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ANTIMICROBIAL ACTIVITIES OF SOME *STREPTOMYCES* ISOLATED FROM GARDEN SOIL SAMPLES AND FISH POND WATER IN FUTA

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Abstract

Context: *Streptomyces* are potential sources for secondary metabolites possessing a variety of biological activities with antimicrobial activity, which is used for human and animal treatment. It is estimated these bacteria synthesize more than 7,000 metabolites. About 80% of these are made by members of the genus *Streptomyces*. *Streptomyces* spp. is known as producers of several bioactive metabolites which has antibiotic, antiparasitic, antitumor, insecticide, herbicide, etc.
Objectives: Determine the antimicrobial activities of Streptomyces isolates and compare the efficacy of the antimicrobial activities of Streptomycetes with selected commercial antimicrobial agents.
Materials and methods: Collection of samples and test organisms; physicochemical screening; antimicrobial assay (co-culture method); antibiotics sensitivity test (disc-diffusion assay).
Result: *Streptomyces griseoflavus, Streptomyces parvus* and *Streptomyces albidus* were isolated from dry soil while *Streptomyces vinaceus* and *Streptomyces globiosporus* from moist-fresh soil and fish pond water used for cultivation of cat fish *Clarias garienpinus* respectively. Out of these five (5) isolates isolated, 3 isolates (60%) exhibited antibacterial activity against the following pathogenic, nosocomial organisms: *Escherichia coli, Klebsiella pneumoniae, Salmonella typhi, Pseudomonas aeruginosa, Staphylococcus aureus, Candida* spp, *Aspergillus fumigatus*, *Aspergillus flavus*, *Aspergillus saprophyticus*, and *Trichoderma* spp respectively. *S. griseoflavus* demonstrated antibacterial activity against *K. pneumoniae*, *Trichoderma* spp and *Candida spp. S. albidus* against *S. typhi* and *A. fumigatus* while *S. parvus* was inhibitory to *S. aureus* and *A. fumigatus*. The active isolates inhibited bacteria growth (0 to 39.33 mm). Commercial antimicrobials used also demonstrated inhibitory effects against the test pathogens.
Conclusion: The garden soil of FUTA farm contains *Streptomyces* spp. with antibacterial and antifungal activities.

Key words: Antibacterial, Antifungal, Nosocomial, Novel drugs.

Introduction

Streptomycetes are soil-resident mycelial bacteria that make numerous secreted proteins and many vital secondary metabolites with useful antibiotics inclusive. Chitin serves as the foremost nutrient source for majority of Streptomycetes; apart from the fact that they have also developed extracellular system that facilitates its timely utilization (Chater *et al.* 2009). Streptomycetes are armed with discrete features such as DNA with a high Guanosine + Cytosine content (Anderson and Wellington 2001).

The Streptomyces genus is designated in nature by the vast amount of species coupled with the conspicuous diversities among their order. They demonstrate substantive variations in their morphology, physiology and biochemical actions, producing most known enzymes (Suneetha and Zaved 2011). More than 500 *Streptomyces* spp. and subspecies have been described, the most vast number of any bacterial genus (Lee *et al*. 2005). Several methods have been employed to identify *Streptomyces* species include culturing methods using the selective technique, construction of genetic marker systems, a combination of

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chemical markers and the presence of LL-diaminopimelic acid plus the absence of characteristic sugars in the cell wall (Sujatha *et al.* 2005). *Streptomyces* species have been the most copious and easily recovered actinomycetes group in soil. Streptomyces produce secondary metabolites with varied bioactivities that are not limited to antibacterial, antifungal, antiviral, antitumoral, and enzyme inhibitory compounds (Àarthi *et al.* 2009). It is estimated these resourceful bacteria synthesize more than 7,000 metabolites (Berdy 2005). More than 9,000 biologically active molecules have been isolated from *Streptomyces* of which over 60 are applicable in medicine, agriculture and research (Demain 2009).

