



(RESEARCH ARTICLE)



## Isolation of antibiotics producing bacteria from marine soil and comparative analysis of same with commercially available drugs

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### Abstract

The isolation of antibiotic producing bacteria from marine soil and comparative analysis of same with ciprofloxacin and amoxicillin against *staphylococcus aureus* and *Escherichia coli* was carried out in a Microbiology Laboratory of Chukwuemeka Odumegwu Ojukwu University, Uli. This was done to isolate antibiotic producing bacteria and compare same with existing commercially available antibiotics with a view to using marine soil in the treatment of common bacterial infections. Soil samples were collected from Bonny Island Sea, Port Harcourt. One gram of mixed soil sample was serially diluted and spread-plated on nutrient agar plates. The representative isolates obtained were sub-cultured to get a pure culture. Morphological, biochemical, physiological characteristics of the bacteria were analyzed. Agar well diffusion was carried out. One isolate had a substantial antibacterial activity with 3.5mm zone of inhibition against two test bacteria used in the preliminary screening. The isolate was marked as *Streptomyces* (STR I) and was identified as *Streptomyces griseus* while other isolates did not show any antibacterial activity. Ciprofloxacin showed the highest antibacterial activity to both *Staphylococcus aureus* and *Escherichia coli* of 3.7mm and 4.0mm respectively while Amoxicillin showed antibacterial activity of 3.5mm and 2.7mm respectively. This reveals that antibiotic producing bacteria from marine soil are also effective in antimicrobial activity and could be used for antimicrobial chemotherapy.

**Keywords:** Antibiotics; Marine; Soil; Bacteria; Isolate; Antibacterial

### 1. Introduction

The oceans cover more than 70% of the earth's surface and contain about 80% of the world's plant and animal tissues. Oceans have plenty of structurally unique metabolites and other resources in the living and dead forms. About 10,000 metabolites have been isolated from different marine organisms. Among them 37% has been isolated from sponges, 21% from coelenterates, 18% from microorganisms, 9% from algae, 6% from echinoderms, 5% from tunicates, 2% from molluscs and 1% from bryozoans [1].

The marine environment is a prolific resource for the isolation of less exploited microorganisms. In recent years microorganisms have become important in the study of novel microbial products exhibiting antimicrobial, antiviral, and antitumor as well as anticoagulant and cardioactive properties. These active compounds may serve as model systems in the discovery of new drugs. Many organisms had developed complex adaptive and self-protecting mechanisms to survive, often associated with the production of structurally unique bioactive compounds. Many such compounds have been extracted from various marine organisms such as bacteria, cyanobacteria, seaweeds, sponges, cnidarians, tunicates, soft corals, bryozoans, molluscs, echinoderms, fish and sea snakes. Marine bacteria produce broad-spectrum classical antibiotics and a variety of toxins such as tetratoxins, saxitoxin, cigualoxins and brevetoxins which are useful in neuro physiological and neuro pharmacological studies. Production of antimicrobial compound seems to be a general

