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2015

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Journal of Applied & Environmental Microbiology, 2015, Vol. 3, No. 3, 82-94
Available online at http://pubs.sciepub.com/jaem/3/3/4
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DOI:10.12691/jaem-3-3-4

Prevalence and Antimicrobial Resistance Phenotype of Enteric Bacteria from a Municipal Dumpsite

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Received August 02, 2015; Revised August 21, 2015; Accepted August 26, 2015

Abstract The objective of the study was to determine the prevalence and antibiotic resistance phenotype of enteric bacteria from the municipal dumpsite. A qualitative survey of the dumpsite was conducted to identify types of solid wastes and nature of interaction on the 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, sequenced 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* spp was from Biom. 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 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 multidrug resistant. *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*

Cite This Article: Kilaza Samson Mwaikono, Solomon Maina, and Paul Gwakisa, "Prevalence and Antimicrobial Resistance Phenotype of Enteric Bacteria from a Municipal Dumpsite." *Journal of Applied & Environmental Microbiology*, vol. 3, no. 3 (2015): 82-94. doi: 10.12691/jaem-3-3-4.

1. Introduction

Antibiotic resistant bacteria are extremely important to human and animals health, as it has become a major public health challenge globally [1,2,3]. 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 [4]. Inappropriate use of antibiotics has been pointed out as one of the reasons which leads to selection and hence development of drug resistant microbes [5,6,7].

Poor solid waste management in many municipalities in developing countries [8,9,10] is associated with the accumulation of unsorted garbage in both undesignated areas and in common 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 favors bacterial changes. 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 [11,12,13]. Enteric bacterial isolates from the

dumpsite were reported to be resistant to commonly used antibiotics [12]. 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 [14,15], 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*., [16], multiple resistant bacteria causing surgical site infection by Manyahi *et al.,* [17], nasal carriage of methicilin resistant *Staphylococcus* by under-five in Tanzania [18] 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.

2. Materials and Methods

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 nearby river (Riv, n = 10). 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. About 5g 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°C until further processing

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).

2.3. Extraction of Total DNA and PCR Amplification

Total genomic DNA was extracted from about 250 mg of sample using PowerSoil™ DNA extraction kit (MOBIO Laboratories, Carlsbad, CA) 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') [19,20,21]. 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°C for 5 min (initial denaturation), 35 cycles of 94°C for 30s, 57 °C for 30 s (annealing), 68°C for 1min (initial extension) and final extension at 68°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). The PCR products were purified using Qiagen kit (Qiagen, Valencia, CA) following manufacturer's protocol

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 °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. 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') [22] and the AccuPower® Taq PCR PreMix as explained above. PCR programme run in GeneAMP™ PCR system 9700 (Applied Biosystems) set at 95°C for 3 min (initial denaturation) and 35 cycles of 94°C for 1 min, 55°C for 1 min, 72°C for 2 min and final extension at 72°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 of 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).

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 [23] was used for sequence alignment, chimera detection, 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/ [24]. 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 KM 24477 to KM 244949.

2.6. Phylogeny of Enteric Bacteria from the Dumpsite and Similarity to Known Pathogens

The MEGA6 software [25] 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 [26] and the evolutionary history was inferred using the Neighbor-Joining method [27]. The evolutionary distances were computed using the Jukes-Cantor method [28]. 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 ≥ 99% were considered highly similar to particular known bacteria.

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. 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 [29]. 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. 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 of amplicons, sequencing and identification of isolates through sequence similarity was done as previously explained

2.8. Antimicrobial Susceptibility Testing

The Kirby-Bauer disk diffusion technique [30] was used to study the antimicrobial susceptibility of bacteria isolates from the dumpsite. The commercially prepared antibiotic discs, Cefotaxime (CTXM, 30µg), Cefoxitin (FOX, 30ug), Penicillin G (P, 10µg), Amoxycillin / Clavulanic acid (AMC, 20/10µg) and Ceftazidime (CAZ, 30µg) in group of β-lactam antibiotics; and Ciprofloxacin (CIP, 5µg) and Nalidixic acid (NA, 30µg) in group of quinolones; and Gentamicin (CN, 10µg) 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 a sterile Peptone water (HiMedia Laboratories Pvt, India). Interpretation of antimicrobial resistance phenotype was performed as per Clinical Laboratory Standards Institute guide [31]. Isolates were categorized as resistant (R), intermediate resistant (IR) and susceptible (S). Excel program was used to prepare summary plots of resistance profile of different enteric bacteria isolates.

