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Full Length Research Paper

Biofertilising, plant-stimulating and biocontrol potentials of maize plant growth promoting rhizobacteria isolated in central and northern Benin

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Plants constantly interact with a multitude of microorganisms that they select among other things through their roots. Some bacteria, known as plant growth promoting rhizobacteria (PGPR), are able to stimulate growth and control plant diseases, thanks to the expression of a wide range of beneficial properties to the plant. The aim of this work was to search for biofertilizing, plant-stimulating and biocontrol potentials in PGPR in central and northern Benin. To achieve this goal, the metabolic properties, especially phosphate solubilization, the production of indole acetic acid, hydrogen cyanide, ammonia, exopolysaccharides, certain enzymes and antifungal activity were investigated on nine rhizobacteria strains: *Bacillus polymysa, Bacillus anthracis, Bacillus circulans, Bacillus thuringiensis, Bacillus panthothenicus, Pseudomonas cichorii, Pseudomonas putida, Pseudomonas syringae* and *Serratia marcescens*. The results reveal that the three genera of rhizobacteria were producers of hydrogen cyanide, indole acetic acid, catalase and solubilized phosphate. All *Pseudomonas* and *Serratia* isolates were producers of exopolysaccharides, protease and lipase while 80% of *Bacillus* strains were lipase producers and 60% produced exopolysaccharides and protease. As regards the production of ammonia by rhizobacteria, 100% by *S. marcescens*, 78% of *Pseudomonas* strains and 80% of *Bacillus* strains produce them. These results show the possibility of using these rhizobacteria as biological fertilizers to stimulate growth, control fungal diseases and improve crop productivity in Benin.

Key words: Rhizobacteria, Bacillus, Pseudomonas, Serratia, enzyme production, P-solubilizing bacteria, indole acetic acid (IAA).

INTRODUCTION

After a long dependence on plant protection products and synthetic fertilizers, today's global agriculture is hit by a current trend that favors more sustainable and more environmentally friendly practices. To meet these New demands, farmers need to turn to the exploitation and profitability of natural resources through agricultural practices that combine performance and crop protection at a lower environmental cost.

In this context, inoculation of plants with rhizobacteria is a very popular technology in organic farming. This biotechnology is gaining attention and is seen as an alternative to reducing the amounts of mineral fertilizers that are used without affecting crop yields; and therefore a component to be assessed in integrated management strategies in agriculture (Adesemoye et al., 2009).

Indeed, plant growth promoting rhizobacteria (PGPR) can increase plant growth. Their secondary metabolites present an unavoidable source of various compounds, involved in several fields: industrial, medical and agricultural (Donadio et al., 2002). Rhizobacteria beneficially influence the plant by stimulating its growth (direct pathway) and/or by protecting it against infections of phytopathogenic agents (indirect pathway) (El Houda, 2011). Directly, they promote the growth of plants by producing phytohormones (auxins and cytokinins), increasing nutrient absorption (phosphates solubilization, fixation of atmospheric nitrogen) and they also promote tolerance to salt stress and drought (Antoun, 2013). Indirectly, they can increase plant growth by inducing resistance in plants, producing anti-pathogenic molecules. They are also able to compete for space and nutrients (Antoun, 2013).

It is in the light of all the above that we undertook to search for biofertilizing, plant-stimulating and biocontrol potentials in PGPR in central and northern Benin, in order to select the best performing ones for the formulation of bio-fertilizer as a substitute for synthetic fertilizers, to overcome the problems of declining fertility and environmental pollution, while improving the productivity of crop.

MATERIALS AND METHODS

The present work has been performed *in vitro* at the laboratory of biology and molecular typing in microbiology. Nine rhizobacteria strains isolated from the maize rhizosphere of different agroecological zones of central and northern Benin by Agbodjato et al. (2015): *Bacillus polymyxa*, *Bacillus panthothenicus*, *Bacillus anthracis*, *Bacillus thuringiensis*, *Bacillus circulans*, *Pseudomonas cichorii*, *Pseudomonas putida*, *Pseudomonas syringae* and *Serratia marcescens* were characterized.

Four reference fungal strains: Aspergillus parasiticus (CMBB 20), Aspergillus ochraceus (CMBB 91), Aspergillus fumigatus (CMBB 89) and Aspergillus clavatus (NCPT 97); known for their phytopathogenic power were employed for the evaluation of antifungal activity.

Characterization of the isolated rhizobacteria strains

Phosphate solubilization

The phosphate solubilization was studied on Pikovskaya solid medium (PVK). The medium was divided into petri dishesandseeded by the disc method. The incubation was performed at 28°C for five days, according to Jang (2006) and Harrison et al. (1972). The development of a transparent halo around the colony reflects a positive result. The experiences have been achieved in three repetitions. The solubilization index (SI) was calculated using the following formula used by Shakeela et al. (2017):

$$\text{Solubilization index (\%)} = \frac{\text{Diameter of the halo(mm)}\pm\text{Diameter of the colony (mm)}}{\text{Diameter of the colony(mm)}} \times 100$$

Indole acetic acid production (IAA)

The production of IAA was demonstrated on Luria Bertani liquid medium (LB) supplemented with 0.1% L-tryptophan. Bacterial colonies were cultured on the medium and then incubated at 28°C. After 48 h, 1 mL was recovered by centrifugation of samples at 10000 rpm for 10 min. The obtained supernatant was mixed with the same volume of Salkowski's reagent (1 mL of 0.5 mol/L iron chloride III ($FeCl_3$) and 49 mL of perchloric acid ($HClO_4$) at 35%) according to Gumiere et al. (2014) and Patten and Glick (1996). The appearance of a pink color after 10 to 30 min reveals the production of IAA. According to the color intensity, three point scale (+ = low intensity; ++ = Average intensity and +++ = high intensity) were used for analyze the results.

Production of exopolysaccharides (EPS)

The production of EPS was investigated using the method described by Leveau et al. (1991). The rhizobacteria strains were streaked onto agar hypersaccharosed cast in a Petri dish. After incubation at 37°C for 24 h. The production of exopolysaccharides was manifested by the appearance of large and sticky colonies.

Production of compounds with antibiotic effect

Volatiles (hydrogen cyanide, ammonia)

The production of hydrogen cyanide (HCN) was demonstrated bythe method described by Lorck (1948). On agar nutrient containing glycine (4.4 g/L), cast in a Petri dish, bacterial streaks were performed. The agar was covered with a Wattman No.1 filter paper previously soaked in a 2% sodium carbonate and 0.5% picric acid solution. The Petri dish was then sealed with paraffin paper and incubated at 36 ± 2°C for four days. The appearance of an orange or red color indicates the production of HCN.

The production of ammonia (NH3) was investigated according to the method of Cappuccino and Sherman (1992). Fresh bacterial colonies were cultured in 10 mL of peptone water and incubated at36 ± 2°C for a period of time between 48 and 72 h. After incubation, 0.5 mL of Nessler's reagent was added. The tum from brown to yellow indicates the production of ammonia.

Antifungal activity of the characterized rhizobacteria

The antifungal activity of rhizobacteria tested was evaluated by the double cropping method described by Kumar et al. (2002) on four

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Table 1. Average solubilization index of PGPR isolates.

