Antibiotic Sensitivity Pattern of Organisms isolated from Diseased Insects

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Abstract: Organisms isolated from diseased insect were investigated for their susceptibility to conventional antibiotics. Five bacteria were investigated out of which four were typed cultures which include *Paenibacillus popilliae* (NRRL B- 4223) isolated from diseased grub hemolymph, *Lysinibacillus sphaericus* (NRRL B- 23338), *Serratia marcescens* (NRRL B-3401) isolated from hornworm with septicemia and *Bacillus subtilis subspecies spizizenii* (NRRL B- 14472). All these were imported from the United States Department of Agriculture, Agricultural Research Service Culture Collection Centre. The last bacterium was isolated from diseased *Macrotermes bellicosus* in Ondo state, Nigeria. Antibiotics tested on the bacteria include GEN=Gentamicin (10µg), COT= Cotriomoxazole (25µg), ERY=Erythromycin (5µg), TET=Tetracycline (10µg), CHL=Chloramphenicol (10µg), AMX=Amoxicillin (30µg), CXC=Cloxacillin (5µg), STR=Streptomycin (10µg), AUG=Augmentin (30µg), CPR=Ciprofloxacin (10µg), OFL=Ofloxacin (5µg), NIT=Nitrofurantin (300µg), AMP=Ampicillin (10µg), CAZ=Ceftazidime (30µg), CRX=Cefuroxime (30µg). Positive discs were used for *Bacillus* sp while negative discs were used for *S. marcescens* and *P. aeruginosa.* Results showed that Gentamycin, Ciprofloxacin and Ofloxacin were all able to inhibit the growth the five bacteria. Ciprofloxacin had the highest antimicrobial activity on *B. subtilis* with an inhibition zone of 36.00 ± 1.00^{d} mm. Results also showed that all the organisms are resistant to Cloxacillin, Amoxicillin, Augmentin, Ampicillin, Ceftazidime and Cefuroxime. All of the bacteria are sensitive to at least four of the antibiotic used.

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Introduction

Antibiotics refer to those medicines used to combat infections caused by microorganisms which can equally be regarded as parasites. They are also referred to as antibacterials or antimicrobials (Volkmar *et al.*, 2010) with various formulations which can be taken orally (liquid, tablet or capsule), applied as ointments or given intravenously (Patzer *et al.*, 2010). Classification of antibiotics is based on their mechanism of action and the type of bacteria or parasites they combat (Patzer *et al.*, 2010). Organisms of interest usually targeted by antibiotics are those categories of medically important microbes known to cause infections in humans and his animals.

Microorganisms known to cause diseases in insects are often referred to as entomopathogens (Van Zyl and Malan, 2014). They are candidates and potential agents of biological control (Tanada and Kaya, 1993). Most times, they are mass produced on organic materials which are usually byproducts from certain industrial processes and introduced into the environment to control the proliferation of particular insect pests (Senthilraja *et al.*, 2010).

Under normal circumstances, they are generally benign, safe and nonpathogenic to humans and other non-target organisms in the environment (Laird *et al.*, 1990). In fact, during the early days of biological control and especially microbial control, there were no much concern for the possible side effects, toxicity or safety considerations of biocontrol organisms. Steinhaus (1957) was possibly the first to raise concerns about the possible side effects of microbial control products for humans as well as other vertebrates and even crops. He very carefully discussed the different aspects of the scientific knowledge at that time. Although he concluded that microorganisms pathogenic to insects are in general harmless to man, animals and plants, he recommended that biocontrol products made from such microorganisms be subjected to appropriate tests and regulations. This is because once released into the environment for biological control processes, the behaviour of microbes cannot be predicted. They may undergo mutation which may lead to changes or alteration in the nucleotide sequence of their genome (Sharma *et al.*, 2015). This can be brought about spontaneously through the process of molecular

evolution (Chen *et al.,* 2014) or when the microbes make contact with mutagens (Rodgers *et al.,* 2016). These alterations and change in the genetic makeup may lead to a previously nonpathogenic agent becoming pathogenic. As a result, it becomes imperative to find possible antibiotics which are able to inhibit such organisms in case they undergo such harmful changes in the environment and start to cause disease in non-targeted organisms.