3. Results

3.1. Qualitative Survey of the Dumpsite

A survey of the dumpsite found different types of solid wastes from domestic, industries, markets and hospitals/pharmaceuticals thrown on the same dumpsite without prior sorting. Wastes comprised of biomedicals such as used syringes, swabs, expired drugs and used catheters; diapers, dead animals, food remnants, cosmetics and torn clothes from domestic; used bottles, package material and other industrial wastes. Domestic animals such as pigs, goats, and cattle, dogs, as well as chickens were scavenging on dumpsite. Wild animals such as rodents, snakes and birds like crows were seen on dumpsite. Humans, apart from the dumpsite workers; women and children were seen searching for recyclable materials on the dumpsite. Close to the dumpsite there is river Burka, to which garbage and non-solid waste leaches during rains. Figure 1 shows the dumpsite scenery.

Figure 1. Dumpsite interaction and types of solid waste on the dumpsite. A - Truck offloading garbage on the dumpsite and people searching for valuable recyclable materials; B - domestic free range pigs scavenging on garbage; C - Diapers from domestic waste; D - used syringes from hospitals; E - cattle drinking water from the river near the dumpsite

3.2. 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 (Supplementary file 1).

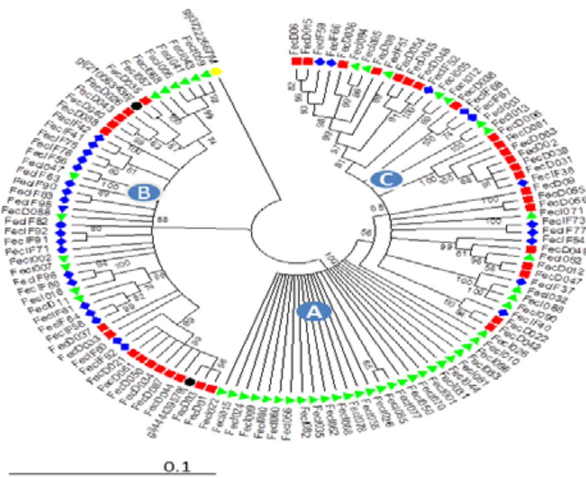

Figure 2. Phylogenetic tree of faecal bacteria from pigs under different management system. Evolutionary relationship of faecal bacteria of pigs under different management system was established using Mega6 software. The bootstrap values (expressed as percentages of 100 replications) are shown at branch 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

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 (Figure 2) 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 bacterial 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 1) all of them known as important human and animal pathogens.

Table 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	*S. sonnei*	NR_074894.1	99	[32]
KM244773	5	*E. furgosonii*	NR_074902.1	99	[33]
KM244781	3	**E. faecium*	NR_102790.1	99	[34]
KM244796	3	*E.coli O157:H7*	NR_074891.1	99	[35]

*E - Enterococcus, E- Escherichia, S – Shigella.