Among the actinomycetes, the genus *Streptomyces* has continued to provide a larger number and wider variety of new antibiotics than any other genus, signifying that a considerable number of *Streptomyces* spp. with novel antibiotic production capabilities exist in nature. These bacteria produce about 75% of commercially and medically useful antibiotics (Berdy 2005). Streptomyces are also well recognized as biological control agents that hamper several soil and airborne plant pathogenic fungi (Og et al. 2008; Silva *et al.* 2008). *Streptomyces* spp. are important source of abundant secondary metabolites, enzymes and antibiotics (Ahmed *et al.* 2008) chiefly due to their much shorter generation time, and the ease of genetic and environmental stage management. Although thousands of antibiotics have been isolated from *Streptomyces*, these represent only a small fraction of their catalogue of bioactive compounds potentials (Watve et al. 2001). The emergence of antifungal resistant fungi is a problem of growing significance in dermatological and surgical wound infections (Panacek *et al.* 2006). In general, the most important resistance problems in the management of wounds have been observed.

Therefore, isolation of new *Streptomyces* from natural resources, niches with promising potentials and characterization of their secondary metabolites is a valuable venture. The isolation of novel *Streptomyces* spp. is in great urgent need in this era of constantly evolving antimicrobial resistance, as they are very potent producers of innovative secondary metabolites (Mellouli *et al.* 2003).

Materials and Methods

Collection of samples and test bacteria

Fish pond water and soil samples were collected separately from agric farm and the fish pond within the Federal University of Technology Akure (FUTA) respectively. The following clinical pathogenic bacteria strains were also collected from the Ondo State Specialist Hospital, Akure and University college hospital Ibadan: *Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella typhi*, *Pseudomonas aeruginosa* and *Staphylococcus aureus, Candida* spp*. Aspergillus fumigatus, Aspergillus flavus, Aspergillus saprophyticus,* and *Trichoderma* spp. Their identities were confirmed and maintained at 4^{o}C till further analysis. All the laboratory reagents and chemicals used were of analytical standards.

Analysis

The parameters for water analysis are: pH, turbidity, Nitrate, phosphate, Sulphate, Calcium, Magnesium, heavy metal, total dissolved solids, electrical conductivity, biochemical oxygen demand (BOD) and chemical oxygen demand (COD) while that of soil sample include: particle size analysis, pH, organic carbon, calcium, magnesium, potassium, sodium, phosphorus and nitrogen according to Association of Analytical Chemists (AOAC 2007) standards.

Isolation and identification of Streptomycetes

With a sterile 1-ml pipette, 0.5 ml from the dilutions 10^{-7}, 10^{-5} and 10^{-2} obtained from the fish pond water, moist fresh soil and dry soil samples respectively were aseptically transferred to already labeled Macbeth scales starch mineral agar (Soluble starch:10g, $CaCO_3$:3g, K_2HPO_4:1g, $(NH_4)_2SO4$:2g, $MgSO_4.7H_2O$:1g, NaCl:1g, Agar:25g, pH:7.0) plates supplemented with 1ml of liquid Nystatin to inhibit fungi and spread with

the aid of sterile glass spreader. The plates were incubated upside down in the dark at 28^{o}C for seven days. Observed colonies on the plates were further examined microscopically to confirm their conformities to typical filamentous Streptomycetes. Isolates of different colonies were sub- cultured repeatedly by streaking on both pridham's medium (Glucose:10g, Starch:2g, $(NH_4)_2SO4$:2g, $CaCO_3$:1g, K_2HPO_4:1g, $MgSO_4$:1g, NaCl:1g, Agar:12g, pH:7.0) and Streptomycetes growth medium (Glucose:10g, Peptone:1g, KNO_3:1g, K_2HPO_4:0.1g, Agar:15g, pH:7.0) until pure cultures were obtained. These different pure cultures were then introduced into a starch mineral agar slant and incubated for seven days at 28^{o}C in order to ensure proper growth. The bottles were kept as stock cultures maintained at 4^{o}C after identified after applicable morphological and biochemical tests with robust reference to Bergey's manual of determinative bacteriology and colour codes for exact description of colours of Streptomycetes according International Streptomycetes Project – ISP methods (Shirling & Gottlieb 1966).

Antimicrobial bioassay by co-culture method

Agar plug method

The selected pathogenic microorganisms; *E. coli*, *K. pneumoniae*, *S. typhi*, *P. aeruginosa*, *S. aureus Candida* spp*. A. fumigatus, A. flavus, A. saprophyticus,* and *Trichoderma* spp were grown in Mueller Hinton broth for twenty four hours (Bacteria) and Potato dextrose agar for forty eight hours (Fungi). Inoculum containing approximately 1.5×10^{8} number of cells were swabbed on the surface of separately well labeled Mueller Hinton agar plates for bacteria and 0.5ml of the standardized inoculum was seeded into the Potato dextrose agar for fungi by pour plate method. Isolated *Streptomyces* strains were previously grown on Streptomycetes growth medium for Seven days to allow antibiotic production in the agar. Colonies of *Streptomyces* were cut using a sterile cork borer (8mm in diameter) and placed on the plate already inoculated with test organism and incubated at 37^{o}C for 24 hours for bacterial and 28^{o}C for 48hrs for fungi, growth inhibition of the indicator organisms was observed by measuring and recording the diameters of circular zones of inhibition in millimeters (Fokkema 1973)**.**

