Global Scientific JOURNALS

GSJ: Volume 6, Issue 10, October 2018, Online: ISSN 2320-9186
www.globalscientificjournal.com

# ASSESSMENT OF ANTIMICROBIAL ACTIVITY OF PLECTASIN AGAINST LOCAL STRAINS OF GRAM NEGATIVE AND POSITIVE BACTERIA

***O. O. Afolabi[1], M. O. Adigun[1], R.F. Peters[2] , C. Okonofua[1], U. Umar[3] and O.E. Afuwape[1]**

*[1]Department of Biological Sciences, Crawford University, Faith City, Igbesa, Ogun State, Nigeria*
*[2]University Teaching Hospital, Idi-Araba, Lagos, Nigeria*
*[3]Department of Microbiology, Abubakar Tafawa Balewa University, Bauchi, Nigeria*
**d_afolabi@yahoo.com//+234 8023123868*

## ABSTRACT

Bacterial infection remains a leading cause of mortality worldwide and this is worsened by the continuous emergence of antibiotic resistance. Defensins show broad spectrum antimicrobial activity and Plectasin 4431-s an organic peptide defensin that has not had its potential negative effects and extent of antimicrobial action clarified was screened against genetically diverse clinical isolates including *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Acinectobacter baumanii*, *Salmonella typhi*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Enterococcus faecalis* and its antibiotic potential compared against other antibiotics which include levofloxacin-15µg, ceftriaxone-30µg, ciprofloxacin-5µg, imipenem-30µg, cefuroxime-30µg. The range of Minimum Inhibitory Concentration (MIC) of plectasin was between 0.007mg/ml to 1.8mg/ml. MIC for *Enterococcus faecalis* was at 0.056mg/ml, MIC for *Staphylococcus aureus* was at 0.113mg/ ml and MIC for *Klebsiella pneumoniae* was at 1.8 mg/ml. There was no inhibitory effect on *Escherichia coli* between plectasin concentration 0.007mg/ml to 1.8mg/ml while similarly *Proteus mirabilis*, *Acinectobacter baumanii*, *Salmonella typhi*, *Pseudomonas aeruginosa* MIC was nil. Results show that Plectasin 4431-s showed significant antimicrobial activity against some Gram positive bacteria such as *Staphylococcus aureus* at concentration 0.113mg/ml to 1.8mg/ml and *Enterococcus faecalis* at concentration of 0.056mg/ml to 1.8mg/ml. For Gram-negative bacteria, Plectasin 4431-s showed insignificant inhibitory effect on the growth of *Escherichia coli*, *Proteus mirabilis*, *Acinectobacter baumanii*, *Salmonella typhi*, *Pseudomonas aeruginosa*. The minimum bactericidal concentration of plectasin against *Enterococcus faecalis*, *Staphylococcus aureus* and *Klebsiella pneumoniae was* 0.056mg/ ml, 0.113mg/ ml and 1.8 mg/ ml respectively. Results obtained suggest that plectasin could be an alternative antibiotic for clinical applications against bacterial infection mostly against Gram-positive bacteria on which they have significant antibacterial activity. This will be a novel strategy that can have major clinical implications in the fight against bacterial infections.

***Keywords:*** Plectasin, gram-positive, gram-negative, bacterial infection, antibiotic

## INTRODUCTION

The epidemic of antimicrobial resistance is a growing public health threat. Bacteria have a high intracellular pressure, they must protect themselves from osmolysis with a rigid peptidoglycan, a meshwork of strands of glycan and peptide covalently cross-linked. Gram-negative bacteria, such as *Escherichia coli* and *Pseudomonas aeruginosa,* and Gram-positive bacteria, such as *Staphylococcus aureus* and *Streptococcus pneumoniae,* both have a peptidoglycan layer as part of their cell-wall structure. The peptidoglycan layer of Gram-positive bacteria is generally much thicker than that of Gram-negative bacteria, and constitutes the outermost layer. The peptidoglycan undergoes cross linking of the glycan strands by the action of transglycosidase, and of the peptide strands by the action of transglycosidase, and of the peptide strands by action of transpeptidase. In addition to Beta-lactams, vancomycin, considered a last resort among antibiotics, also targets peptidoglycan to block cell-wall biosynthesis. It binds to the D-$Ala_4$-D-$Ala_5$ moiety of pentapeptides and thereby keeps the substrates away from transpeptidase and transglycosidase. This substrate sequestion leads to the failure of peptidoglycan crosslinks, making the cell wall susceptible to osmolysis (Walsh, 2010). Although a large number of antibiotics are used clinically, the variety of targets that they inhibit is limited. Antibiotics are usually classified on the basis of chemical structure and mode of action. To understand how antibiotics work and why bacteria become resistance to them, a brief description of the targets of the main classes of antibiotics is required.



The main classes of antibiotics inhibit four classical targets; cell wall biosynthesis, protein biosynthesis, DNA and RNA biosynthesis, and (IV) folate biosynthesis (Walsh, 2009).