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phenomenon for most bacteria. An antimicrobial is a substance that kills or inhibits the growth of microbes such as bacteria, fungi or viruses. The discovery of antibiotics has revolutionized the world of medicine. The decreasing rate of discovery of novel drugs from established terrestrial sources has motivated the evaluation of new sources of chemically diverse objective compounds [2]. Microbes are known to form a highly specific and symbiotic relationship with filter feeding organisms like sponges, algae, with their nutrient-rich and host associated environments forming unique niche for microbial exploitation. The sponge-microbe association has attracted a number of researchers both for their diversity and secondary metabolite production which have been associated with antimicrobial, antifouling, HIV protease inhibitory, HIV-reverse transcriptase inhibitory, immune suppressant and cytotoxic activities. Epibiotic bacteria growing on the surfaces of marine algae and other organisms live in a highly competitive environment and can produce secondary metabolites which inhibit the settlement of potential competitors such as invertebrate larvae and can antagonize other bacteria [3]. Microalgae are significant resource for bioactive metabolites, particularly cytotoxic agents with an application in cancer chemotherapy. The algal extracts with antibacterial activity can be used as antibiotics which are good for health and fails to cause side effects. [10]. Thus, it is widely used for pharmaceutical purposes. Various strains of cyanobacteria produce intracellular and extracellular metabolites with diverse biological activities such as antifungal, antibacterial, ant algal and antiviral activity. *Streptomyces* is the largest antibiotic producing genus in the microbial world. Most *Streptomyces* and other *Actinomycetes* produce a diverse array of antibiotics including aminoglycosides, anthracyclines, glycopeptides, -lactams, macrolides, nucleosides, peptides, polyenes and tetracyclines [4]. These metabolites a wide range of applications as it possesses various properties like antibacterial and antifungal activities inhibit the growth of leukemia cell lines, and can be used to treat the immunosuppressive patients suffering from a several opportunistic pathogens. Many useful chemicals like Gliovictin have been derived from marine fungi. The oceans represent an under explored environment for microbial discovery. The marine bioactive compounds or marine natural products (MNPs) are known to be produced by many heterotrophic bacteria.

These antibacterial compounds are inhibitory to terrestrial as well as indigenous bacterial strains, which is of considerable ecological significance [5]. The search for new antimicrobial agent is a field of almost importance. Marine environment provides the most effective drugs used in human therapy. The prevalence of antimicrobial resistance among key microbial pathogen is increasing at an alarming rate worldwide. As bacteria are continuously overcoming the tools with which humans have to fight, there is a need for search for new antibiotics that affect the target. The full wealth of microbial diversity in the sea is yet to be revealed. A large fraction of marine bacteria has not been cultured yet, and novel cultivation methods need to be developed in order to culture them. Marine microorganisms have received very little attention in drug discovery mainly due to the cultural difficulty. Competition among microbes for space and nutrients in marine environment is a powerful selection pressure that endows marine microorganisms to produce marine natural products. Today, both academic and industrial interest in marine microorganisms is on the rise. Marine microorganisms have become an important point of study in the search for novel microbial product. In the process of exploitation of marine microorganisms, the following steps are to be followed: Isolation of microorganisms from marine source, screening the isolated organism for our desired characteristics, identification of organisms for biochemical or molecular methods like 16S rRNA sequencing, DNA-DNA hybridization. By considering the scope of marine bacteria and the less exploited nature of marine microorganisms. This study was carried out to isolate antibiotic producing bacteria from marine soil and compare with Ciprofloxacin and Amoxicillin against pathogenic bacteria *Staphylococcus aureus* and *Escherichia coli*.

### Objectives

- To isolate antibiotics producing bacteria from marine sea.
- To compare the activities of the marine antibiotic with commercially used antibiotics (Amoxicillin and Ciprofloxacin).
- To test the activities of these marine isolates against *Staphylococcus aureus* and *Escherichia coli*.

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## 2. Material and methods

### 2.1. Sample Collection

Wet soil samples were collected from Bonny Island Sea, Port Harcourt, Nigeria. Three samples (surface, middle and deep down) soil samples were mixed together and used for the isolation of bacteria which have antibacterial activity. The samples were collected in freshly purchased polythene bags and were brought to laboratory by preventing any contamination on the way. The samples were then stored at a temperature of 40°C until used, to minimize the metabolic activities of the microorganisms and to keep them in the exact qualitative and quantitation level of population.

## 2.2. Sterilization of Glass wares and Medium

The glassware were thoroughly washed with detergent and rinsed with sterile water, after which they were sterilized by autoclaving at 121°C for 15 minutes at 15psi. Please delete this sentence.

## 2.3. Culture Media

The culture media used include Nutrient agar to determine the total viable bacterial count. Cultures were prepared according to the respective manufactures specification and sterilized in are autoclave at 121°C at 15 psi for 15 minutes.