Perpendicular streaking method

A single streak of *Streptomyces* from pure culture was made down the edge of Streptomycetes assay media. The plates were then incubated for about seven days in order to allow antibiotics to be produced; after which a single streak of each test organism was put perpendicularly to the Streptomyces without touching it for bacterial while agar plugs of test fungi already in confluence growth were aseptically placed next to the *Streptomyces* streak without touching it. After incubation, inhibition zones were measured and compared with the control experiment. Standard antimicrobial agents were also used against the test organisms as positive control and comparative purposes (Fokkema 1973).

Table 1. Physicochemical characteristics of fish pond water sample used.

Physicochemical characteristics			
pH	6.800	Calcium (mg/l)	17.000
Conductivity (Ms/cm)	0.006	Chloride (mg/l)	28.000
Turbidity (NTU)	140.000	Sulphate (mg/l)	0.600
TDS (mg/l)	790.000	Phosphate (mg/l)	1.400
COD (mg/l)	159.000	Nitrate (mg/l)	2.240
BOD (mg/l0	1.690	Copper (mg/kg)	0.050
Magnesium (mg/l)	1.800	Zinc (mg/kg)	0.080

Results

Physicochemical Analysis of samples

The physicochemical traits of the fish pond water suggest the water was very low in some vital nutrients on which the growth of Streptomycetes is predicated. For instance, Calcium was 17.00mg/l and Nitrate 2.240mg/l (Table 1). The soil was fairly higher in Calcium and Magnesium contents when compared with that of fish pond water above (Table 2).

Table 2. Physicochemical characteristics of soil sample used.

Physicochemical characteristics	
% Silt	25.70
% Clay	17.60
pH: 1:2 H_2O (29^oC)	5.80
Organic carbon (Mg/l)	2.3×10^{-5}
Calcium (Mg/l)	200400.00
Magnesium (Mg/l)	24305.00
Potassium (Mg/l)	37925.06
Sodium (Mg/l)	8736.20
Phosphorus (Mg/l)	205977.10
Nitrogen (Mg/l)	1.9×10^{-5}

Table 3. Colonial, cellular, physiological and biochemical characteristics of Streptomycetes isolates.

Macroscopic characteristics	Isolate B	Isolate J	Isolate E	Isolate H	Isolate F
Colony shape	Undulate	Circular	Irregular	Circular	Circular
Colony colour	White	Brown	Grey	Orange	Yellow
Colony edge	Rough	Smooth	Undulate	Rough	Round
Elevation	Papillary	Umbonate	Raised	Convex	Convex
Microscopic characteristics					
Gram reaction	+	+	+	+	+
Shape/structure	Filamentous	Filamentous	Filamentous	Filamentous	Filamentous
Spore staining	+	+	+	+	+
Biochemical investigations					
Catalase	+	-	-	-	-
Starch hydrolysis	+	+	+	+	+
Carbon sources utilization					
Glucose	+	+	+	+	-
Lactose	+	+	+	+	-
Fructose	+	+	+	+	-
Mannitol	+	+	+	-	+
Sucrose	-	-	-	+	-
Plausible Actinomycete	*Streptomyces griseoflavus*	*Streptomyces parvus*	*Streptomyces albidus*	*Streptomyces vinaceus*	*Streptomyces globiosporus*

Streptomycetes isolates

Streptomycetes colony forming units obtained are 5.0×10^7, 5.0×10^7 and 1.0×10^8 for fish pond water, moist-fresh soil and dry soil samples respectively. It was observed that dry soil has the highest colony forming units. Five different Streptomycetes species were isolated from fish pond water, moist-fresh soil and dry soil samples respectively. Physical and biochemical details of these isolates can be seen in table 3. These isolates were grouped according to their sources: *Streptomyces globiosporus* was isolated from the fish pond water. *Streptomyces vinaceus* was isolated from moist-fresh soil. *Streptomyces griseoflavus*, *Streptomyces parvus* and *Streptomyces albidus* were isolated from soil sample that was air-died for two weeks.