Materials and methods

Importation of Potential Biocontrol organisms

The suitable bacteria isolated from soil and diseased insects where ordered from the United States Department of Agriculture, Agricultural Research Services culture collection centre after due approval from the Federal Ministry Of Agricultural and Rural Development, Nigerian Agricultural Quarantine Services. The typed strains included NRRL B- 4223 *Paenibacillus popilliae* isolated from diseased grub hemolymph, NRRL B- 23338 *Lysinibacillus sphaericus*, NRRL B-3401 *Serratia marcescens* isolated from hornworm with septicemia and NRRL B- 14472 *Bacillus subtilis subspecies spizizenii.* Bacteria were sent through courier in freeze-dried form and kept inside glass vials.

Revival of Bacterial freeze-dried cultures

The typed strains were preserved in a dormant state by drying a heavy suspension of cells in sterile bovine serum. The cells were brought back to active state of growth by transfer to a suitable liquid medium. A file scratch was made in the centre of the glass tube vials. The tube was wiped with cotton moistened with 70% alcohol and broken. The open end was lightly flamed and pellets were transferred into broth. Incubation was done for 24 hours and growth occurred. Organisms from broth were transferred to solidified media and put on slant for storage and further studies.

Isolation of organism from diseased termite (worker)

Termites were sourced from outside traps, brought to the laboratory after harvesting and subjected to 'near-natural' treatment under laboratory conditions. They were supplied with plastic cages and moistened cellulosic materials as food source. Cages were kept in dark corners and watched for individuals showing morbid and mortal symptoms in the form of death, reduced activities, lethargy and colour change. Resulting cadavers were macerated and bacteria were isolated and purified from the macerate using standard methods. Characterisation of bacteria was done in accordance with standard methods (Oyeleke and Manga, 2008). Identification of bacteria was done using standard procedures (Cowan and Steel, 1993).

Conventional Antibiotic Sensitivity Test

Muller hinton media was used for the antibacterial sensitivity. Media was prepared and sterilized according to standard methods and dispensed into Petri dishes. McFarland turbidity standards of each bacteria was prepared according to standard methods (Cheesbrough, 2000). The turbidity of the suspension was adjusted visually adjusted by adding sterile physiological saline to each suspension. Each of the suspension was seeded evenly onto the agar surface using sterile swab stick, allowed to dry for 30 minutes before antibiotics discs were placed on the surface using sterile forceps.

The Kirby - Bauer test also known as disc diffusion method was used to determine the effect of standard antibiotics on the organisms (Marie, 2005). This method involved the use of the commercially available paper disc that had been impregnated with antibiotics of known concentration. The commercial antibiotic discs (Abtek Biologicals Limited) used included Gentamicin (10µg), Cotriomoxazole (25µg), Erythromycin (5µg), Tetracycline (10µg), Chloramphenicol (30µg), Amoxicillin (30µg), Cloxacillin (5µg), Streptomycin (10µg), Augmentin (30µg), Ciprofloxacin (5µg), Ofloxacin (5µg), Nitrofurantin (300µg), Ampicillin (10µg), Ceftazidime (30µg) and Cefuroxime (30µg). After placing the antibiotics discs, plates were incubated for 24 hours at 37^{o}C.

After incubation, clear areas around the discs were measured, which represents the zones of inhibition and the areas without clear zones were also observed. Seeded agar plate without antibiotics served as the control experiment. The zones of inhibition were measured in millimeter (mm). The experiment was carried out in triplicate.