## 2.4. Isolation of Bacteria

The serial dilution and spread method were followed to isolate bacteria from the soil samples. 1ml of the water sample collected was added to 9 ml of sterile distilled water and was serially dilute up to  $10^{-7}$  dilution, 1g of wet soil sample was added to 9 ml of sterile distilled water and serially diluted up to  $10^{-7}$  dilution. The bacteria were isolated by spread plating 0.1ml of each of the dilution on nutrient agar plates.

## 2.5. Identification of Isolates

### 2.5.1. Morphological identifications

The colony of the pure culture of each bacterium isolates were observed for morphological features using methods of [6]. Cell's shape was determined under x 100 objective of the height microscope after Gram staining procedure [7].

### 2.5.2. Gram Staining

In this staining process, a thin smear of the culture was prepared on a clean grease free slide, air dried and heat fixed. The smear was flooded with crystal violent solution for 60 seconds and rinsed with water, gram iodine solution for 60 seconds and rinsed with water. Alcohol (95%) was used to decolorize the slide content for 10 seconds and rinsed with water. The smear was then counter stained with safranin solution for 30 seconds, rinsed and air-dried. The stained smear was used to decolorize the slide content for 10 seconds and rinse with water. The smear was then counterstained with safranin solution for 60 seconds, rinse and air dried. The stained smear was then observed under light microscope oil immersion objective lens. [7].

### 2.5.3. Maintenance of organisms

The isolated test organisms were used for the antibacterial sensitivity testing, prior to the test, the organisms were sub-cultured on nutrient agar plates at 37°C for 24 hours. Then the 24 hours culture was transferred into nutrient broth and incubated at 37°C for 24 hours [8].

### 2.5.4. Catalase Test

As stated in Prescott, hydrogen peroxide was the reagent used. A hopeful of the bacteria from a 24-hour old pure culture was transferred to a clean glass slide and a drop of the hydrogen peroxide was added to the bacteria on the slide.

Effervescence caused by the liberation of free oxygen as gas bubbles revealed positive results, indicating the presence of Catalase in the culture. Catalase is the enzyme capable of decomposing hydrogen peroxide to water and oxygen [10],[12].

### 2.5.5. Slide Agglutination test

As stated, UK Standards for Microbiology Investigations, this test is used to identify coagulase positive and negative bacteria These organisms that can produce the enzyme coagulase. A drop of distilled water was placed on each end of the slide. Then was emulsified by a colony of test organisms.

A drop of plasma was added to top of the suspensions and mixture gently. Clumping of the organisms within 10 minutes indicating a positive result.

### 2.5.6. Oxidase Test

The Oxidase test is used to identify organisms that produce cytochrome C oxidase, an enzyme of the bacterial electron transport chain. A filter paper staked with the substrate tetramethyl-P-phenylene diamene dihydrochloride and

addition of sterile distilled water. Pick a colony of the 18-24 hours colony and smear on the filter paper. A color change from white to deep blue or purple within 10-30 seconds indicates a positive result [5].

#### 2.5.7. Motility test

This test is used to determine the ability of an organism to move by itself. The help of flagella.

A semisolid agar medium is prepared and dispensed into test tubes, a straight wire was used to make a single stab down the center of the tube to about half the depth of the medium and incubated at 37°C for 24-48 hours.

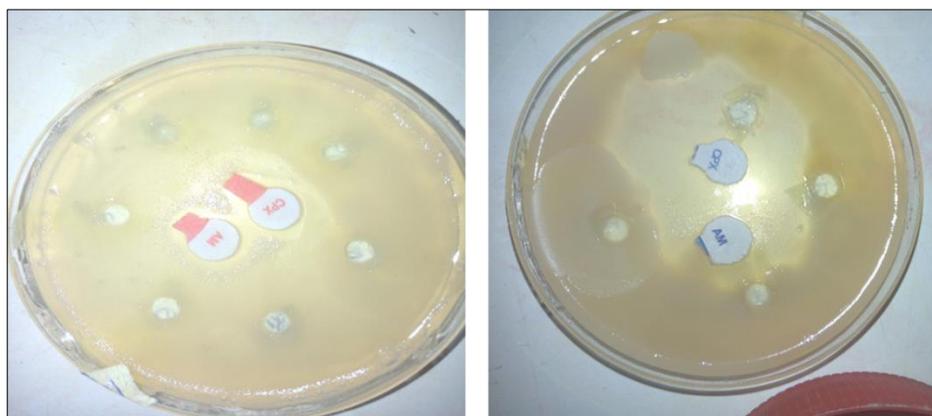
The Hazy growth that spread throughout the medium and the stab line shows a positive result [8].