### Inhibition of Bacterial Cell Wall Biosynthesis

Since bacteria have a high intracellular pressure, they must protect themselves from osmolysis with a rigid peptidoglycan, a meshwork of strands of glycan and peptide covalently cross-linked. Gram-negative bacteria, such as *Escherichia coli* and *Pseudomonas aeruginosa,* and Gram-positive bacteria, such as *Staphylococcus aureus* and *Streptococcus pneumoniae,* both have a peptidoglycan layer as part of their cell-wall structure. The peptidoglycan layer of Gram-positive bacteria is generally much thicker than that of Gram-negative bacteria, and constitutes the outermost layer. The peptidoglycan undergoes cross linking of the glycan strands by the action of transglycosidase, and of the peptide strands by the action of transglycosidase, and of the peptide strands by action of transpeptidase. In addition to Beta-lactams, vancomycin, considered a last resort among antibiotics, also targets peptidoglycan to block cell-wall biosynthesis. It binds to the D-$Ala_4$-D-$Ala_5$ moiety of pentapeptides and thereby keeps the substrates away from transpeptidase and

transglycosidase. This substrate sequestion leads to the failure of peptidoglycan crosslinks, making the cell wall susceptible to osmolysis (Walsh, 2010).

**Inhibition of Protein Biosynthesis**

Protein biosynthesis, a central reaction for cellular function, is catalyzed by ribosomes, composed of two nucleoprotein subunits,30s and 50s subunits with about two-thirds RNA and one- thirds protein, in prokaryotes (Ban *et al.,* 2010*).*This machinery of prokaryotes differs substantially from the eukaryotic counterpart, which explains the effectiveness and selective toxicity of many clinically important antibiotics. The X-ray structure of 30s and 50s subunits (Schluenzen *et al.,* 2010*)* and of 70S ribosome reveals the architecture of this gain protein biosynthesis machinery in detail, and the peptidyltransferase activity deriving from the catalytic ribozyme domain of the 23S rRNA with no obvious assistance from protein subunits. Due to the large number of steps involved in protein biosynthesis, initiation, elongation and termination, it is not surprising that there are many steps to be blocked, or that many clinically useful antibiotics that interact with the conserved sequences of 16S rRNA of the 30S subunits (Aminoglycoside and tetracycline) and the 23S rRNA of the 50S subunit (macrolides, chloramphenicol, lincosamides, and quinupristin- dalfopristin) have been found (Carter *et al.,* 2010).

**Inhibition of DNA And RNA Biosynthesis**

DNA replication is an essential process for all organisms. Bacteria chromosomal DNA is packed in a highly twisted (supercoiling) state in the cell, and dynamically changes its structure during the replication process (Sherratt, 2003). The enzymes involved in interconversion of a topologically different form of the DNA are called topoisomerases. The DNA topoisomerase are classified into type I and type II type I topoisomerase transiently breaks either one strand at a time, while type II topoisomerase breaks both strands at the same time (Maxwell, 2009). Due to the essential character of DNA replication, it is natural that microorganisms such as S*treptomycetes* produce metabolites such as novobiocin and coumermycin that kill their neighbours by inhibiting the DNA replication process, the target of which has been found to be type II topoisomerase, specifically DNA gyrase. Nalidixic acid, the prototype quinolone, was discovered to possess antibacterial activity in 1962 and was approved for urinary tract infections in 1964. This finding led to more potent quinolone antibiotics, such as norfloxacin, ciprofloxacin, ofloxacin, and gatifloxacin, called Fluoroquinolones due to the presence of a fluorine substituent. These quinolones target the

DNA gyrase and affect the double-strand cleavage double-strand religation equilibrium in the gyrase catalytic reaction, in such a way that the cleaved complex accumulates by stabilization of the complex in the presence of quinoles. A second type II topoisomerase, known as topoisomerase IV, is also implicated in DNA replication and decatenation of knotted daughter chromosomes at the end of bacterial DNA replication, and thus is an important target, probably the primary one in *Staphylococcus aureus.* Transcription is an essential process for decoding genetic information from DNA to RNA in all organisms. RNA polymerase of bacteria composed of different subunits with a stoichiometry to form the core enzyme catalyzes transcription Rifampicin is a semisynthetic version of rifamycin B, isolated from a strain of *Amycolatopsis mediterranea* (formerly known as *Streptomyces mediterranea or Nocardia mediterranea*) (Masceretti, 2003).