Results

In this study, it was discovered that Ciprofloxacin had the highest antimicrobial activities on *Bacillus subtilis* with an inhibition zone of 36.00 ± 1.00^{d} while the least susceptibility was noticed on P. aeruginosa by Erythromycin with an inhibition zone of 7.67 ± 0.58^{b}. All the microorganisms studied used were susceptible to Gentamycin, Ciprofloxacin and Ofloxacin. Out of the 15 antibiotics used, *B. subtilis* was susceptible to 8 while *B. popilliae* and *B. sphaericus* are susceptible to only 4. All the organisms are resistant to Cloxacillin, Amoxicillin, Augmentin, Ampicillin, Ceftazidime and Cefuroxime.

B. popilliae, B. sphaericus and *P. aeruginosa* are resisitant to Cotrimoxazole. Erythromycin is able to inhibit the growth of *B. subtilis, P. aeruginosa* and *S. marcescens. B. subtilis, B. popilliae* and *B. sphaericus* are all susceptible to tetracycline. Chloramphenicol is only able to inhibit B. subtilis while *B. popilliae, B. sphaericus, B. subtilis* are all susceptible to

tetracycline while Streptomycin is effective only against P. aeruginosa. Nitrofurantin is able to inhibit *B. sphaericus* and *B. subtilis.* All these are represented in table one below:

Table 1. Sensitivity of entomopathogenic bacteria to Conventional Antibiotics

Organism	GEN	COT	ERY	TET	CXC	CHL	AMX	STR	CPR	OFL	AUG	NIT	AMP	CAZ	CRX
B. Subtilis NRRL-14472	23.67 ±0.58^{e}	27.33 ±1.15^{c}	20.67 ±0.58^{d}	15.67 ±0.58^{c}	0.00 ±0.00^{a}	20.67 ±0.58^{b}	0.00 ±0.00^{a}	-	36.00 ±1.00^{d}	35.33 ±0.58^{d}	0.00 ±0.00^{a}	24.33 ±0.58^{c}	0.00 ±0.00^{a}	0.00 ±0.00^{a}	0.00 ±0.00^{a}
P. aeruginosa	10.67 ±0.58^{a}	0.00 ±0.00^{a}	7.67 ±0.58^{b}	-	0.00 ±0.00^{a}	-	0.00 ±0.00^{a}	7.33 ±0.58^{b}	25.00 ±1.00^{b}	15.33 ±0.58^{a}	0.00 ±0.00^{a}	-	0.00 ±0.00^{a}	0.00 ±0.00^{a}	0.00 ±0.00^{a}
S. marcescens NRRL-3401	15.00 ±1.00^{b}	21.00 ±1.00^{b}	16.33 ±0.58^{c}	-	0.00 ±0.00^{a}	-	0.00 ±0.00^{a}	0.00 ±0.00^{a}	24.67 ±0.58^{b}	26.67 ±0.58^{c}	0.00 ±0.00^{a}	-	0.00 ±0.00^{a}	0.00 ±0.00^{a}	0.00 ±0.00^{a}
B. Popilliae NRRL-4223	21.67 ±0.58^{d}	0.00 ±0.00^{a}	0.00 0±0.00^{a}	10.67 ±0.58^{b}	0.00 ±0.00^{a}	0.00 ±0.00^{a}	0.00 ±0.00^{a}	-	30.33 ±0.58^{c}	26.67 ±0.58^{c}	0.00 ±0.00^{a}	0.00 ±0.00^{a}	0.00 ±0.00^{a}	0.00 ±0.00^{a}	0.00 ±0.00^{a}
B. Sphaericus NRRL-23338	19.33 ±0.58^{c}	0.00 ±0.00^{a}	0.00 ±0.00^{a}	16.67 ±0.58^{d}	0.00 ±0.00^{a}	0.00 ±0.00^{a}	0.00 ±0.00^{a}	-	21.67 ±0.58^{a}	17.33 ±0.58^{b}	0.00 ±0.00^{a}	19.33 ±0.58^{b}	0.00 ±0.00^{a}	0.00 ±0.00^{a}	0.00 ±0.00^{a}