Screening of isolates for antimicrobial activity

As depicted in figure 1, *Streptomyces griseoflavus* showed distinct antibacterial activities against *Klebsiella pneumoniae* and *Salmonella typhi* with zone of inhibitions ranging from 32.00 to 39.33mm respectively while

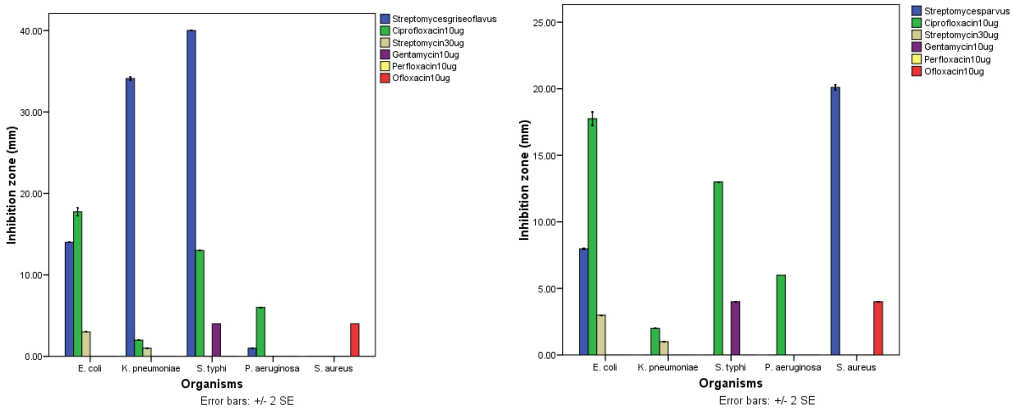


Fig. 1. Comparative antibacterial activities of *Streptomyces griseoflavus* and commercial antibiotics on selected pathogenic organisms. **Fig. 2.** Comparative antibacterial activities of *Streptomyces parvus* and commercial antibiotics on selected pathogenic organisms.

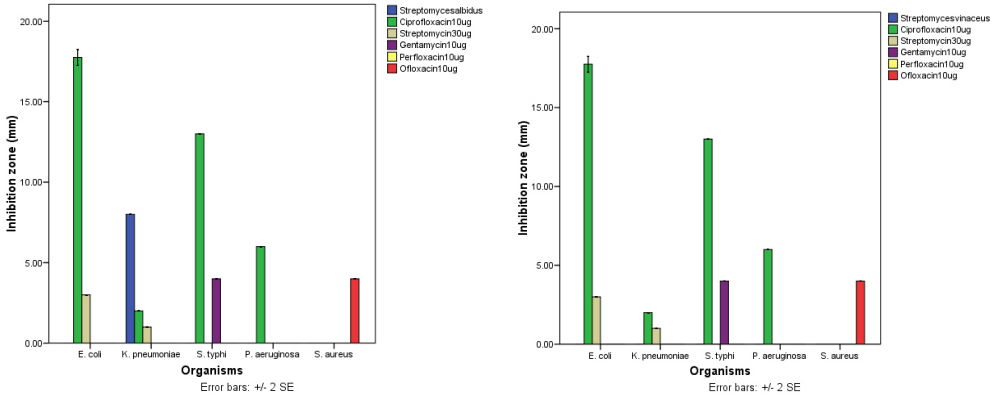
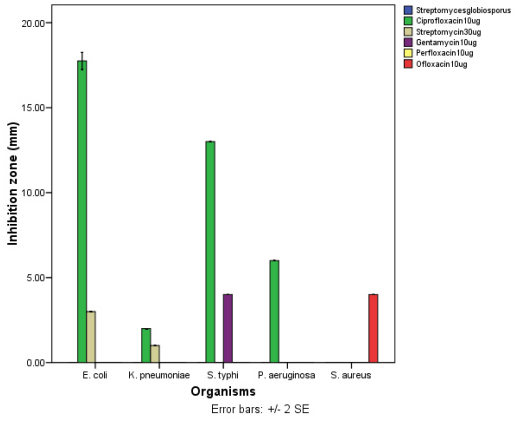


Fig. 3. Comparative antibacterial activities of *Streptomyces albidus* and commercial antibiotics on selected pathogenic organisms. **Fig. 4.** Comparative antibacterial activities of *Streptomyces vinaceus* and commercial antibiotics on selected pathogenic organisms.