**Other Targets**

**Folic Acid Metabolism**

The longest-used antibacterial agents are the sulfa drugs, or sulfonamides, first introduced in the 1930s (Walsh, 2009). The current generation of sulfa drugs is sulfamethoxazole, which is used in combination with trimethoprim. Each antibacterial agent is bactericidal when used together. This effect makes the combination recipe remains clinically relevant in the treatment of many bacterial infections, such as urinary and respiratory tract infections. Each of the drugs inhibits distinct steps in folic acid metabolism. Sulfamethoxazole inhibits dihydropteroate synthase in the biosynthetic pathway of folate in a competitive manner with higher affinity for the enzyme than a natural substrate, *p*-aminobenzoic acid .The resulting dead-end product cannot be a substrate for the next enzyme, dihydrofolate synthase. Since humans do not have dihydropteroate synthase, sulfa drugs achieve high selectivity against bacteria. Trimethoprim inhibits dihydrofloate reductase, a key enzyme providing a one carbon carrier, tetrahydrofolate, for the synthesis of deoxythymidylate, purine nuecleotides, and the amino acids glycine and methaionine. A combination of trimethoprim and sulfa drugs acting at distinct steps on the same biosynthetic pathway shows synergy and a reduced mutation rate for resistance as compared to the use of each drug alone provides validation of combination chemotherapy (Masceretti, 2003).

**Cell Membranes**

The integrity of cell membranes including the outer membrane of Gram-negative bacteria is essential to all organisms. Cationic peptides exemplified by the polymyxins, a group of cyclic peptides with a fatty acid chain attached to the peptide, attack the cytoplasmic membrane of Gram-positive bacteria, and Gram negative membrane and the outer membrane of Gram-negative bacteria. The interaction of the cationic peptide and the membrane disrupts membrane organization and causes increased permeability of cell

components, which eventually leads to cell-killing. The toxicity of polymycin B due to its membrane- active nature restricts its use in tropical applications. A second set of an bacterial peptides is bacteriocins produced by both Gram-positive and Gram- negative bacteria to compete with neighbouring bacterial cells with some exceptions, most of these bacteriocins, such as nisin, permeabilize targets cell membranes (Hancock and Chappie, 2007).

Studies on the mechanism of action of nisin show that it binds with high affinity to docking molecules, lipid II, a precursor of cell wall biosynthesis, leading to the hypothesis of the dual functionality of nisin. Recent structural analysis of the nisin-lipid II complex reveals that a novel lipid II recognition motif of nisin and related antibiotics (lanthionine-containing antibiotic peptides) binds to the pyrophosphate moiety of lipid II. This provides a basis for rational design of future antibiotics (Hsu *et al.,* 2010).

Unfortunately, few drug classes have been identified and brought to market in the last decade. Antibiotics are compounds that can effectively inhibit the growth of microorganisms. They have been used for the treatment of bacterial diseases since the early 20th century. After the introduction of penicillin, many classes of antibiotics were discovered and most infectious diseases were brought under control. However, the increased use of antibiotics in clinical practice was soon followed by the emergence of antibiotic resistance. Indeed, resistance started appearing in target organisms within a few years of introduction of antibiotics into medical practice (Scheffler *et al.,* 2013). Antibiotics tend to lose their efficacy over time owing to the emergence of new strains with resistance to multiple antibiotic classes. One recently reported novel antibacterial compound is from the plectasin class. Plectasin antibiotics are defensin-like peptide antibiotics of fungal origin which supposedly exhibit broad-spectrum activity against gram-positive bacteria, including potency against multiple drug-resistant strains. It specifically bind a target molecule and interferes with bacterial biosynthesis, resulting in rapid cell death.

Plectasin is a secondary metabolite with a strong affinity for Gram-positive bacterium cell wall subunit Lipid II, inhibits the formation of bacterium cell wall, and present bacterial breeding. Structurally, this 40-amino acid residue peptide can fold into a CSαβ structure, resembling potassium channel-blocking scorpion toxins together with same disulfide bridge patterns. Of concern is the fact that bacteria resistance to antibiotic is now recognized as a major global health, security issue that threatens a return to the pre-antibiotic era, with potentially catastrophic economic, social and political ramifications. Although bacteria naturally adapt to outsmart antibiotics, human actions accelerate the development and spread of resistance (Schneider *et al*., 2010).

Basically, Plectasin has strong affinity to Gram-positive bacterium cell wall subunit Lipid II, inhibits the formation of bacterium cell wall, and present bacterial breeding. Structurally, this 40-amino acid residue peptide can fold into a cysteine-stabilized alpha-beta (CSαβ) structure, resembling potassium channel-blocking scorpion toxins together with same disulfide bridge patterns. Similarly, fungal defensin-like peptides (fDLPs) with plectasin as a member of the family are emerging as attractive anti-infective agents due to their therapeutic efficacy, low toxicity and high serum stability. On the basis of combined analyses of sequence, structural, and phylogenetic data, plectasin classified as ancient invertebrate-type defensins (AITDs), have been structurally and functionally characterized. The plectasin specifically bind a target molecule and interfere with bacterial biosynthesis, resulting in rapid cell death (Schneider *et al*., 2010). Plectasin is the first defensin-like antimicrobial peptide isolated from a black saprophytic ascomycete (*Pseudoplectania nigrella*) and it shows potent activity against gram-positive bacteria in vitro and in vivo. It consists of 40 amino acids with three intramolecular disulfide bridges between C1 and C5, C2 and C4, and C3 and C6 that stabilize its alpha helix-beta motif tertiary structure (Mandell *et al*., 2010).