3.3. Antimicrobial Sensitivity Test

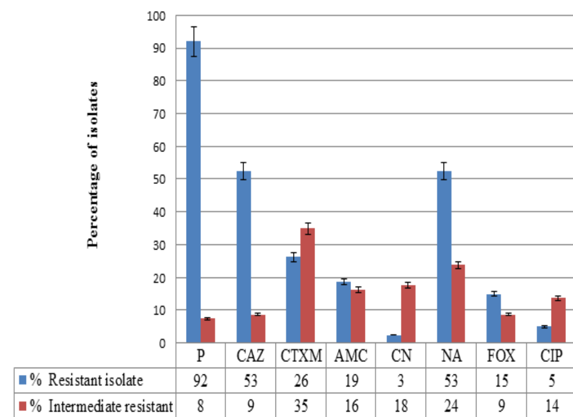

Figure 3. Antimicrobial resistance phenotypic profile of bacteria isolates. Percentage of enteric bacterial isolates with different degrees of resistance; P - Penicillin G, CAZ - Ceftazidime, CTXM - Cefotaxime, AMC - Amoxycillin /Clavulanic, CN - Gentamicin, NA - Nalidixic Acid, FOX- Cefoxitin, CIP - Ciprofloxacin. Blue bars represent resistant isolates and red bars represent isolates with intermediate resistance

Eighty pure bacteria isolates from different solid wastes were used for antimicrobial sensitivity test. Phenotypic profile analysis revealed that, over 50% of all the isolates were resistant to Penicillin G, Ceftazidime and Nalidixic Acid antibiotics (Figure 3). While for penicillin G most bacteria showed resistance (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).

Table 2. Multidrug resistance profile of bacteria from the dumpsite

Escherichia sp		
# of antibiotics	Resistance pattern	#of isolates
2	P, NA	4
3	P,CAZ, NA	2
3	CIP, P, NA	1
4	CIP,CAZ, P,NA	1
4	P, CAZ, CTXM, NA	1
5	AMC, P, CAZ, CTXM, FOX	1*
6	CN, AMC, P, CAZ, FOX, NA	1*
Shigella sp		
2	P, NA	1
3	P, CAZ, NA	1
4	P, CAZ, CTXM, NA	1*[r]
5	AMC, P, CAZ, CTXM, NA	1*[a]
6	AMC, CAZ, P, CTXM, FOX ,NA	1*[b]
Pseudomonas sp		
2	P, NA	1
3	P, CAZ, CTXM	1
3	CIP, CAZ, NA	1
4	CIP, P, CAZ, NA	2**
5	AMC, P, CAZ, CTXM, NA	1 ***
6	AMC, P, CAZ, CTXM, FOX, NA	1***
Serratia sp		
3	P, CAZ, NA	1
7	CIP, CN, AMC, P, CAZ, CTXM, NA	1*[y]
Enterococcus sp		
2	CIP, NA	1
3	P, CAZ, NA	1*[z]
Enterobacter sp		
4	P, CAZ, CTXM, FOX	1
Bacillus sp		
2	P, CAZ	1
2	P, NA	2
3	P, CAZ, CTXM	1
3	P, CAZ, NA	1
4	P, CAZ, CTXM, FOX	4
5	AMC, P, CAZ, CTXM, FOX	1[x*]
5	P, CAZ, CTXM, FOX, NA	1[x*]
5	AMC, P, CAZ, CTXM, FOX	1[x*]
6	AMC, P, CAZ,CTXM, FOX, NA	1[x*]

Multidrug resistance expressed by bacterial isolates from different solid waste. Isolates expressing resistance to more than four antibiotics are shown with an asterisk; * *Escherichia coli* isolated from faecal matter 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.

Further, phenotypic profiling revealed prevalence of multidrug resistant bacteria on the dumpsite (Table 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.

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 [36,37,38] 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 [39,40]. 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. 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 [40,41,42], 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.*, [43] in effluent of municipal waste water treatment plant. *Pseudomonas* is associated with diseases in humans and animals, for example, Casalta *et al.,* [44] 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* [45]. 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 (Figure 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 high risk 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 [46,47]. The fact that *Bacillus* sp is associated with several diseases of humans and animals [48,49,50,51], their prevalence and 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 [52,53]. 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 [54], antimicrobial resistance in urinary isolates [55], and antibiotic resistant bacteria in diabetic women's [16], nasal carriage of methicillin resistant *Staphylococcus* to under 5 children [18] and antimicrobial resistant isolates from blood stream [56]. 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* [57,58]; nosocomial infections by *Enterococcus* [59,60] as well as various food-borne diseases by Escherichia coli [61,62] . 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. 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.