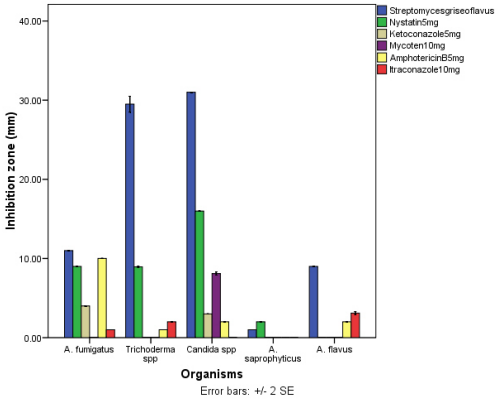
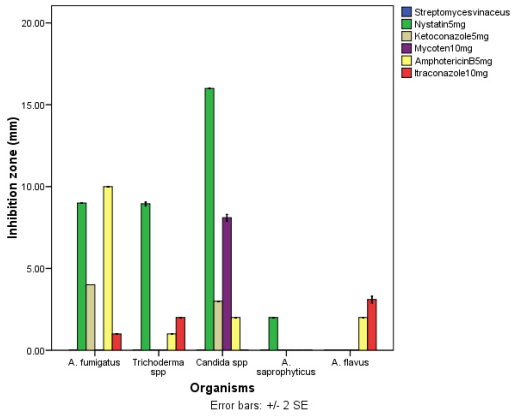


Fig. 5. Comparative antibacterial activities of *Streptomyces globiosporus* and commercial antibiotics on selected pathogenic organisms. **Fig. 6.** Comparative antifungal activities of *Streptomyces griseoflavus* and commercial antifungal drugs on selected pathogenic organisms.



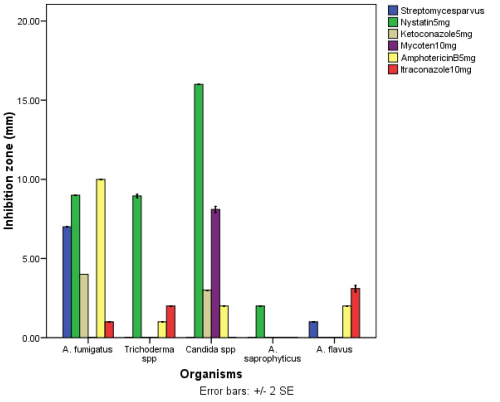


Fig. 7. Comparative antifungal activities of *Streptomyces parvus* and commercial antifungal drugs on selected pathogenic organisms. **Fig. 8.** Comparative antifungal activities of *Streptomyces albidus* and commercial antifungal drugs on selected pathogenic organisms.

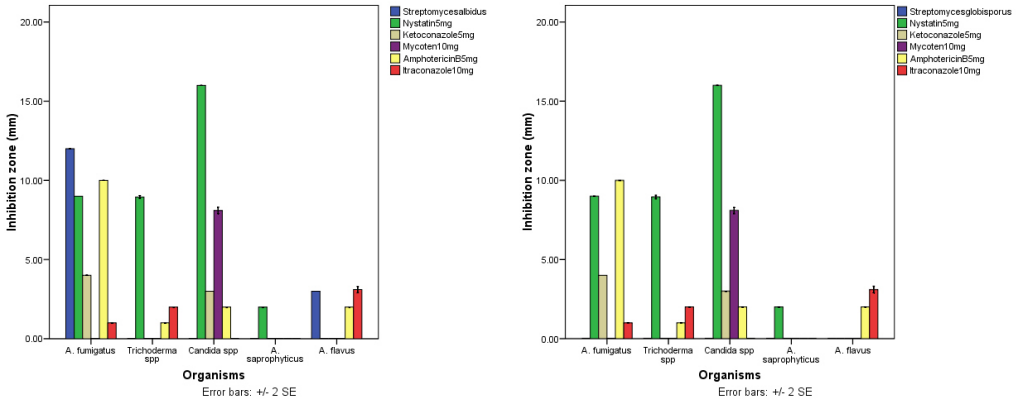


Fig. 9. Comparative antifungal activities of *Streptomyces vinaceus* and commercial antifungal drugs on selected pathogenic organisms. **Fig. 10.** Comparative antifungal activities of *Streptomyces globiosporus* and commercial antifungal drugs on selected pathogenic organisms.