Plectasin antibiotics are defensin-like peptide antibiotics of fungal origin, these compounds exhibit broad-spectrum activity against gram-positive bacteria, including potency against multiply drug-resistant strains. The plectasin specifically bind a target molecule and interfere with bacterial biosynthesis, resulting in rapid cell death (Mygind *et al.,* 2005). These Fungal defensin-like peptides exhibit activity against several antibiotic-resistant clinical isolates with significant therapeutic potential. Some efforts have been taken to improve antimicrobial efficacy and to reduce undesirable side effects of fungal defensin-like peptides. Defensins are a family of evolutionary related antimicrobial peptides, they are part of the second defence system, innate immunity that was discovered in the early 1980s in the higher organisms also defensins have been reported across prokaryotic and eukaryotic kingdoms, including Bacteria, Fungi, Plantae, and Animalia. Furthermore, due to the action of Plectasin it can be considered as the best candidate for antimicrobial drug development for clinical application due to its low toxicity, immunogenicity, and high stability. To solve this problem, identifying and developing new and organic antibiotics is an urgent need (Silva *et al.*, 2014).

## MATERIALS AND METHODS

### Study Area

Clinical isolates were obtained from the bacteriology (Medical microbiology and parasitology laboratory) of the Lagos University Teaching Hospital (LUTH).

### Identification and Confirmation Clinical Isolates

Samples are taken from urine, wound and ear swab and blood culture. Macroscopy, microscopy and culture was done on every sample.

### Culture

The urine samples are cultured on MacConkery agar and chocolate agar which is done by taking an inoculating loop full and streaking on the agar.

### Microscopy

The microscopic examination of the organisms was carried out according to the method described by Cheesbough (2006).

### Preparation of smear

1-2 drops of normal saline was dropped on a clean slide. A loopful of the test organism was then transferred onto the normal saline and spread over a small area and allowed to air-dry. The dried smear was heated fix by passing the slide through the Bunsen flame 2-3 times.

### Gram staining of the isolates

A thin smear of each isolate was made and allowed to dry at room temperature and passed over a bursen flame to fix. The fixed smear was then stained with crystal violet for 30 min and washed under running water. Lugol's iodine was applied for 30 s tipped off and decolorized very rapidly using 70 % alcohol and washed with running tap counter stain was done by carbon fuschin for 30 s and washed on running tap. Slides are blotted and examined under microscope using oil immersion.

### Isolation of *Staphylococcus aureus*

A colony of suspected *S. aureus* which appeared off-white on nutrient agar was picked and inoculated using sterile inoculating loop on Manitol salt Agar plate and incubated at $37^0$C for 12-18 h. Yellow appearance on the plate depicts *S. aureus*.

### Isolation of *Escherichia coli*

To yield a pure discrete colony of *E.coli,* a colony of suspected *E. coli* which appears pink on MacConkey agar was picked using a sterile inoculating loop and streaked on Eosin methylene blue agar (EMBA) which had been prepared according to manufactures' instruction and incubated at $37^0$C for 12-18 h. Green metallic sheen on the line of streaking on Eosin methylene blue agar (EMBA) plates shows *E. coli.*

### Isolation of *Klebsiella*

*Klebsiella* formed typical pink colonies which were wet and raised on MacConkey agar. Colonies suspended to be for *Klebsiella* were subjected to biochemical tests for confirmation.

### Microbact identification system

#### Isolation

An 18-24 h pure culture of the organism to be identified was obtained. Agars such as MacConkey, blood or chocolate were used to grow the organisms.

Inoculation

The wells of individual substrate are expoterile by slowly peeling the tag slowly. Four drops of the bacterial suspension using a pipette is added to each well. Using a sterile pipette or dropper bottles, sterile mineral oil was added to wells (1, 2, 3) for 12A/12E and added for well 8 (12B), well 20 (24E), well 12 (12B), well 24 (24E) and well 12 (12B), well 24 (24E), for miscellaneous gram negative bacilli.

Incubation

The inoculated rows resealed with the adhesive seal and the specimen identification number was written on the end tag with a marker pen. It is incubated at $35^0 \pm 2^0$C for 18 to 24 h. To determine the purity of the inoculums, it was advisable to inoculate a solid non-selective medium with the test suspension to act as a culture purity check.

Reading of the test strip

1. The 12A/12E strip was read at 18-24 h. The 12B was read at 24 h in identifying enterobacteriaceae.
2. The strip is removed from the incubator, the seal was peeled back. All possible result was recorded. The result was evaluated as positive or negative by comparing them with the colour chat given in the kit. The result was recorded under the heading on the report form.
3. For 12A/12E or 24E, the following reagents is added:

Well 8 (indole production) - two broth of indole was added and read within 2 min of the addition.