KEY: GEN=Gentamicin (10µg), COT= Cotriomoxazole (25µg), ERY=Erythromycin (5µg), TET=Tetracycline (10µg), CHL=Chloramphenicol (30µg), AMX=Amoxicillin (30µg), CXC=Cloxacillin (5µg), STR=Streptomycin (10µg), AUG=Augmentin (30µg), CPR=Ciprofloxacin (5µg), OFL=Ofloxacin (5µg), NIT=Nitrofurantin (300µg), AMP=Ampicillin (10µg), CAZ=Ceftazidime (30µg), CRX=Cefuroxime (30µg)

- Not tested, antibiotic not on the G+ve or G-ve pack

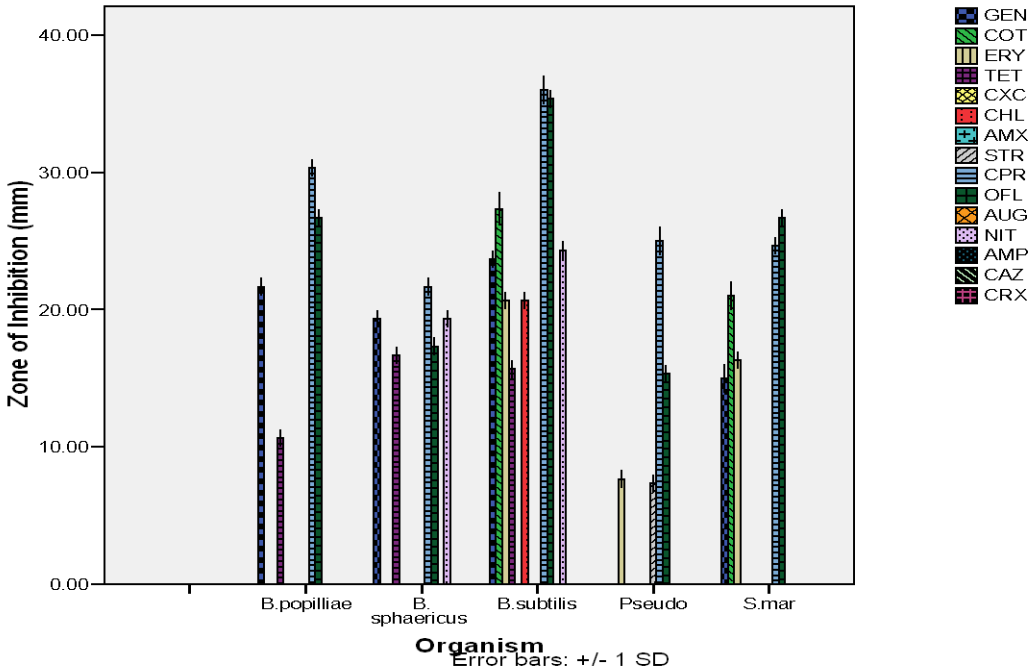

Figure 1. Illustrates the Graphical Representation of Zone of Inhibition by Antibiotic Sensitivity Discs

Discussion

Till date, information on the susceptibility of entomopathogens to antimicrobial agents is rather limited due to the assumption that they are generally benign and safe to other animals in the environment thus neglecting the fact that once released into the environment; microbes can acquire traits and characteristics they were not known to posses before.

This study reveals the pattern of the antibiotics susceptibility of bacteria isolated from diseased insects. Usage of antibiotics becomes necessary when patients are plagued with infectious diseases. Choice of drugs given is often subjected to the outcomes of susceptibility tests carried out on the bacteria responsible for such ailment. (Anibijuwon *et al.*, 2012).

P. aeruginosa was resistant to some of the conventional antibiotics used. Past studies has attested to the existence of antimicrobial resistance strains of Pseudomonas aeruginosa and these strains sometimes requires the synergy of more than one antibiotic or extracts from plants before they can be combated. Resistance of *P. aeruginosa* might also be due to the fact that Gram negative bacteria tend to have higher inherent resistance to antimicrobial agents as shown in similar reports by Ndukwe *et al.*, (2005).