#### 2.5.8. Starch hydrolysis Test

Starch agar medium (Starch 20.0g/l, peptone 5.0g/l, yeast extract 3.0g/l, agar 15.0g/l pH 7.0) was inoculated with isolated fungal cultures. The plates were incubated at 25°C in inverted position for 5 to 7 days. The surface of the plates was flooded with iodine solution for 30 seconds. Examined the disappearance of starch agar media plates by observing the disappearance of clear zones around the fungal growth [7],[12].

#### 2.5.9. Agar well diffusion method

After screening by point inoculation for the antimicrobial substance, production of marine isolates were tested by agar well diffusion method. The overnight cultures of the chemical isolates in nutrient broth were uniformly swabbed on the surface of the nutrient agar plates using sterile cotton swabs. Five wells of 6mm sizes were made with sterile cork borer on the seeded plates. Around 100µl of overnight culture of the marine bacterial isolates in nutrient broth, was added to each of the wells aseptically. The plates were incubated without invert for 24 hours at 37°C and the zone of inhibition was noted and recorded. The marine strains showing promising activities against chemical isolates were selected for further studies.



**Figure 3** Agar well diffusion method

### 3. Results

#### 3.1. Isolation of Bacteria

To isolate new type of antibacterial compounds active against resistant organisms, marine bacteria were isolated from soil samples of Bonny Island Sea, Port Harcourt, Nigeria. The bacteria were isolated by serial dilution by spread plate method. After 24 hours of incubation, isolated colonies were obtained. 8 bacteria were isolated based on their morphological and structural characteristics as shown in table 3 and 4.

**Table 2** The source of marine samples collected

Source	Marine sample
Bonny-Island sea	Surface marine sample
Bonny-Island sea	Middle marine sample
Bonny-Island sea	Deep down marine sample

**Table 3** The total number of organisms isolated

Isolates	Marine sample source
isolate 1	Wet soil sample
isolate 2	Wet soil sample
isolate 3	Wet soil sample
isolate 4	Wet soil sample
isolate 5	Wet soil sample
isolate 6	Wet soil sample
isolate 7	Wet soil sample
isolate 8	Wet soil sample

**Table 4** The morphological and cultural characteristic of the marine isolation

Marine isolates	Shape	Margin	Size	Texture	Appearance
M <sub>1</sub>	Entire	Raised	Small	Smooth	Shiny
M <sub>2</sub>	Regular	Curled	Large	Rough	Dull
M <sub>3</sub>	Circular	Entire	Distinct	Smooth	Shiny
M <sub>4</sub>	Circular	Curled	Small	Rough	Shiny
M <sub>5</sub>	Circular	Curled	Moderate	Rough	Dull
M <sub>6</sub>	Circular	Entire	small	Smooth	Shiny
M <sub>7</sub>	Irregular	Curled	Moderate	Smooth	Shiny
M <sub>8</sub>	Circular	Entire	Small	Smooth	Shiny

Key: M= Marine

**Table 5** Biochemical characterization of the marine isolates

Biochemical characteristics	Marine isolates							
	M <sub>1</sub>	M <sub>2</sub>	M <sub>3</sub>	M <sub>4</sub>	M <sub>5</sub>	M <sub>6</sub>	M <sub>7</sub>	M <sub>8</sub>
Gram staining	+	+	-	+	-	+	+	+
Oxidase	+	+	+	-	+	-	-	+
Starch hydrolysis	-	-	-	-	-	-	+	-
Catalase	+	-	+	+	+	+	-	-
Coagulase	+	-	-	+	+	-	-	-
Motility	-	-	+	-	+	+	-	+