Rhizobacteria	Solubilization index (%)	
	m	Cv
Bacillus ***polymysa***	263.28[f]	9.70
Bacillus ***anthracis***	258.43[f]	2.68
Bacillus ***circulans***	260.65[f]	1.07
Bacillus ***thuringiensis***	282.17[e]	1.42
Bacillus ***panthothenicus***	251.90[f]	10.09
Pseudomonas ***cichorii***	440.16[c]	1.71
Pseudomonas ***putida***	483.89[a]	0.73
Pseudomonas ***syringae***	470.24[b]	4.85
Serratia;***marcescens***	366.93[d]	4.19
Probability	0.000	
Meaning	***	

*** = p < 0.001 (very highly significant). Averages with the same letters in the same column are not significantly different. m = average; cv = coefficient of variation.

phytopathogenic fungal strains: *A. parasiticus* (CMBB 20), *A. ochraceus* (CMBB 91), *A. fumigatus* (CMBB 89) and *A. clavatus* (NCPT 97). A disk (5 mm diameter) was cut out from a young culture of the fungal pathogen and placed in the middle of a Petri dish of Potato Dextrose Agar (PDA). Ten microliter of a rhizobacteria suspension about 10^8 CFU/mL were spotted at approximately 2 cm from and opposite sides of the fungus infected disk. The control of the Petri dishes were realized by monoculture of each pathogen. The Petri dishes were then incubated at 26 ± 1°C and checked for zones of inhibition of mycelium growth after seven days when the fungal mycelium had reached the edge of the Petri dish. The experiences have been achieved in three repetitions. When the pathogen grew over the rhizobacteria, we concluded that the rhizobacteria did not have antifungal activity. On the other hand, when fungal growth was restricted by the rhizobacteria, the rhizobacteria's antifungal activity (percentage of inhibition) was calculated by the following formula used by Noumavo et al. (2015):

$$\% \text{ growth inhibition } = \frac{d1 - d2}{d1} \times 100$$

Where: d1 = Growth diameter of the pathogen in monoculture (control) andd2 = Growth diameter of the pathogen in dual culture.

Enzyme productionby the rhizobacteria tested

The production of catalase was sought according to the method described by Riegel et al. (2006). With a sterile Pasteur pipette, a bacterial colony was dispersed in a drop of hydrogen peroxide previously deposited on a clean dry slide. The positive reaction is shown by an immediate release of oxygen bubbles forming a foam solution.

Proteolytic activity was determined according to the Smibert and Krieg (1994) method by culturing under incubation for two days at 28°C of the isolated agar skimmed milk. The development of a transparent halo around colonies indicates a positive reaction (Naik and Sakthivel, 2006). The lipase investigation was carried out according to the method described by de Groot et al (1991), by culturing the isolates on nutrient agar supplemented with 1% oleic acid. A positive reaction is characterized by the appearance of a halo around the colonies.

Statistical analysis

To determine the most solubilizing rhizobacteria and the rhyzobacteria that inhibited more the growth of each mold category, an analysis of variance followed by the student Newman and Keuls test was performed on solubilization index and inhibition rate values of the growth of the molds for all the replicates of the different rhizobacteria. In addition, to determine metabolites production levels per rhyzobacteria and specific groups with similar or opposite production levels or of metabolites, a dataset containing rhyzobacteria on lines, metabolites and enzymzes in columns and the corresponding values established and then subjected to principal component analysis (PCA) in R statistical software with the FactoMineR package (R Core Team 2016).

RESULTS AND DISCUSSION

Phosphate solubilization

One of the important properties of PGPR that is directly influencing plant growth is the solubilization of phosphate. Phosphorous (P), an essential nutrient element is the second most important element after nitrogen. In the soil, phosphate usually exists in insoluble forms, which reduces its availability for plants (Satyaprakash et al., 2017). This is the reason why a bacterium that has a phosphate solubilizing power is important.

In the present study, the nine isolates tested were able to solubilize phosphate. The solubilization index (SI) by its variance analysis results showed that there was a very high significant difference (P <0.001) between the solubilization capacities of the different strains (Table 1). Where the three isolates of *Pseudomonas* was very effective compared to the others, with an SI from 440.16 to 483.89%. *Bacillus* strains presented the lowest solubilizing efficiencies; SI g from 251.90 to 282.17%. As for *S. marcescens* strains was moderately effective with an SI of 366.93%. The good solubilizing efficiency of

Table 2. Estimation of the production of some metabolites by the nine rhizobacteria

Rhizobacteria	Qualitative production of metabolites			
	IAA	EPS	HCN	NH_3
Bacillus polymysa	+++	+	+	+
Bacillus anthra	+	-	++	++
Bacillus circulans	+	-	++	++
Bacillus thuringiensis	++	+	+++	++
Bacillus panthothenicus	+++	+	+	-
Pseudomonas cichorii	+++	++	+++	+
Pseudomonas putida	+++	++	+++	+++
Pseudomonas syringae	+++	++	+	-
Pseudomonas marcescens	++	+++	++	+++

NH_3: Ammonia; HCN: Hydrogen cyanide; IAA: Indole Acetic Acid; EPS: Exopolysaccharides; + = Low; ++ = average; +++ = Strong, - = no production.

Pseudomonas obtained in our study was also reported in the works done by Sulbaran et al. (2008) and Chibani (2017). Messele and Pant (2012) observed improved biological yield and P uptake in chickpea (*Cicer arietinum* L.) by phosphate-solubilizing *Pseudomonas*. In addition, the evaluation of the solubilizing activity by an Iranian team in different Rhizobium strains revealed an SI ranging from 141 to 248% (Alikhani et al., 2006). Rajkumar et al. (2006) indicated that phosphate solubilization by *Pseudomonas* sp. PSA4 and *Bacillus* sp. BA32 stimulated the proliferation of plant roots and improved the absorption of soil minerals such as iron and phosphate by the host plant. Yazdani et al. (2009) explained that the application of phosphate solubilizing bacteria can reduce phosphorus application to 50% without affecting the yield maize seeds. Exploitation of the phosphate solubilizing bacteria as bio inoculants will increase the available P in the soil; therefore, it will minimize the application of P fertilizers, and so, reduce environmental pollution and promote sustainable agriculture (Zennouhi et al., 2018)

Indole acetic acid production

The synthesis of plant growth hormones, of which IAA is the most effective, is a very common phenomenon with root exudates of rhizosphere bacteria. All tested strains were found to produce IAA at varying rates. The highest yields were observed in all *Pseudomonas* and some of *Bacillus* strains; while mean productions were observed in *B. thuringiensis* and *S. marcescens* and finally the lowest productions in *B. anthracis* and *B. circulans* (Table 2). The production of IAA by PGPR depends on species and strains and is also influenced by the culture conditions, stage of development and availability of substrates in the rhizosphere (Ashrafuzzaman et al., 2009). Indiragandhi et al. (2008) showed that strains of *Serratia* spp. PRGB11 are capable of producing IAA. As far as Cherif (2014) is concerned, he showed in his works that some *Bacillus* strains synthesize IAA. The work of Abbas et al. (2018) showed that all five selected endophytic bacterial strains produced indole acetic acid. Indeed, IAA functions as an important signal molecule in the regulation of plant development, acting on organogenesis, trophic responses, cellular responses such as cell expansion, division, differentiation and regulation of cells (Ryu and Patten, 2008; Mezaache, 2012). As a result, rhizobacterial IAA is identified as an effector molecule in plant–microbe interactions, both in pathogenesis and phytostimulation (Spaepen and Vanderleyden, 2011).