All the three gram positive bacteria tested against Tetracycline showed susceptibility and this can be attributed to its broad spectrum ability (Clayton, 1993). Similar ability is also noticed with Ciprofloxacin, Ofloxacin and Gentamicin.

This study also shows that each of the bacteria is susceptible to at least four of the antibiotics used. Therefore, these organisms can be considered suitable to be employed in further biocontrol studies. They are considered suitable because in the event that they affect human or other nontargets in the environment upon their release, they can be combated with the usage of antibiotics.

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References

1. Anibijuwon II, Adetitun DO, Kelly BA, Bankefa EO. Antibiotic sensitivity pattern of bacteria isolated from ear discharge. International Journal of Biology, Pharmacy and Allied Sciences. 2012;1(6): 795-805.
2. Cheesbrough M. District Laboratory Practice in Tropical Countries. Part 2: Cambridge, 2000.
3. Chen J, Miller BF, Furano AV. "Repair of naturally occurring mismatches can induce mutations in flanking DNA" *eLife*. 2014;3(30):13
4. Clayton LT. Taber's Cyclopedic Medical Dictionary (17th ed.). F. A. Davis Co. ISBN 0-8036 8313.
5. Cowan ST, Steel KJ. Manual for the Identification of Medical Bacteria. 2nd Edition. Cambridge University Press, London. (1993):205-209.
6. Laird M, Lacey LA, Davidson EW. Editors. Safety of microbial insecticides. Boca Raton, FL: CRC Press. (1990):259.
7. Maria GC, Mary BS, Debashree B, Lacie F-Cubley. Antibacterial activity of native California medicinal plant extracts isolated from Rhamnus californica and Umbellularia californica. Annals of Clinical Microbiology and Antimicrobials. 2015;1(4):29. DOI 10.1186/s12941-015-0086-0.
8. Marie BC. Manual of Antimicrobial Susceptibility Testing. American Society for Microbiology. 2005. ISBN 1-55581-349-6.
9. Ndukwe KC, Okeke I, Lamikanra A, Adesina SK, Aboderin O. Antimicrobial Activity of Aqueous Extracts of Selected Chewing Sticks. J. Contemp. Dent. Pract. 2005;6(3): 86-94.
10. Oyeleke SB, Manga BS. Essentials of Laboratory Practical in Microbiology, 1st Edition. Tobest Publishers, Minna, Nigeria. (2008):28 – 62.
11. Patzer SI, Volkmar B. Gene cluster involved in the biosynthesis of griseobactin, a catechol-peptide siderophore of Streptomyces sp. *Journal of Bacteriology*. 2010;19(2):426–435.
12. Rodgers K, McVey M. "Error-Prone Repair of DNA Double-Strand Breaks" *Journal of Cellular Physiology*. 2016; 23(11): 15–24.
13. Senthilraja G, Ananda T, Durairaj C, Kennedy JS, Suresh S, Raguchandera T, Samiyappan R. A new microbial consortia containing entomopathogenic fungus, *Beauveria bassiana* and plant growth promoting rhizobacteria, *Pseudomonas fluorescens* for simultaneous management of leafminers and collar rot disease in groundnut. Biocontrol Science and Technology. 2010;20(5):449-464.
14. Sharma S, Javadekar SM, Pandey M, Srivastava M, Kumari R, Raghavan SC. "Homology and enzymatic requirements of microhomology dependent alternative end joining". Cell Death & Disease. 2015;6(3): 58.
15. Steinhaus E. Concerning the harmlessness of insect pathogens and the standardization of microbial control products. *Journal of Economic Entomology*. 1957; (50)715-720.
16. Tanada Y, Kaya HK. *Insect pathology.* Academic Press, San Diego. (1993).
17. Van Zyl C, Malan AP. The Role of Entomopathogenic Nematodes as Biological Control Agents of Insect Pests, with Emphasis on the History of Their Mass Culturing and *in vivo* Production. *African Entomology.* 2014;22(2):235-249.

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