Well 10 (vogues-proskauer reaction) one drop was added each of the v-p 1&11 reagents and read15mins to 30mins after the addition.

Well 12 (tryptone deaminase) one drop of TDA was added and read immediately after addition.

4. For 12B/24E

The gelatine well was read at 24-48 h. Hydrolysis of gelatine is indicated by dispersal of the back particles throughout the walls. The arginine reaction is interpreted differently at 24 h and 48 h of incubation.

Computer aided identification package

The microbact Computer aided identification package is used for identification. The percentage figures shown against the organism name is the percentage share of the probability for that organism as a part of the total for all choices.

Antimicrobial Susceptibility Tests

The susceptibility of isolates to various antibiotics was tested using Kirby-Bauer disc diffusion method. Mueller Hinton agar was used. Gram negative multiple discs were used. Inoculums were prepared by touching a colony of the test organism with a wire loop and the growth transferred into a bijou bottle containing Mueller Hinton broth and the incubated for 2 h. Mueller-Hinton plates were dried in the hot air oven by inverting Petri dishes containing the media to remove surface moisture. Plates are inoculated using a sterile cotton swab dipped into the suspension of each isolate and rotated against the side of the bottles above fluid level to remove surplus fluid. The Medias were then inoculated by streaking of the swab over the entire surface of the plates in three directions at $60^0$ of each other. On each plate was placed antibiotic discs for different antibiotics using forceps and pressed gently to

ensure contact with the media. .The antibiotic discs used include, levofloxacin-15µg, ceftriaxone-30µg, ciprofloxacin-5µg, imipenem-30µg, cefuroxime-30µ
The discs were placed with enough space (about 24 mm apart) between each disc measured from the centre of each disc. The plates were then incubated overnight at $35^0$C.
After the period of incubation, zones of inhibition around each disc was measured in mm with the aid of a ruler from one end to the other including the diameter of the disc.

## RESULT AND DISCUSSION

Antibiotics Susceptibility Testing

The isolates which were characterized using Microbact® kit were *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Acinectobacter baumanii Salmonella typhi, Klebsiella pneumoniae and Proteus mirabilis.* The antibiotic discs used include, levofloxacin-15µg, ceftriaxone-30µg, ciprofloxacin-5µg, imipenem-30µg, cefuroxime-30µg. Some were resistant while others were sensitive to these antibiotics. The antibiotics susceptibility pattern is as shown on Table 1.

**Table 1: Antibiotic Susceptibility Pattern of the Isolates**

| | levofloxacin-15µg, | ceftriaxone-30µg, , | ciprofloxacin-5µg | imipenem-30µg, | cefuroxime-30µg |
|---|---|---|---|---|---|
| *Escherichia coli* | 33S | 30S | 26S | 33S | 6R |
| *Staphylococcus aureus* | 24S | 25S | 26S | 21I | 31S |
| *Pseudomonas aeruginosa* | 22S | 6R | 29S | 24S | 6R |
| *Acinectobacter baumani* | 12R | 12R | 22.5S | 25S | 6R |
| *Salmonella typhi* | 22S | 22S | 17I | 26S | 15I |
| *Klebsiella pneumoniae* | 17S | 30S | 22S | 24S | 10R |
| *Proteus mirabilis* | 6R | 36S | 6R | 6R | 6R |

S- SENSITIVE R- RESISTANT I-INTERMEDIATE

**Table 2: Details of the antibiotics that were used in the study to test for antibiotics resistance.**

| Group | Antibiotics | Abbreviation | Generally accepted antibiotics disc concentrations (µg) | Resistant (mm) | Intermediate Resistant (mm) | Susceptible (mm) |
|---|---|---|---|---|---|---|
| Cephems | Ceftriaxone | CRO | 30 | 13 | 14-20 | 21 |
| Quinolones | Levofloxacin | LEV | 5 | 13 | 14-16 | 17 |
| Carbapenems | Imipenem | IMP | 30 | 19 | 20-22 | 23 |
| Cephems | Cefuroxime | CAZ | 30 | 17 | 18-20 | 21 |
| Quinolones | Ciprofloxacin | CIP | 5 | 15 | 16-20 | 21 |

Minimum Inhibitory Concentration and Plectasin Dilution Results

To confirm that plectasin has an inhibitory effect against several species of gram-positive bacteria, an antimicrobial assay was performed first. As shown in table 3, growth of *Staphylococcus aureus* was markedly inhibited, suggesting that plectasin is a biologically active agent. Gram-negative bacteria, including *Pseudomonas aeruginosa,* were considerably more resistant to plectasin.