Production of exopolysaccharides (EPS)

With the exception of *B. circulans* and *B. anthracis*, all strains tested were found to produce EPS at variable rates (Table 2). The absence of production in some strain can be explained by the fact that the tested rhizobacteria does not have the gene producing the exopolysaccharides. Previous studies showed that bacterial EPS under salt stress can bind sodium ions and reduces its toxicity in the soil (Arora et al., 2010). EPS bind to Na^+ cations and, in particular, decrease its content, thereby helping to reduce salt stress in plants (Ashraf et al., 2004). EPS-producing PGPR strains induce tolerance to soil salinity, promote the growth of soybean (*Glycine maxima*) plants (Bezzate et al., 2000) and limit the uptake of Na^+ by wheat roots (Ashraf et al., 2004). Sandhya et al. (2009) argue that EPS participate in the formation of bacterial aggregates and consequently improve soil aeration, water infiltration and root growth. In salt stress condition, the EPS chelate cations available in the root zone, thus contributing to reduce the salinity of the rhizosphere. The bacterial EPS in conditions of water

Table 3. Estimation of the production of some enzyme by the nine rhizobacteria.

Rhizobacteria	Qualitative production of enzyme		
	Cat	Pro	Lip
Bacillus polymysa	+	-	+
Bacillus anthra	+	++	+
Bacillus circulans	+	+	+
Bacillus thuringiensis	+	-	-
Bacillus panthothenicus	+	++	+
Pseudomonas cichorii	+	++	+++
Pseudomonas putida	+	+	+++
Pseudomonas syringae	+	+	+++
Pseudomonas marcescens	+	+++	++

Cat: catalase, Prot: protease; Lip: lipase; + = Low; ++ = average; +++ = Strong, - = no production.

stress in the soil can limit or delay the middle of desiccation (Heulin and Achouak, 2012). Conversely, in case of excess water (rain, floods), EPS contribute to avoid dispersion of soils clayey (Henao and Mazeau, 2009). According to many authors, PGPRs producing EPS have a selective advantage over other bacteria during biotic stress (Wang et al., 2000) and abiotic stress (Mayak et al., 2004).

Production of compounds with antibiotic effect

Volatile substances are also involved in the suppression of different pathogens. Hydrocyanic acid is one of those metabolites synthesized by certain rhizobacteria as a means of avoiding predation or competition (Heydari et al., 2008). All strains produced HCN at varying rates where *B. thuringiensis, P. cichorii* and *P. putida* strains recorded the highest yields; *B. anthracis, B. circulans,* and *S. marcescens* produced moderate HCN, and *B. polymysa, B. panthothenicus* and *P. syringae* produced only a small amount (Table 3). Contrary to the work done by Cherif, (2014), this work has shown that the majority of these isolated strains do not have the capacity to produce HCN. HCN production is a common activity in *Bacillus* (50%) in rhizosphere soils (Ahmad et al., 2008). *Pseudomonas* strains producing HCN are used in biological control against bacterial canker of tomato (Lanteigne et al., 2012). HCN production by the *P. fluorescens* CHA0 strain reduces the pathogenicity of fungi such as *Thielaviopsis basicola*, a black rot agent in tobacco (Mercado-Blanco and Bakker, 2007).

Another secondary metabolite produced by some rhizobacteria which indirectly influences plant growth is ammonia (NH_3). The results obtained in our study show that *Pseudomonas* and *Bacillus* isolates and *S. marcescens* produced ammonia at 78, 80, and 100%, respectively. Strains of *P. putida* and *S. marcescens* produced high amounts of ammonia, whereas *B. anthracis, B. circulans* and *B. thuringiensis* recorded average ammonia production. In contrast to *P. cichorii* and *P. syringae*, which had low ammonia production, *B. polymysa* and *B. panthothenicus* did not produce ammonia (Table 2). These ammonia production rates are lower than the 95% and 94% obtained by *Bacillus* and *Pseudomonas* isolates respectively was agree in the work done by Joseph et al. (2007). Yadav et al. (2010) where they also found higher rates of ammonia production by *Bacillus* spp. and by *Pseudomonas* spp on chickpea (*Cicer arietinum*) in India.

Antifungal activity

Regarding to the results of variance analysis on inhibition levels of mycelial growth by the rhizobacteria tested, there were showed highly ($p<0.01$) or extremely significant ($p<0.001$) differences between the rhizobacteria (Table 4) for all the reference molds. With the exception of *P. cichorii*, the other *Pseudomonas* isolates did not express their potentiality to inhibit fongus growth. For of *Serratia* and *Bacillus* strains, the inhibitory activity was on the other hand very remarkable. Indeed, the rhizobacteria *B. polymysa*, *B. panthothenicus* and *S. marcescens* were the ones which showed more inhibitory effect on the mycelial growth of *A. parasiticus* at average inhibition rates of 75.55, 73.33 and 70.73% respectively. *B. anthracis* had the lowest average rate of inhibition (60%). For the *A. ochraceus* mold, *B. panthothenicus*, *B. anthracis* and *B. circulans* rhizobacteria strongly inhibited mycelial growth with mean inhibition rates of 70.50, 68.85 and 67.14% respectively and were significantly different. *P. cichorii* is the rhizobacterium that showed the least inhibitory effect on the growth of *A. ochraceus* with an average rate of 58.57%. As for the *A. fumigatus and A. clavatus* molds, the *B. polymysa* rhizobacteria followed by *B. panthothenicus* showed more inhibitory effect on their mycelial growth. The results also show that *B.*

Table 4. Antifungal activity of rhizobacteria on fungal plant pathogens.

Rhizobacteria	Inhibition of mycelial growth (%)							
	***A. parasiticus* CMBB 20**		***A. ochraceus* CMBB 91**		***A. fumigatus* CMBB 89**		***A. clavatus* NCPT 97**	
	cv	**cv**	**m**	**cv**	**m**	**cv**	**m**	**cv**
Bacillus polymysa	75.55^{a}	6.02	66.00^{abc}	7.39	68.79^{a}	3.83	70.71^{a}	1.01
Bacillus anthracis	60.00^{d}	1.86	68.85^{ab}	2.28	-	-	-	-
Bacillus circulans	68.33^{bc}	2.44	67.14^{ab}	2.13	-	-	-	-
Bacillus thuringiensis	65.00^{cd}	2.56	63.14^{abc}	4.07	-	-	-	-
Bacillus panthothenicus	73.33^{ab}	3.68	70.50^{a}	1.32	57.86^{b}	10.97	68.57^{a}	3.08
Pseudomonas cichorii	63.89^{cd}	4.35	58.57^{c}	4.28	52.86^{b}	7.02	53.57^{b}	3.87
Pseudomonas putida	-	-	-	-	-	-	-	-
Pseudomonas syringae	-	-	-	-	-	-	-	-
Serratia marcescens	70.73^{ab}	1.93	60.35^{bc}	10.14	54.39^{b}	7.17	55.65^{b}	6.65
Probability	0.000		0.005		0.008		0.000	
Meaning	***		**		**		***	

** = p < 0.01 (highly significant); *** = p < 0.001 (very highly significant). Averages with the same letters in the same column are not significantly different. - = no inhibition, m= average; cv= coefficient of variation.

polymysa had the highest inhibitory effect on the mycelial growth of all molds. No antagonism was observed between the rhizobacteria *P. putida and P. syringae* and the four fungal strains. It is also to be noted that all the rhizobacteria strains that showed antagonistic effect to *A. parasiticus* also had same effect on *A. ochraceus*. Saranya and Sowndaram (2014) revealed a complete inhibition of mycelia growth of *Rhizotonia solani* (85%) and partial inhibition of *Sarocladium oryzae* (45%) against two rhizobacteria as Singh et al. (2017), whose showed in their results that *Bacillus* spp. possess antifungal activity against the spore forming fungi of *Alternaria* spp., *Fusarium* spp., *Bipolaris* spp. and *P. fluorescens* did not show any inhibition against the spores of the phytopathogenic fungi.