Key: - Negative; + Positive

**Table 6** The result of agar well diffusion of the marine isolation and commercially used antibiotics (Ciprofloxacin and Amoxicillin)

Clinical isolates	M <sub>1</sub>	M <sub>2</sub>	M <sub>3</sub>	M <sub>4</sub>	M <sub>5</sub>	M <sub>6</sub>	M <sub>7</sub>	M <sub>8</sub>
<i>Staphylococcus aureus</i>	R	3.5 mm	R	R	R	R	R	R
<i>Escherichia coli</i>	R	R	R	R	R	R	R	R
+++++++								
Clinical isolates	Antibiotics							
	Ciprofloxacin	Amoxicillin						
<i>Staphylococcus aureus</i>	3.7mm	3.5mm						
<i>Escherichia coli</i>	4.0mm	2.7mm						

Key: R - Resistance

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#### 4. Discussion

In this study, eight isolates were obtained from Bonny Island, sea Portharcourt, these isolates include, *Staphylococcus aureus*, *Escherichia coli*, *Clostridium spp* and *Streptomyces griseus*. [5]. isolated antibacteria against *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *vibro cholorea* were isolated.

In this present study, one out of eight isolates exhibited substantial antibacterial activities against *Staphylococcus aureus* and it was initial found to be *Streptomyces spp*. Antibiotic production by marine bacteria had been screened using sensitivity method, out of the 633 isolates, 170 strains (26.8%) were found to be antibiotic 224 epiphytic bacteria strains from intertidal seaweeds, out of which 38 strains (16.9%) displaced antibacterial activity [3].

Large numbers of heterotrophic bacteria of disease morphology are reported from the marine soil. It has been shown that often bacteria occupy more volume than the marine organisms even up to 60% of the mesophyl region [11].

In the present study, it was found that *Staphylococcus aureus* was more prevalent in the marine wet soil sample. This was recorded that presence of *Staphylococcus aureus* were prevented in soil samples making it more pathogenic organisms [5].

During antimicrobial spectrum determination from marine bacteria, it was recorded that the *Escherichia* was resistant to all the marine isolates while *Staphylococcus aureus* showed resistant to all the marine isolates except M2 (*Streptomyces griseus*). This was recorded in studies carried out by [9]. out of isolated 51 strains from marine algae, *Focus resicolosus* and in that, 13 (25%) exhibited activity against methicillin resistance *Staphylococcus aureus* (MRSA) out of 13 strain, only the exhibited good activity against MRSA. [5].

Ciprofloxacin and Amoxicillin were used against *Staphylococcus* and *Escherichia coli*. Ciprofloxacin exhibited more antibacterial. I action to both organics, (3.7mm and 4.0m) while amoxicillin recorded about (3.mm and 2.7) m respectively. The study further recalls that the commercially used antibiotics. Ciprofloxacin and Amoxicillin shared more antibacterial activity than the marine isolates. Antagonistic interaction and comparison among marine isolates were studied by [5]. Each of the 86 marine bacteria isolates were examined for their inhibition of growth by agar diffusion assay and about 53 of these isolates exhibited antagonistic properties against other pathogenic bacteria, hence understanding antibacterial activities of these marine isolates may allow for more focused search for antibiotics that are more active against bacteria species.

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#### 5. Conclusion

Understanding antibiotics at the phylogenic level may allow a more focused search for antibiotics. The techniques employed in the present investigation yielded reproducible results. Marine (M<sub>2</sub>) isolates 2 (*Streptomyces griseus*) should further investigation since it showed strong inhibitory action against *Staphylococcus aureus*. Further testing of the spectrum of antibiotic produce by M2 should be undertaken in clinical trials, perhaps by pharmaceutical company, to determine its efficacy against the many humans and animal pathogenic microorganisms that have developed resistance to the currently widely used antibiotic.

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#### Compliance with ethical standards

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##### *Disclosure of conflict of interest*

I state that there is no conflict of interest in this article, the authors are in full agreement.

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