All well 10 ⟶ Growth control turbid (shows organism are viable)

All well 11 ⟶ Broth control clear (shows broth was sterile)

A ⟶ *Enterococcus faecalis sp* MIC at well 6 concentration 0.056mg/ ml

B ⟶ *Staphylococcus aureus* MIC at well 5 concentration 0.113mg/ ml

C ⟶ *Escherichia coli* MIC Nil between plectasin concentration 1.8mg/ml to 0.007mg/ml

D ⟶ *Klebsiella pneumoniae* MIC at well 1 concentration 1.8 mg/ ml

E, F, G, H ⟶ *Proteus mirabilis, Acinectobacter baumanii, Salmonella typhi, Pseudomonas aeruginosa* NIL MIC between plectasin concentrations is greater than 1.8mg/ml to 0.007mg/ml

**Table 3: The minimum inhibitory concentration and plectasin dilution**

| | | 1.8 mg/ ml | 0.9 mg/ ml | 0.45 mg/ ml | 0.225 mg/ ml | 0.113 mg/ ml | 0.056 mg/ ml | 0.028 mg/ ml | 0.014 mg/ ml | 0.007 mg/ ml | MIC growth control | Broth control |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | *Enterococcus facealis* | - | - | - | - | - | - | + | + | + | + | - |
| B | *Staphylococcus aureus* | - | - | - | - | - | + | + | + | + | + | - |
| C | *Escherichia coli* | + | + | + | + | + | + | + | + | + | + | - |
| D | *Klebsiella pneumoniae* | - | + | + | + | + | + | + | + | + | + | - |
| E | *Proteus mirabilis* | + | + | + | + | + | + | + | + | + | + | - |
| F | *Acinectobacter baumani* | + | + | + | + | + | + | + | + | + | + | - |
| G | *Salmonella typhi* | + | + | + | + | + | + | + | + | + | + | - |
| H | *Pseudomonas aeruginosa* | + | + | + | + | + | + | + | + | + | + | - |



No growth-(-)

Presence of growth-(+)

**Table 4: Minimum Bactericidal Concentration**

| | WELL 1 1.8 mg/ ml | WELL 2 0.9mg/ ml | WELL 3 0.45 mg/ ml | WELL 4 0.225 mg/ ml | WELL 5 0.113 mg/ ml | WELL 6 0.056 mg/ ml | WELL 7 0.028 mg/ ml | WELL 8 0.014 mg/ ml | WELL 9 0.007 mg/ ml |
|---|---|---|---|---|---|---|---|---|---|
| *Enterococcus Facealis* | - | - | - | - | - | - | + | + | + |
| *Staphylococcus aureus* | - | - | - | - | - | + | + | + | + |
| *Klebsiella pneumoniae* | - | + | + | + | + | + | + | + | + |

No growth-(-)

Presence of growth-(+)



**Table 5: Preliminary identification of bacterial isolates**

| COLONIAL MORPHOLOGY AFTER 24 HRS AT $37^{O}$C | GRAM REACTION | PRESUMPTIVE IDENTIFICATION |
|---|---|---|
| Golden yellowish appearance on Mannitol Salt Agar | Gram-positive cocci in clusters | *Staphylococcus aureus* |
| Pink coloration on Mac Conkery agar and small green metallic sheen on Eosin Methylene Blue Agar | Gram-negative | *Escherichia coli* |
| Smooth circular, mucoid with pink coloration and blue on simmon's citrate slant. | Gram-negative rod | *Klebsiella pneumoniae* |
| Black coloration on salmonella shigella Agar(SS) | Gram-negative rod | *Salmonella typhi* |
| Swarming on agar | Gram-negative rod | *Proteus mirabilis* |

| | | |
|---|---|---|
| Coccobacillary on non-selected agar. Rods predominate in fluid media | Gram-negative rod | *Acinectobacter baumanii* |
| Non-hemolytic colonies on sheep blood agar | Gram-negative commercial bacterium | *Enterococcus facealis* |
| | Gram-negative gamma proteobacteria | *Pseudomonas aeruginosa* |

**Table 6: Biochemical test and identification of isolates**

| Isolate | *Pseudomonas aeruginosa* | *Staphylococcus aureus* | *Proteus mirabilis* | *Klebsiella pneumoniae* | *Escherichia coli* | *Acinectobacter baumanii* | *Salmonella typhi* | *Enterococcus facealis* |
|---|---|---|---|---|---|---|---|---|
| Oxidase | - | - | - | - | - | + | - | - |
| Motility | - | - | - | - | - | - | - | - |
| Nitrate | - | - | - | - | + | - | - | - |
| Lysine | + | + | - | + | + | + | + | + |
| Ornithine | - | - | + | - | - | - | - | - |
| $H_2S$ | - | - | - | - | - | - | - | + |
| Glucose | + | + | + | - | + | - | + | + |
| Mannitol | + | + | - | - | + | - | + | + |
| Xylose | - | - | + | - | + | - | - | + |
| ONPG | - | - | + | + | + | - | - | + |
| Indole | - | - | - | - | - | - | - | - |
| Urease | + | - | + | + | - | - | - | + |
| V-P | - | - | + | + | - | - | - | + |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Citrate | + | - | - | + | - | - | - | + |
| TDA | + | - | + | - | - | - | - | + |
| Gelatin | - | - | - | - | - | - | - | - |
| Malonate | - | - | - | - | - | + | - | + |
| Inositol | - | - | - | - | - | - | - | - |
| Sorbitol | - | + | - | - | + | - | + | + |
| Rhamnose | - | - | - | - | + | - | - | + |
| Sucrose | - | - | - | - | - | - | - | + |
| Lactose | - | - | - | - | - | - | - | - |
| Arabinose | - | - | + | - | + | + | - | + |
| Adonitol | - | - | - | - | - | - | - | - |
| Raffinose | - | - | - | - | - | - | - | + |
| Salicin | - | - | - | - | - | - | - | - |
| Arginine | + | - | + | - | + | - | - | - |