Hydrolytic enzyme production

Some PGPR strains have the ability to degrade fungal cell walls through the production of hydrolytic enzymes. All the strains tested produced catalase. Protease production was high in *S. marcescens,* while a medium production was recorded in *P. cichorii, B. panthothenicus* and *B. anthracis*. The *B. polymysa* and *B. thuringiensis* strains did not produce the protease. All strains of *Pseudomonas* had a high lipase production, while this production was average in *S. marcescens* and it was low in *Bacillus* strains with the exception of *B. thuringiensis* which did not produce lipase (Table 3). Similar results were observed by Noumavo et al. (2015) as well as Preeti et al. (2012) on the production of catalase. The bacterial strains producing catalase are potentially very advantageous because of their resistance to environmental, mechanical and chemical stresses (Preeti et al., 2012). *Bacillus* species are catalase positive and able to form endospores that allow them to survive for long periods under adverse environmental conditions (Alizadeh and Ordookhani, 2011).

Relationship between the different metabolites and the nine tested rhizobacteria

Estimation of the production of different metabolites by rhizobacteria was analyzed through a principal component analysis (Figure 1). The main results obtained indicated that the first two main components account at 65.04% for all the variability linked to the production of metabolites by rhizobacteria. The results showed that the different rhizobacteria do not have the same production potential of the metabolites evaluated. The projection of metabolites and rhizobacteria in the factorial axis plane formed by the two main components shows that *P. cichorii, P. syringae* and *P. putida* solubilized more phosphate, produced more indole acetic acid, exopolysaccharides, hydrogen cyanide, ammonia and lipase In contrast *B. anthracis, B. circulans, B. thuringiensis, B. panthothenicus* and *B. polymysa* have a strong antifungal activity (AAF) and produce less ammonia, hydrogen cyanide indole acetic acid, exopolysaccharides, lipase and solubilize less phosphate.*S. marcescens* produced more proteases, exopolysaccharides and ammonia with strong antifungal activity (AAF) and in contrast *B. polymysa B. circulans* and *P. syringae* produce more indole acetic acid and vice

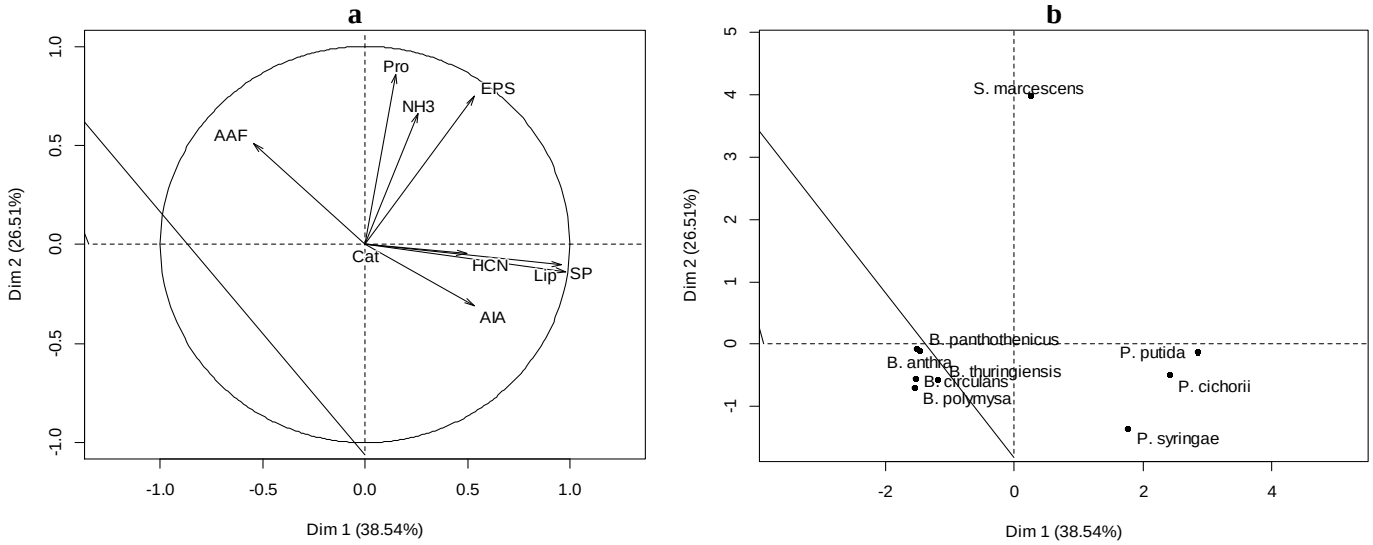

Figure 1. (a): Projection of metabolites in the correlation circle formed by the first two major components; (b): Projection of rhizobacteria in the axis plane formed by the first two principal components.
NH_3: ammonia ; HCN : Hydrogen cyanide; AIA : Indole Acetic Acid; EPS : Exopolysaccharides ; SP : Phosphate solubilization, Cat : catalase, Prot : protease ; Lip : lipase ; AAF : Antifungal activity. *B*.= *Bacillus* ; *P*.= *Pseudomonas* ; *S*.= *Serratia*.

versa. In addition, all rhizobacteria produced catalase in high proportion.

The results of this study showed that isolates were heterogeneous; some strains have remarkable phytostimulatory and biofertilising capacities. Indeed, the solubilization of phosphates and the simultaneous production of IAA has been demonstrated (Weller and Thomashow, 1994). While others had exclusive biocontrol abilities (antifungal activity). According to many authors, a correlation exists between these different activities (Mehta et al, 2010).

