arusha, Tanzania. The estimated mean of 9399 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 [6,11,29]. 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 [12,13]. 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.*, [6], Anitha *et al*., [11] and Oyeleke *et al*., [14] 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*., [29] found mostly the same groups of bacteria: *Pseudomonas aeruginosa, Salmonella spp., Klebsiella pneumoniae, Serratia marcescens, Acinetobacter baumannii, Staphylococcus aureus, Staphylococcus epidermidis, Enterococcus faecalis, 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. Our 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 [30,31,32], 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 [33]; industrialy used in synthesis of ectoine which is used as a stabilizer for enzymes and cell protectant in skin and health care [34]. Predominance of *Acinetobacter spp* (9.2% of all sequences) is in agreement with findings of Saini *et al.*, [10], Hossain *et al*., [29] and Muntasir *et al*., [35] 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 [36,37]. The detection of

Pseudomonas spp (1.7%) in solid biomedical waste is also reported by [6,29,38,39] based on culture methods. Apart from this bacteria being associated with multidrug resistance [40,41,42,43] 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 [44,45]. *Pseudomonas* is used in bioremediations of environmental pollutants [46] and importantly to degrade polyethylene materials [47]. *Paracoccus spp* (1%) was another prdominat genera. This genera 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 [48,49].

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 [6,11,14]. The importance of this genus to animal and human health as well as its applications in biotechnology is well known [50,51,52]. 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, e.g. 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%. Since this genus has been reported in ruminants [53] and tannery waste water [54], 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 [55] and in cold and alkaline environment from which novel enzymes with industrial potential were discovered [56]; 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 [57], bioremediation of petroleum refinery effluents [58] and in production of *chitinase* enzymes of potential use in biotechnology and agro-industry [59]. 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 a common 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.

5. Conclusion

There's 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. Theres 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 reports on bacteria of industrial use from 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.

List of Abbreviations

MEGAN- MetaGenome ANalyser;
OTUs – Operational Taxonomic Units
BecA –ILRI - Biosciences eastern and central Africa – International Livestock Research Institute

Competing Interest

The authors declare no conflict of interest.

Authors' Contributions

PSG, KSM, VK, designed the study; PSG Supervised the study, KSM performed sample collection and DNA extraction; KSM, AS and SM performed sequence library preparation, sequencing and data analysis; KSM, AS, VK and PSG were responsible for the draft and final version of the manuscript. All authors read and approved the final draft of the manuscript

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