This study revealed the effect of plectasin 4431-s on eight bacterial isolates comprising of *Staphyloccus aureus, Klebsiella pneumoniae, Escherichia coli, Salmonella typhi, Proteus mirabilis, Acinectobacter baumanii, Pseudomonas aeruginosa, Enterococcus faecalis* . Five of the isolates are Gram-negative and three of isolates organisms are Gram-positive.

Different classes of antibiotics such as levofloxacin-15µg, ceftriaxone-30µg, ciprofloxacin-5µg, imepenem-30µg, cefuroxime-30µg were tested one each of the isolated bacteria. ( *E.coli* 33S, *S.aureus* 24S,*P.aeruginosa* 22S, *S.typhi* 22S, *K.pneumoniae* 17S*)* were susceptible to levofloxacin while, *(A.baumanii* 12R and *P.mirabilis* 6R*)* were resistant. *(E.coli* 30S, *S.aureus* 25S, *S.typhi* 22S, *S.aureus* 36S, *K.pneumoniae 30S*) were susceptible to ceftriaxone while *(P.aerugino sa* 6R, *A.baumanii* 12R*)* were resistant. *(E.coli* 26S, *S.aureus* 26S, *A.baumanii* 29S , *P.aeruginosa* 22.5S, *K.pneumoniae* 22S*)* were susceptible to ciprofloxacin, (*P.mirabilis* 6R and *S.typhi* 17I*)* resistant. *(E.coli* 33S, *S.typhi* 26S, *P.aeruginosa* 24S, *K.pneumoniae* 24S, *A.baumanii* 25S*)* were susceptible to imipenem while *(S.aureus* 21I and *P.mirabilis* 6R*)* resistant. (*E.coli* 6R, *S.typhi* 6I, *P.aeruginosa 6R, A.baumanii 6R,*

*K.pneumoniae 6R, P.mirabilis 6R)* were resistant to cefuroxime, *S.aureus 31S* was susceptible.

Plectasin is a defensin that has shown promise but has not had its potentially negative effects clarified (Quiros, 2011). Investigations ( MIC and MBC) were performed to test against genetically diverse clinical isolates of *Enterococcus faecalis, Staphylococcus aureus, Klebsiella pneumoniae, Escherichia coli, Proteus mirabilis, Acinectobacter baumanii, Salmonella typhi, Pseudomonas aeruginosa* on plectasin 4431-s.

Minimum Inhibitory Concentrations (MIC) was carried out and determined by the broth micro dilution method. The Minimum Bactericidal Concentration was also determined by subculturing 0.01ml of the highest concentration of the agent that shows visible growth and the other wells with no visible growth in the MIC dilution wells to sterile media. The MIC is between plectasin concentrations 0.007mg/ml to 1.8mg/ml.

MIC for *Enterococcus faecalis* was at 0.056mg/ml, MIC for *Staphylococcus aureus* was at 0.113mg/ ml, MIC for *Klebsiella pneumoniae* was at 1.8 mg/ ml. There was no inhibitory effect on *Escherichia coli* between plectasin concentration 0.007mg/ml to 1.8mg/ml while similarly *Proteus mirabilis, Acinectobacter baumanii, Salmonella typhi, Pseudomonas aeruginosa* MIC was nil. Results show that Plectasin 4431-s showed significant antimicrobial activity against some gram positive bacteria such as *Staphylococcus aureus* at conc. 0.113mg/ml to 1.8mg/ml and *Enterococcus faecalis* at conc. of 0.056mg/ml to 1.8mg/ml. For gram-negative bacteria, Plectasin 4431-s showed negligible inhibitory effect on the growth of *Escherichia coli, Proteus mirabilis, Acinectobacter baumanii, Salmonella typhi, Pseudomonas aeruginosa*. The minimum bactericidal concentration of plectasin against *Enterococcus faecalis, Staphylococcus aureus* and *Klebsiella pneumoniae was* 0.056mg/ ml, 0.113mg/ ml andconc.1.8 mg/ ml respectively.