Conclusion

The responses of isolated bacteria to the various tests inherent to the promotion of plant growth allow the demonstration of the natural potentialities of each strain. The present study is interested in determining properties with direct beneficial effects on plants through the provision of nutrients or indirect mechanisms by way of protection against plant pathogens. Indeed, the search for pro-PGPR properties on the different rhizobacteria under study, revealed that all *Pseudomonas* species solubilized more phosphate, produced more Indole acetic acid, exopolysaccharides, hydrogen cyanide, ammonia and lipase, while all species of *Bacillus* had a strong antifungal activity, As *S. marcescens* produced more proteases, these results could exploit these PGPR strains as beneficial multi-effect bioinoculants to increase productivity and improve plant and soil health, which will likely reduce the problems associated with the use of toxic fertilizers in agricultural practices.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Antimicrobial resistance patterns of *Enterobacteriaceae* recovered from wastewater, sludge and dumpsite environments in Kakamega town, Kenya

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Enteric bacterial resistance to antibiotics and the emergence of resistant pathogens in the environment is a global threat to public health. In Kenya, sewage treatment plants are not designed to eliminate enteric microbes, whereas domestic, medical and other hazardous wastes are all discarded in common solid-waste dump sites. Arising from these practices, waste treatment sites in developing countries may be important selection sites for antimicrobial resistant microbes. This study, present information on the levels and patterns of antimicrobial resistance among members of the *Enterobacteriaceae* family, in dump sites and sewage treatment plants in Kakamega town, Kenya. We employed Standard Microbiological Methods to recover microbes and identify enteric bacteria. Disc diffusion was used in performing the susceptibility profiling of the enteric bacteria identified. Members of *Enterobacter, Citrobacter, Klebsiella, Escherichia, Serratia, Shigella* and *Proteus* were recovered at varied rates, with *Enterobacter* recording the highest occurrence at 37.14% in dumpsites, where *E.coli* recorded the highest occurence at 36.11 and 38.71% in wastewater and sludge, respectively. *Serratia, Shigella* and *Proteus* species were the least recovery (n < 2). Amoxicillin recorded the highest level of resistance at 72%, Cephalexin at 54%, while Amikacin showed the smallest level of antibiotic resistance at 2%. The highest multi-drug resistance was 9 out of 10 different antibiotics tested. All antibiotics tested except for Sxt-Amc and Cip-C showed positive correlations ($p<0.05$) with bacteria resistant to amoxicillin, being more resistant to other antibiotic with a percentage relative resistance frequencies ranging from 64 to 100%. Finding show high levels of multiple antibiotic resistance commonly used as antibiotics. The study further depicts waste treatment sites in tropics, as important sites for recruitment of antibiotic resistance traits.

Key words: Antibiotic resistance, *Enterobacteriaceae*, wastewater, dumpsite.

INTRODUCTION

Enteric bacterial resistance to antibiotics and the emergence of resistant pathogens in the environment is a threat to public health and of great global concern. The resistance develops when bacteria are not affected by antibiotics that previously inhibited or destroyed them and cured infection. The emergence and persistence of antimicrobial resistance is driven by varied factors including the indiscriminate use of antibiotics and variable drug efficacy, which presents a major threat to the control of infectious diseases (Omulo et al., 2015). Infections

caused by resistant bacteria result in illnesses, lasting for longer periods as well as higher mortality rates. Antibiotic resistances in bacteria have increased risk of spreading infection in patients which incur higher costs as, seek alternative antibiotic treatment. The rapid emergence of antimicrobial resistance by microbial pathogens, threatens to reverse the public health gains made since widespread use of antibiotics was adopted (Saga and Yamaguchi, 2009).

The emergence of antibiotic resistant pathogens and especially the enteric type in the environment, is a threat to public health and of great global concern (Kümmerer, 2009a). In developing countries, information on antibiotic resistance in the environment is least understood. The emergence and persistence of antimicrobial resistance is driven by varied factors such as the indiscriminate use of antibiotics (Omulo et al., 2015), the overuse and misuse of these antibiotics exposures microbes to antimicrobial compounds in their environment, leading to the emergence of antibiotic resistant strains (Houndt and Ochman, 2000). This phenomenon therefore presents a major threat to the control of infectious diseases, as this may lead to an increased number of treatment failures, morbidity and mortality of infectious diseases.

In Kenya, several studies have reported incidences of resistance to antimicrobials in clinical cases (Muhonja, 2012; Sang et al., 2012; Juma et al., 2015; County et al., 2016). Kakamega County, the second most populous in Kenya (KNBS, 2010) has reported high incidences of multidrug resistant diarrhoea, causing microbes that have been implicated in a number of the outbreaks (DREF Annual Report, 2009; Muhonja et al., 2012). However, there is little or no information on the prevalence and antibiotic resistance patterns, displayed by these enteric bacteria in the environment at waste deposit sites in Kenya. Sewage treatment, a process aimed at removing contaminants from wastewater includes physical, chemical, and biological processes which result in production of environmentally safer treated wastewater or treated effluent.

In the developed world, several studies have reported the presence of antibiotics in wastewater (Ferreira et al., 2007; Kümmerer, 2009b; Martinez, 2009a) and the commonly used three-step treatment process of wastewater treatment plants is, insufficient to remove all the pharmaceutical residues (Al-Bahry et al., 2014). This therefore, makes waste treatment sites environmental hotspots, for the development and spread of antibiotic resistance (Boyle, 2013). Antibiotic resistance in such environments has previously been reported to develop, through direct transmission of already resistant strains and/or via environmental matrices such as manure in soils of the farm and exposure of microorganisms to low concentrations of antibiotics which eventually selects for resistance (Achudume and Olawale, 2007; Ikpeme et al., 2011; Boyle, 2013). The availability of variety of pharmaceuticals in waste treatment sites has been shown to create selection pressure on microbes and generation of resistant strains (Mwaikono et al., 2015) therefore, if waste treatment sites are poorly managed they may pose a great danger to human health (Mwaikono et al., 2015; Karak et al., 2012).

Nonetheless, the continued lack of knowledge on the pathogenic microbes especially the enteric type occurring in waste treatment sites in developing countries deprives communities living around these sites or eking a living from these sites an understanding of occupational health risks that may be associated with the sites. Furthermore the limited information on characteristics of waste treatment site microbes delinks clinical management of enteric infections from the effects and outcomes on the environment, in developing countries. This is because, solid wastes present in landfills and waste water treatment sites have been found to contain pharmaceutical residues emerging from domestic and biomedical wastes, favouring bacterial changes resulting from complex interaction of diverse microbes in the waste treatment sites (Ferreira et al., 2007; Adekanle et al., 2014; Mwaikono et al., 2015). Therefore, an understanding of antibiotic resistance patterns occurring in the environment presents a good representation of emerging antibiotic resistance patterns, which may occur in the environment but may have not been reported in clinical situations.

The objective of this study therefore was to establish members of the *Enterobacterieceae* family, occurring in waste treatment sites namely dumpsites and wastewater treatment plants in Kakamega town; and further understanding the antibiotic susceptibility levels of these microbes against ten commonly used antibiotics representing the various groups of antibiotics; Tetracycline (tetracyclines), Amoxicillin (penicillins), Cephalexin and ceftriaxone (cephalosporins), Co-amoxiclav (beta-lactamase inhibitors), Amikacin and Gentamicin (Aminoglycosides), Chloramphecol (phenicols), Ciprofloxacin (Fluoroquinolons), and Sulphamethoxazole-Trimethoprin (Sulphonamides) used to treat diarrheal and respiratory infections locally (Christabel et al., 2012; Omulo et al., 2015).