Acknowledgements

We acknowledge the Government of Tanzania through The Nelson Mandela African Institution of Science and Technology for sponsoring this research; The Africa Biosciences Challenge Fund (ABCF) for a fellowship to execute laboratory work at Biosciences eastern and Central Africa – International Livestock Research Institute (BecA- ILRI hub) in Nairobi, Kenya. Dr. Rob Skilton for official facilitation while at BecA-ILRI hub, Ms Lucy Muthui for her assistance with sequencing, the Genome Science Centre at Sokoine University of Agriculture for assistance with Microbiology and sampling techniques are highly acknowledged.

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Supplementary file 1

Table S1. Bacterial genera identified in solid biomedical waste from the dumpsite

S/N	Sample/Clone	Genera	% ID
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	Biom16	*Derxia*	33
14	Biom6	*Derxia*	56
15	Biom2	*Enterococcus*	100
16	Biom24	*Flavisolibacter*	98
17	Biom19	*Flavobacterium*	88
18	Biom56	*Luteimonas*	100
19	Biom142	*Lysinibacillus*	97
20	Biom143	*Lysinibacillus*	97
21	Biom146	*Lysinibacillus*	100

22	Biom149	*Lysinibacillus*	71
23	Biom39	*Massilia*	100
24	Biom83	*Micrococcineae*	96
25	Biom22	*Oligella*	100
26	Biom53	*Parapusillimonas*	25
27	Biom78	*Peptoniphilus*	66
28	Biom3	*Planomicrobium*	83
29	Biom15	*Proteiniclasticum*	100
30	Biom35	*Proteiniclasticum*	99
31	Biom37	*Proteiniclasticum*	100
32	Biom38	*Proteiniclasticum*	100
33	Biom61	*Proteiniclasticum*	100
34	Biom66	*Proteiniclasticum*	100
35	Biom69	*Proteiniclasticum*	100
36	Biom74	*Proteiniclasticum*	100
37	Biom80	*Proteiniclasticum*	99
38	Biom12	*Pseudomonas*	99
39	Biom77	*Rhodoplanes*	55
40	Biom68	*Roseicyclus*	11
41	Biom122	*Salirhabdus*	40
42	Biom86	*Stenotrophomonas*	100
43	Biom14	*Thauera*	100
44	Biom30	*Tissierella*	61

Table S2: Bacterial genera identified in domestic solid waste from the dumpsite

S/N	Sample	Genera	% ID
1	Dom16	*Acinetobacter*	100
2	Dom23	*Acinetobacter*	100
3	Dom44	*Acinetobacter*	100
4	Dom7	*Allochromatium*	99
5	Dom52	*Atopostipes*	100
6	Dom113	*Bacillus*	100
7	Dom114	*Bacillus*	100
8	Dom132	*Bacillus*	100
9	Dom37	*Candidatus Hydrogenedens*	100
10	Dom47	*Clostridium XI*	99
11	Dom11	*Fusibacter*	100
12	Dom28	*Kurthia*	58
13	Dom40	*Leuconostoc*	100
14	Dom38	*Meniscus*	31
15	Dom5	*Mesorhizobium*	100
16	Dom54	*Mesorhizobium*	58
17	Dom48	*Oceanibaculum*	58
18	Dom30	*Phascolarctobacterium*	94
19	Dom36	*Pontibacter*	100
20	Dom8	*Pontibacter*	100
21	Dom35	*Proteiniclasticum*	100
22	Dom111	*Pseudomonas*	100
23	Dom12	*Saccharofermentans*	75
24	Dom31	*Saccharophagus*	18
25	Dom19	*Sphingomonas*	100
26	Dom26	*Sporacetigenium*	100
27	Dom129	*Staphylococcus*	100
28	Dom34	*Thalassolituus*	100
29	Dom4	*Tindallia*	74
30	Dom39	*Treponema*	100