This shows that plectasin can be synthesized, and fully processed active plectasin can be effectively produced at high yields (Mygind *et al.,* 2005). The results indicate that plectasin offers promise as a new antibiotic against Gram-positive bacteria without side effects for systemic use. It is also noteworthy that similar activities have been demonstrated against Gram-negative bacteria (*Escherichia coli and Pseudomonas aeruginosa)* and other antibiotics which include levofloxacin-15µg, ceftriaxone-30µg, ciprofloxacin-5µg, imipenem-30µg, cefuroxime-30µg. Some were resistant while others were sensitive to these antibiotics.

Result suggested that plectasin could be an alternative antibiotic for clinical application and fight against bacterial infection mostly gram-positive bacteria that plectasin have antibacterial activity on.

## RECOMMENDATION

This is a novel organic antibiotic that can have major clinical implications in fight against bacterial infections. The development of effective antimicrobial agents to treat these infections is an area of intense research. Peptide antimicrobial agents represent a promising new class of compounds which collectively act at a number of different bacterial targets and have demonstrated potency against these emerging pathogens. It is therefore recommended that more research should be done on the peptide plectasin and tested on fungi and more bacteria, which may include a new therapeutic concept of the conventional anti-bacterial therapy, used as an alternative antibiotic for clinical application and fight against bacterial infection. It is also recommended that the drug concentrations should be increase in other to check for the antimicrobial effect of plectasin on the organisms.

## REFERENCES

Ban N., Nissen P., Hansen J., Moore P.B., Steitz T.A. (2010). The complete atomic structure of the large ribosomal subunit at 2.4 A resolution.*science*,289,905-920.

Carter A.P., Clemons W.M., Brodersen D.E., Morgan-Warren R. j., Wimbwerly B. T., Ramakrishnan V.(2010). Functional insights from the structure of 30S ribosomal subunit and its interactions with antibiotics . *Nature* 407 340-348.

Cheesbough M.(2006). District Laboratory Practice in Tropical Countries. Cambridge University. Pp. 62

Hancock R.E.W., Chappie D.S. (*2007).* Peptide Antibiotics. *Antimicrob Agents Chemother., 43,1317-1323.*

Hsu S.T.D., Breukink E., Tishenko E., Lutters M.A.G., De Kuijuff B., Bonvian A. N.J.J., Van Nuland N.A.J. (2010). The nisin- lipid II complex reveals a pyrophosphate cage that provides a blue print for novel antibiotics. *Nat. struct. mol.biol* 11,963-967.

Mandell *Douglas, and Bennett. (*2010*).Enterobacteriaceae. Mandell,Principles and Practice of Infectious Diseases. 7th ed. Churchill Livingstone, An Imprint of Elsevier.*

Mascaretti O.A. (2003). ”Bacteria versus Antibacterial Agents, an Integrated Approach*,”ASM Press, Washington, DC*,pp 97-105.

Maxwell A. (2009). DNA gyrase as a drug target. *Trends microbial.*, 5,102-109.

Mygind P. H., Fischer R. L., Schnorr K. M., Hansen M. T., Sonksen C. P., Ludvigsen S., Raventos D., Buskov S., Christensen B., De Maria L., Taboureau O., Yaver D., Elvig-Jorgensen S. G.,Sorensen M. V., Christensen B. E., Kjaerulff S., Frimodt-Moller N., Lehrer R. I., Zasloff M., and Kristensen H. H.(2005). Plectasin is a peptide antibiotic with therapeutic potential from a saprophytic fungus. *Nature* 437:975–980

Scheffler R.J., Colmer S., Tynan H., Demain A.L., Gullo V.P. (2013) Antimicrobials, drug discovery, and genome mining. *Appl. Microbiol. Biotechnol.*, (97) 969–978.

Schluenzen F., Tocilj A., Zarivach R., Harns J., Gluehman M., Janell D., Bashan A., Bartels H., Agmon I., Franceschi F., Yonath A. (2010). Structure of functionally activated small ribosomal subunit at 3.3 A resolution.*cell*, 102, 615-623.

Schneider T., Kruse T., Wimmer R.,Wiedemann I.,Sass V.,Pag U.,Jansen A., Nielsen, A.K.; Mygind, P.H.; Raventos, D.S. (2010). Plectasin, a fungal defensin, targets the bacterial cell wall precursor Lipid II. *Science*, 328, 1168–1172.

Sherratt D.J. (2003). Bacaterial chromosome dynamics.*Science*, *301,* 780-785.

Silva P.M.,Goncalves S.,Santos N.C. (2014).Defensins:antifungal lessons from eukaryotes. *Front. Microbiol*.5, (97). D,eds. Molecular aspects of chemotherapy. *Newyork perganman.:*205-213 .

Walsh, C. (2009). "Antibiotics, Action, Orgins, Resistance," *ASM Press, Washington*, DC. Pp.3-49.

Walsh, C. (2010). Where will new antibiotics come from? *Nat. Rev. Microbiol.* 1:65–70