MATERIALS AND METHODS

Study area and study design

This study was conducted in Kakamega town, in Western Kenya. Soil recovered from dumpsite, sludge recovered from wastewater

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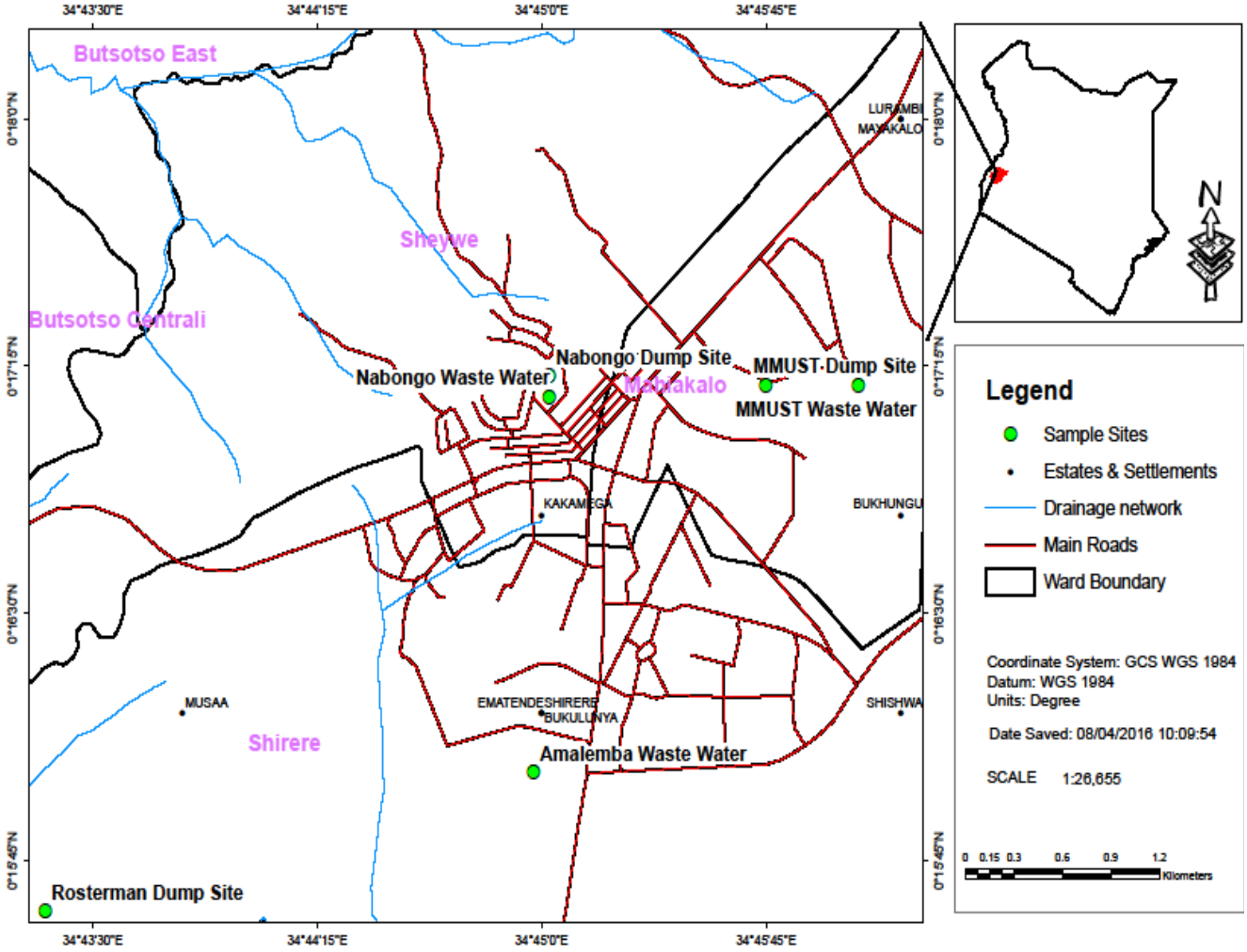

Figure 1. Map of study area.

treatment plants and water samples recovered from wastewater treatment plants were collected from three dumpsites and three waste waters namely Rosterman dumpsite, Nabongo dumpsite, Masinde Muliro University and Technology (MMUST) dumpsite, Amalemba wastewater, Nabongo waste water and MMUST wastewater as shown in Figure 1.

The study was based on a repeat cross–sectional study design. The samples were collected from April to October, 2015.

Geographical Information System (GIS), mapping coordinates

Rosterman dumpsite
133 36 N: 0691850- Longitude
0028313- Latitude

Amalemba Wastewater Treatment Plant
136 36 N: 0694603-longitude
0029431-latitude

Nabongo dumpsite
138 36N 0694552-longitude
0031624-latitude

Nabongo wastewater treatment plant
139 36N 0694405-longitude
0031624-latitude

Masinde Muliro University (MMUST) Dumpsite
140 36N 0696200-longitude
0031691-latitude

Masinde Muliro University (MMUST) Wastewater treatment plant
141 36N 0696511-longitude
0031634-latitude

Sample collection and processing

A total of 60 samples (24 soils recovered from dumpsites, 12 sludge recovered from wastewater and 24 wastewater recovered from wastewater treatment plants) were collected during the study period. Sampling at the dumpsites adopted the random multistage sampling.

Soil samples were collected aseptically using a sterilized spoon at each point while wastewater was collected aseptically using a

Table 1. Levels (%) of members of *Enterobacteriaceae* recovered from wastewater, sludge and dumpsite soils**.**

Bacterial Isolates	Recovery (%)		
	Dumpsite (n=35)	Wastewater (n=36)	Sludge (n=31)
Enterobacter	37	12	16
Citrobacter	29	11	32
E.coli	23	36	39
Klebsiella	6	31	13
Serratia	3	3	nil
Proteus	3	nil	nil
Shigella	nil	3	nil

bottle fitted with a handle. On the other hand, samples from the sludge were collected aseptically using a spoon filled with a handle. All the samples were transported on ice in insulated containers to Masinde Muliro University of Science and Technology, Microbiology laboratory for analysis.

Isolation of enteric bacteria

Soils recovered from dumpsites and sludge recovered from wastewater was processed as described by van and Smalla (1997). This was done by transferring 2 spoonfuls of soil and sludge into a pre-sterilized Whirl Pak bag, 100 ml of sterile phosphate–buffered water was added and mixed for 2 min. The mixture was then filtered through a pre-sterilized 28 micron –pore size nylon filter (membrane solutions). The filtrate was then used in subsequent experiments.

To recover *Shigella ssp* and *Salmonella ssp*, 10 ml of sample (filtrate recovered from dump site soil or sludge; or wastewater) were enriched in 90 ml of selenite cystene (Himedia Lab. Pvt. Mumbai, India) and incubated at 37°C overnight. A loop full of the suspension was plated onto xylose lysine desoxycholate (XLD) (Himedia Lab. Pvt. Mumbai, India) and MacConkey agar (Himedia Lab. Pvt. Mumbai, India) plates and incubated at 37°C for 24 to 48 h. Three characteristic *Salmonella* and *Shigella* presumptive colonies were selected and preserved by plate on nutrient Agar (Himedia Lab. Pvt. Mumbai, India) subjected to further tests for identification.

Other enteric bacteria were recovered by direct plating on MacConkey agar (Himedia Lab. Pvt. Mumbai, India) and incubated at 37°C for 18 h. From each plate, 4 distinct colonies (2 non-lactose and 2 lactose fermenters) were picked and plated on nutrient agar (Himedia Lab. Pvt. Mumbai, India) for further identification, based on biochemical tests namely triple sugar iron (TSI) and Citrate utilization and Lysine decarboxylation and deamination, motility and indole tests, the bacteria were classified as described by Cheesbrough (2000). Confirmed isolates were then stored at -80°C in tryptic soy broth with 15% glycerol until used in other experiments.