Table S3: Bacterial genera identified in faecal material of pigs scavenging on the dumpsite

S/N	Sample	Genera	% ID
1	FecD12	*Bacillus*	100
2	FecD128	*Bacillus*	100
3	FecD26	*Bacillus*	84
4	FecD50	*Bacillus*	100
5	FecD60	*Bacillus*	100
6	FecD60	*Bacillus*	100
7	FecD84	*Bacillus*	100
8	FecD85	*Bacillus*	100
9	FecD87	*Bacillus*	86
10	FecD91	*Bacillus*	100
11	FecD99	*Bacillus*	81
12	FecD133	*Brevibacillus*	48
13	FecD16	*Clostridium IV*	45
14	FecD83	*Clostridium sensu stricto*	100
15	FecD17	*Clostridium XI*	99
16	FecD19	*Clostridium XI*	75
17	FecD7	*Clostridium XI*	100
18	FecD120	*Enterococcus*	100
19	FecD144	*Enterococcus*	100
20	FecD35	*Enterococcus*	100
21	FecD77	*Enterococcus*	100
22	FecD86	*Enterococcus*	100
23	FecD1	*Escherichia/Shigella*	100
24	FecD21	*Escherichia/Shigella*	100
25	FecD3	*Escherichia/Shigella*	100
26	FecD3	*Escherichia/Shigella*	99
27	FecD34	*Escherichia/Shigella*	100
28	FecD44	*Escherichia/Shigella*	100
29	FecD48	*Escherichia/Shigella*	100
30	FecD50	*Escherichia/Shigella*	100
31	FecD51	*Escherichia/Shigella*	100
32	FecD61	*Escherichia/Shigella*	100
33	FecD63	*Escherichia/Shigella*	99
34	FecD81	*Escherichia/Shigella*	100
35	FecD82	*Escherichia/Shigella*	100
36	FecD83	*Escherichia/Shigella*	100
37	FecD87	*Escherichia/Shigella*	99
38	FecD93	*Escherichia/Shigella*	100
39	FecD97	*Escherichia/Shigella*	100
40	FecD75	*Fusobacterium*	66
41	FecD37	*Kandleria*	98
42	FecD13	*Lachnospiracea_incertae_sedis*	71
43	FecD10	*Lactobacillus*	100
44	FecD88	*Mitsuokella*	100
45	FecD58	*Oscillibacter*	48
46	FecD33	*Paenibacillus*	99
47	FecD40	*Planococcaceae_incertae_sedis*	96
48	FecD43	*Planococcaceae_incertae_sedis*	94
49	FecD14	*Sporacetigenium*	63

Table S4: Bacteria genera identified in faecal materials of indoor reared pigs

S/N	Sample	Genera	% ID
1	FecI11	*Acetivibrio*	68
2	FecI16	*Anaerorhabdus*	39
3	FecI17	*Bacillus*	100
4	FecI19	*Bacillus*	100
5	FecI2	*Clostridium IV*	80
6	FecI20	*Clostridium IV*	84
7	FecI21	*Clostridium sensu stricto*	100
8	FecI23	*Clostridium sensu stricto*	100
9	FecI27	*Clostridium sensu stricto*	98
10	FecI27	*Clostridium sensu stricto*	98
11	FecI29	*Clostridium sensu stricto*	100
12	FecI30	*Coprobacillus*	9
13	FecI30	*Escherichia/Shigella*	100
14	FecI32	*Escherichia/Shigella*	100
15	FecI34	*Escherichia/Shigella*	100
16	FecI38	*Escherichia/Shigella*	100
17	FecI39	*Escherichia/Shigella*	100
18	FecI41	*Escherichia/Shigella*	100
19	FecI41	*Escherichia/Shigella*	100
20	FecI43	*Escherichia/Shigella*	100
21	FecI43	*Escherichia/Shigella*	100
22	FecI44	*Escherichia/Shigella*	100
23	FecI45	*Eubacterium*	39
24	FecI46	*Gemmiger*	57
25	FecI47	*Lachnospiracea_incertae_sedis*	79
26	FecI48	*Lachnospiracea_incertae_sedis*	97
27	FecI51	*Lachnospiracea_incertae_sedis*	74
28	FecI54	*Lactobacillus*	100
29	FecI58	*Lactobacillus*	100
30	FecI59	*Lactobacillus*	100
31	FecI6	*Lactobacillus*	100
32	FecI61	*Lactobacillus*	100
33	FecI64	*Lactobacillus*	100
34	FecI67	*Lactobacillus*	100
35	FecI68	*Megasphaera*	100
36	FecI7	*Megasphaera*	100
37	FecI72	*Oscillibacter*	45
38	FecI79	*Oscillibacter*	76
39	FecI84	*Oscillibacter*	100
40	FecI86	*Prevotella*	99
41	FecI93	*Roseburia*	100
42	FecI97	*Tannerella*	63
43	FecI98	*Tannerella*	63