The commercial antibiotics Ciprofloxacin showed inhibitory action against *Escherichia coli* with inhibition zone of 15mm. Also, *Streptomyces parvus* exhibited inhibitory action against *Staphylococcus aureus* but was not that effective against *Escherichia coli* when compared with ciprofloxacin (Figure 2). *Streptomyces albidus* seemed to be effective against *Klebsiella pneumoniae* with inhibition zone of 8mm when compared with Ciprofloxacin and Streptomycin with zone inhibitions of 1.33mm and 1.00mm respectively. *Streptomyces vinaceus* and *Streptomyces globiosporus* did not show any antibacterial activities. Ciprofloxacin, a commercial antibiotic inhibited *Escherichia coli, Salmonella typhi* and *Pseudomonas aeruginosa* with inhibition zones 15.67 mm, 13.67 mm and 5.33 mm respectively (Figures 4 and 5).

It was determined that of the 5 isolates, 3 (60%) produced distinct inhibitory substances against at least one indicator fungi. Among the active isolates, 3 inhibited the pathogenic fungi. The active isolates suppressed or inhibited fungal growth with inhibition zones ranging from 1 to 30mm. In general, activity of *Streptomyces griseoflavus* was specifically the highest on the pathogenic fungi than all the isolates and the standard antimicrobials used as positive controls (Figure 6 - 9). *Streptomyces globiosporus* did not have any antifungal effects (Figure 10).

Discussion

Findings from the present study have corroborated the submission of Demain and Davies (1999) with respect to the growth of Streptomyces isolates obtained. The colony forming units (c.f.u) were determined by counting the colonies on the dilution plates. Maximum number of colonies (1.0×10^8 c.f.u/gm of soil) was obtained from the dry soil sample that is chiefly used for maize cultivation. The physicochemical characteristics of the soil sample used indicate a considerable high level of calcium and phosphorus; 200400.00 Mg/l, 205977.10 Mg/l respectively. This we can say has contributed to the presence of the *Streptomyces* in the soil since these inorganic elements are vital components of the isolation media. Physicochemical attributes of the pond water sample showed a comparatively lower amount of calcium and phosphate; 17.000mg/l, 1.400mg/l respectively. These results agree with previous findings of Mustafa, (2009) that *Streptomyces* spp are prevalent in soil that is rich in exchangeable cations. This may likely be responsible for lesser number of *Streptomyces* isolated from the pond water sample. It can also be observed that there is greater number of *Streptomyces* in the dry soil than all other sources; this has been linked to the ability of *Streptomyces* to form spores and bridge soil spaces using mycelium, together with their ability to use a broad spectrum of suitable carbon sources and inorganic nitrogen, enable them to survive long term under extreme conditions (Ka¨mpfer 2006).

Screening the actinomycetes for antibacterial activities revealed that out of the 5 isolates, 3 (60%) produced distinct inhibitory substances against at least one indicator bacterium. Three of the five *Streptomyces* isolates inhibit the test fungi by producing inhibition zones by a mechanism that involves the release of antifungal agents. This shows the antagonistic effect that these strains of *Streptomyces* and the evidence were summarized as follows; clear inhibition zones were seen after 48 hrs, shows wider inhibition zone when compared with the synthetic antifungal agents, spores do not germinate when cultured on the inhibition zone.

Streptomyces griseoflavus and *Streptomyces albidus* inhibited Gram negative bacteria while *Streptomyces parvus* exhibited a broad spectrum antibacterial action against both Gram-negative and Gram-positive bacteria. The latter is at par with previous investigation that showed *Streptomyces* spp had activity against Gram-positive bacteria (Thakur *et al.* 2007). The active isolates generally inhibited bacteria growth with inhibition zones ranging from 0 to 39.33mm and compete favorably with the standard, purified antibiotics. It should also be noted that the pathogenic organisms used were of hospital origin with track records of resistance to commercial antibiotics this was also shown as both the Gram-negative and Gram-positive bacteria were totally resistant to most of these antibiotics.

Conclusion

This study has provided information on the antibacterial activities of some *Streptomyces* spp isolated from fish pond water and garden soil of FUTA.

Recommendation

The garden soil of FUTA has *Streptomyces* with antibacterial activities; this could be an important source of bioactive metabolites that can either serve as substitute or enhancement for the commercial antibiotics. Further research is required to outline this potential. It is therefore suggested that broader clinical studies be done to measure this novel advantage, in order to convert it to improved clinically applicable outcomes.

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