Antimicrobial response tests (AST)

Susceptibility tests with ten antibiotics were performed using the standard Kirby-Bauer disk diffusion method on Muller Hinton media (HIMedia Lab. Pvt. Mumbai, India). The antimicrobials tested were; amoxicillin (30 µg), co-amoxiclav (30 µg), tetracycline (30 µg), sulphamethoxazole-trimethoprin (25 µg), chloromphenicol (50 µg), gentamicin (10 µg), ciprofloxacin (30 µg), cephalexin (30 µg), ceftriaxone (µg) and amikacin (30 µg) (all HIMedia Lab. Pvt. Mumbai, India).

Inoculated plates were incubated at 37°C for 18 to 20 h. Measurement of diameters (in millimetres) of clear zones growth inhibition, adopted the Clinical and Laboratory Standards Institute (CLSI) 2008) procedural method. A standard reference strain of *E. coli* (ATCC 25922) was used as a control. The breakpoints used to categorize isolates as resistant to each antimicrobial agent were those recommended by CLSI (2008).

Data analysis

Data were entered in Ms Excel Windows XP professional 2003 and analyzed by Minitab 13.1 Windows 95. Antimicrobial susceptibility test were recorded as diameters of the zone of inhibition (mm).

Bacterial recovery and antibiotic resistance were expressed in frequencies and percentages, while Kruskal-Wallis test was used to determine differences in zones of inhibition among bacterial species and sampling sites but t-tests and Pearsons' Correlation Analysis was used to determine relationships among the different antibiotics, displaying multiple resistances in the study.

RESULTS AND DISCUSSION

The study set out to establish members of the *Enterobacterieceae* family occurring in waste treatment sites in Kakamega town. Out of 102 isolates of *Enterobacteriaceae* recovered from dumpsite soils, wastewaters and sludge, only 7 genera namely *Enterobacter*, *Citrobacter*, *Escherichia*, *Klebsiella*, *Proteus*, *Serratia* and *Shigella* (Table 1) were recovered during the study period compared to 20 genera as described by Ewing (1986). The frequency and rates of recovery of the members of *Enterobacteriaceae* family in this study varied from one source to the other.

The distribution of enteric bacteria among the different sources investigated could been influenced by two sets of environmental factors, namely factors influencing the survival of bacteria in the external environment and the continuous re-introduction of new bacterial cells in to these environments. The natural life cycle of the enteric bacteria consists of transitions between two distinct habitats (Savageau, 2016), the animal hosts representing the primary habitat, and the environment exterior to the host such as soil, sediment and water representing the secondary habitat. However studies have shown that

Table 2. Levels antibiotic resistance (%) of members of *Enterobacteriaceae* recovered from wastewater, sludge and dumpsite soils.

Bacterial Isolates	Recovery (%)		
	Dumpsite (n=35)	Wastewater (n=36)	Sludge (n=31)
Enterobacter	100	100	100
Citrobacter	100	100	82
E.coli	65	57	80
Klebsiella	100	100	85
Serratia	100	100	nil
Proteus	100	nil	nil
Shigella	nil	90	nil

Table 3. The percentage frequencies of resistance observed among the members of *Enterobacterieceae* recovered from dumpsite, sludge and wastewater (n=102).

Resistance	Frequency (%)	Resistance	Frequency (%)
At least 1 antibiotic	99 (97.1)	2	72 (70.6)
3	49 (48)	4	25 (25
5	18 (18)	7	7 (7)
8	5 (5)	9	2 (2)

Table 4. Antibiotic resistance rates of *Enterobacterieceae* recovered from dumpsite, sludge and wastewater against individual antibiotics.

Bacterial isolates	Antibiotic resistance (%)									
	Te	Amc	Cn	Ctr	Ak	Amx	Gen	C	Cip	Sxt
Citrobacter (n=24)	4(17)	8(33)	16(67)	6(25)	2(1)	15(63)	3(13)	4(17)	2(1)	12(50)
E.coli (n=33)	2(6)	7(21)	7(21)	4(12)	1(3)	13(39)	1(3)	2(6)	4(12)	3(9)
Enterobacter (n=23)	4(17)	9(39)	12(36)	3(13)	1(4)	21(91)	1(4)	4(17)	1(4)	7(30)
Klebsiella (n=17)	2(12)	8(47)	10(59)	9(53)	0(0)	12(71)	1(6)	1(6)	2(12)	8(47)

bacteria have the capability of entering into a viable but nonculturable state (VBNC), when conditions are not favourable like enteric bacteria which may experience the secondary habitat (Martínez-Vaz et al., 2014). By entering VBNC, microbes have the capacity to survive hostile environments (Ramamurthy et al., 2014) but cannot be cutlured on standard growth media, these therefore explain why only 7 out of 20 possible genera of *Enterobacterieceae* family previously reported by Ewing (1986) could have been detected in this study. Nonetheless, the constant arrival of microorganisms from the primary habitat may continuously influences bacterial occurrence and survival in the secondary habitat, and therefore the coliforms reported in this study may have been sustained by the delivery of new coliforms from primary sources such as domestic waste from homes, animals and wildlife (Savageau, 2016).

The recovery of members of genera *Escherichia*, *Enterobacter*, *Klebsiella*, *Citrobacter*, *Proteus*, *Shigella* and *Serratia* directly points to these sites as high risk (Karak et al., 2012), and therefore sites investigated in this study may pose a public health risk if not put under appropriate management. The presence of these microbes in dumpsites has previously been shown to demonstrate the accumulation of unsorted garbage (Karak et al., 2012). According to Mwaikono et al. (2015) such dumpsites have shown to possess a variety of wastes ranging from domestic, biomedical and industrial wastes, a fact which was also observed at the dumpsite investigated in this study.

In this study, antibiotic resistance levels of up to 100% and 9 different antibiotics against the 10 tested is reported as shown in Tables 2, 3, and 4. The emergence, selection, and dissemination of resistant organisms have been reported to occur in areas where antibiotics have been heavily used such as human, veterinary and agriculture (Woolhouse et al., 2015; Martinez, 2009a). Such practices eventually cause large amounts of antibiotics to be released into municipal wastewater probably due to incomplete metabolism in human or due to disposal of unused antibiotics (Bouki et al., 2013; Birosova et al., 2015). Bacteria have shown the capability

Table 5. Antimicrobial resistance patterns displayed by members of *Enterobacterieceae* recovered from dumpsite, sludge and wastewater.