Table S5: Bacterial genera identified in faecal material of pigs shifted from indoor to free range on the dumpsite

S/N	Sample	Genera	% ID
1	FecIF15	*Anaerotruncus*	31
2	FecIF75	*Anaerovorax*	95
3	FecIF76	*Anaerovorax*	90
4	FecIF101	*Bacillus*	100
5	FecIF53	*Bacillus*	100
6	FecIF39	*Clostridium IV*	36
7	FecIF71	*Clostridium IV*	90
8	FecIF91	*Clostridium IV*	48
9	FecIF92	*Clostridium IV*	32
10	FecIF19	*Clostridium sensu stricto*	100
11	FecIF56	*Clostridium sensu stricto*	100
12	FecIF2	*Clostridium XI*	100
13	FecIF35	*Clostridium XI*	64
14	FecIF41	*Clostridium XI*	100
15	FecIF42	*Clostridium XI*	100
16	FecIF46	*Clostridium XI*	100
17	FecIF60	*Coriobacterineae*	72
18	FecIF43	*Escherichia/Shigella*	100
19	FecIF55	*Escherichia/Shigella*	100
20	FecIF58	*Escherichia/Shigella*	100
21	FecIF62	*Escherichia/Shigella*	100
22	FecIF80	*Escherichia/Shigella*	100
23	FecIF92	*Escherichia/Shigella*	100
25	FecIF29	*Oscillibacter*	35
26	FecIF36	*Oscillibacter*	21
27	FecIF95	*Oscillibacter*	93
28	FecIF90	*Papillibacter*	32
29	FecIF86	*Prevotella*	95
30	FecIF96	*Prevotella*	99
31	FecIF3	*Prolixibacter*	10
32	FecIF32	*Rikenella*	46
33	FecIF33	*Rikenella*	46
34	FecIF58	*Rikenella*	34
35	FecIF61	*Rikenella*	22
36	FecIF64	*Rikenella*	72
37	FecIF63	*Roseburia*	39
38	FecIF1	*Ruminococcus*	79
39	FecIF82	*Ruminococcus*	100
40	FecIF83	*Subdivision5_genera_incertae_sedis*	70
41	FecIF12	*Tannerella*	63
42	FecIF14	*Tannerella*	68
43	FecIF47	*Treponema*	94

Table S6: Bacterial genera identified in river sludge near the dumpsite

S/N	Sample	Genera	% ID
210	Riv137	*Bacillus*	99
211	Riv138	*Bacillus*	100
212	Riv1	*Bacillus*	100
213	Riv2	*Bacillus*	100
215	Riv105	*Escherichia/Shigella*	99
216	Riv4	*Escherichia/Shigella*	97
217	Riv5	*Lysinibacillus*	59
218	Riv6	*Lysinibacillus*	100
219	Riv106	*Obesumbacterium*	17