Resistance	Frequency (%)	Resistance	Frequency (%)
Amc	1.12	Amx	10.11
Cn	11.23	Sxt	3.37
Te	1.12	-	-
Amc-Amx	6.74	Amx-Sxt	4.49
Cn-Amx	6.74	Cn-Ctr	1.12
Amc-Cn-Ctr	1.12	Amc-Cn-Sxt	1.12
Amc-Ak-Amx	2.25	Amc-Amx-Sxt	3.37
Amx-C-Sxt	1.12	Cn-Amx-Ctr	1.12
Cn-Amx-Gen	1.12	Cn-Ctr-Amx	1.12
Cn-Amx-Sxt	2.25	Te-Amc-Amx	1.12
Te-Amc-Cn	1.12	Te-Amx-Sxt	2.25
Te-Cn-C-Sxt	1.12	Cn-Amx-Ctr-Sxt	1.12
Amc-Cn-Amx-Ctr	1.12	Amc-Cn-Ak-Amx	1.12
Amc-Ctr-Gen-Sxt	1.12	-	-
Amc-Cn-Ctr-Amx-C	1.12	Amx-Cn-Ctr-Amx-Cip	5.62
Amc-Cn-Ctr-Amx-Sxt	1.12	Amx-Cn-Amx-Sxt-Ctr	1.12
Te-Cn-Ctr-Amx-Cip	1.12	-	-
Amc-Cn-Ctr-Amx-Gen-C-Sxt	1.12	Te-Amc-Cn-Ctr-Amx-C-Cip-Sxt	3.37
Te-Amc-Cn-Ctr-Ak-Amx-Gen-C-Sxt	1.12	Te-Amc-Cn-Ctr-Amx-Gen-C-Cip-Sxt	1.12

Key: Te, tetracycline; Amc, co-amoxiclav; Cn, Cephalexin; Ctr, ceftriaxone; Ak, Amikacin; Amx, Amoxicillin; Gen, Gentamicin; C, Chloramphenical; Cip, Ciprofloxacin; SXT, Sulphamethoxazole-trimethoprin.

of attaching themselves onto surfaces in the wastewater thereby forming biofilms, which enables the bacteria to withstand environmental stresses (Birosoval et al., 2015).

Biofilms are characterized by high bacterial density and diversity, which provide suitable conditions for horizontal gene transfer and genetic exchange of resistant traits. In this study isolates recovered from sludge and dumpsite soils recorded the highest numbers of antibiograms, indicating that they present the best selection sites for antibiotic resistance. Reinthaler et al. (2003) has shown that biofilm formation increases the rate of genetic exchange for antibiotic resistance traits in matrixes such as sludge. Microbes have also been shown to acquire antimicrobial resistance as one of the mechanisms which help them survive in hostile environments (Anatoly et al., 2016).

In this study, a total number of 38 antibiograms was recorded as shown in Table 5, with cephalexin being the most frequent at over 11%, followed by amoxicillin at over 10%. The most common multiple resistance was the combination of co-amoxiclav and amoxicillin and cephalexin and amoxicillin both recorded 6.7% levels. The highest number of antibiotics resisted by one bacterium was 9 with two types of antibiograms Te-Amc-Cn-Ctr-Ak-Amx-Gen-C-Sxt and Te-Amc-Cn-Ctr-Amx-Gen-C-Cip-Sxt. The relative frequencies of antibiotic resistance (Table 6) for individual antibiotics shows that amoxicillin, cephalexin and sulphamethoxazole-trimethoprin appear to be more associated with cross resistances to other antibiotics. This could be as a result of the antibiotic resistance developed against these three drugs being associated with identical mechanisms of transmission such as common integrons, cassettes and transposons (Kümmerer, 2009b; Suzuki et al., 2014). Alternatively the resistance mechanism may be as a result of sharing a common target site as for the cases of amoxicillin, cephalexin, co-amoxiclav and ceftriaxone all which are related with inhibition of cell wall synthesis. Wastewater treatment plants (WWTPs) have been recognized as a reservoir for antibiotic resistant bacteria and antibiotic resistant genes, including plasmids encoding resistance to antibiotics (Allen et al., 2010; Novo and Manaia, 2010; Bouki et al., 2013).

Generally, WWTPS are rich in nutrients, which enhance the multiplication of microorganisms facilitating gene exchange due to cell to cell contact (Sørensen et al., 2005; Dionisio et al., 2002), making waste disposal sites important reservoirs of antibiotics resistance genes that can be exchanged by bacteria from different environmental compartments (Dang et al., 2008). Since majority of the antibiotics showed positive correlation that were significant ($p < 0.05$) with the exception of Cn/Sxt and Ak/Sxt combinations which showed negative correlation, it indicate that the resistance genes in the waste treatment sites may be closely associated and probably common to mobile genetic elements that are circulating and evolving, calling for more investigations. Overall, this study supports the hypothesis that natural

Table 6. Comparison of percentage relative frequencies of resistances and susceptibility levels against the different combinations of antibiotics tested on members of *Enterobacterieceae* recovered from dumpsite, sludge and wastewater.

Atibiotics	% Relative Frequency									
	Te	**Amc**	**Cn**	**Ctr**	**Ak**	**Amx**	**Gen**	**C**	**Cip**	**Sxt**
Tetracycline (Te)										
Resistant (n=14)	100	64	57	36	7	64	14	36	27	71
Susceptible (n=91)	0	14	14	50	93	14	86	57	57	29
Co-Amoxiclav (Amc)										
Resistant (n=37)	24	100	62	43	5	100	11	16	24	43
Susceptible (n=38)	68	0	7	49	92	0	4	81	60	51
Cephalexin (Cn)										
Resistant (n=55)	14	41	100	36	4	66	7	10	16	30
Susceptible (n=38)	75	30	0	46	88	11	89	77	77	41
Ceftriaxone (Ctr)										
Resistant (n=29)	17	45	76	100	4	79	10	24	31	69
Susceptible (n=74)	72	31	24	0	90	14	83	72	62	66
Amikacin (Ak)										
Resistant (n=2)	50	100	100	50	100	100	50	50	0	50
Susceptible (n=95)	50	0	0	0	0	0	50	50	50	50
Amoxicillin (Amx)										
Resistant (n=73)	12	51	51	32	3	100	6	15	14	39
Susceptible (n=21)	77	36	27	62	93	0	90	81	75	58
Gentamicin(Gen)										
Resistant (n=4)	50	100	75	75	0	75	100	50	25	100
Susceptible (n=95)	25	0	25	25	100	25	0	50	50	0
Chloramphenical (C)										
Resistant (n=11)	45	55	82	64	9	91	18	100	27	82
Susceptible (n=85)	27	18	0	27	91	0	82	0	45	9
Ciprofloxacin (Cip)										
Resistant (n=38)	40	90	90	90	0	100	10	30	100	40
Susceptible (n=86)	60	0	10	10	90	0	80	70	0	60
Sulphamethoxazole-trimethoprin (Sxt)										
Resistant (n=38)	26	42	50	26	3	74	11	24	11	100
Susceptible (n=60)	61	34	32	61	82	16	87	68	74	0

environment such as waste treatment sites can act as reservoirs for emerging microbial contaminants including antibiotic resistance genes. Since the rate of gene mobilization at 30°C is higher than 37°C, indicating that climatic factors may contribute to elevated concentrations of antibiotic resistance genes in the tropics (Walsh et al., 2011).

Conclusion

This study demonstrates the occurrence of multidrug resistant enteric microbes in waste treatment sites. It further demonstrates that, in waste treatment sites in Kakamega town amoxicillin, cephalexin and sulphamethoxazole-trimethoprin resistance traits may

play a major role in selection of multiple antibiotic resistances in matrixes of high microbial densities and interactions.

Antibiotic resistance amongst waste water enteric bacterial species is a serious threat in Kenya. Continuous and close monitoring of antibiotic resistance among enteric bacteria associated with waste treatment sites, to understand emerging antibiotic resistance patterns at these sites, form an important interphase for microbes originating from humans and the environment. In addition the finding of this study also highlights on the need of proper management strategies of the different types of wastes to enhance decontamination of the wastes.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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