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Preliminary Assessment of Antibacterial ability of *Chromobacterium violaceum* isolated from a water body in Port Harcourt, Rivers State Nigeria

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ABSTRACT

Background*:* The current global antibiotic resistance crisis has led to an increase in the search for alternative therapies. *Chromobacterium violaceum* has attracted interest due to its antibacterial activity. This activity however differs depending on strain. This study set out to explore the antibacterial capabilities of *C. violaceum* isolated from Rivers State, Nigeria.

Methods*:* Water samples collected from different points of the New Calabar River were assessed for the presence of *C. violaceum* using standard microbiological techniques. Susceptibility testing was carried out using the Kirby Bauer technique and antibacterial activity assessed using the agar well diffusion and spot test.

Results*: C. violaceum* was only detected from a single sample of the six analyzed. This isolate was resistant to 50% of the antibiotics tested (Amoxicillin, Ampicillin, Ceftazidime and Cefuroxime). No antibacterial activity was observed using the spot test. Activity was however noted using the agar well diffusion test against 4 different isolates representing 3 genera, one of which was the Gram negative *Serratia marcescens*, in addition to *Bacillus* sp and *Staphylococcus aureus*.

Conclusion*:* This study therefore reports on a strain of *C. violaceum* isolated from Rivers state which exhibits antibacterial activity not just against Gram positive bacteria but also against a Gram negative bacteria.

***Key words*— antibacterial activity, *Staphylococcus aureus*, *Chromobacterium violaceum*, drug resistant, Gram negative**

1. INTRODUCTION

With the current global antibiotic resistant crisis, studies exploring alternatives have been on the increase [1]. Several naturally occurring compounds exhibiting antibacterial activities have been described over the years, often derived from either plants or other bacteria [2]. Violacein is one such compound. This compound is produced by several organisms key of which is the Gram negative purple pigmented rod, *Chromobacterium violaceum*. This compound has since been purified and its antibacterial activity confirmed [3], [4]. With the recent race for new antimicrobials, attention has once more turned to violacein with a focus on assessing strains of *C. violaceum* for antibacterial activity. The various bacterial strains may differ in the level of violacein produced [5], [6]. Initial reports noted activity of violacein against Gram positive bacteria, particularly *Staphylococcus aureus*. More recent studies have however reported on activity of violacein against some Gram negative such as *Pseudomonas* sp in addition to Gram positive bacteria [7], [8]. *C. violaceum* has been reported as occurring naturally in both soil and water of tropical regions [8], [9]. This organism has also been reported as an uncommon human pathogen responsible for a variety of diseases and generally sensitive to a wide array of antibiotics [10]. Despite the several reports of the antibacterial activity of *C. violaceum*, no such reports have been made from Nigeria. The only reports of *C. violaceum* from Nigeria have been made in association with their ability to cause infection in humans [11], [12], [13], and its presence in various ecosystems [14], [15], [16], [17]. Only one study explored the hydrocarbon degrading potential of this organism [18]. This study therefore was designed not just to isolate *C. violaceum* but also to determine its antibiotic susceptibility pattern and carry out a preliminary assessment of its antibacterial abilities.

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2. MATERIALS AND METHODS

2.1 Materials

2.1.1 Equipment

Instruments used in this study include test tubes, conical flasks, beakers, Petri dishes, measuring cylinder, pipettes, binocular light microscope (Olympus, Japan), autoclave (Autoclave, China), microscopic slides (China), swab sticks, Nutrient Agar (Lab M, UK), MacConkey agar (Lab M, UK), Mueller-Hinton agar (Lab M, UK).

2.1.2 Biological Materials

Water samples were aseptically collected from three different points of the New Calabar River (Choba bridge, Man O'War camp, Omuiheuchi River) using sterile 500 mL screw cap glass bottles with special care to ensure that no contact was made with the water body. Samples were then transported to the laboratory for immediate analysis.

2.2 Methods

2.2.1 Bacterial isolation and identification

Water samples were assessed for the presence of *C. violaceum* using standard microbiological techniques. Inoculation was done by plating samples on nutrient agar to observe for characteristic purple colonies. Additionally, test isolates were obtained from various sources and all isolates identified using standard biochemical tests as previously described [19], [20].

2.2.2 Antibiotic Susceptibility Testing

This was carried out using the Kirby-Bauer disc diffusion method [21]. In brief, a Mueller Hinton agar (MHA) plate was inoculated with an inoculum corresponding to 0.5 McFarland standard using a sterile swab stick. Following a 5 min pre-incubation step at room temperature, the relevant test antibiotic multidisc was placed on the inoculated plate and the set up incubated at 37ºC for 24 hour. The susceptibility of each isolate determined by comparing the zones of inhibition observed following incubation, against a standard reference [22].

2.2.3 Assessment of antibacterial activity of C. violaceum

Preliminary screening for antibacterial activity of *C. violaceum* against several bacterial isolates was carried out using the spot test and agar well diffusion test. In brief, for the spot test, following the uniform streaking of a standardized inoculum on a Mueller Hinton agar plate, *C. violaceum* was spot inoculated on to the lawn of test bacteria. For the agar well diffusion test, 4 mm wells were made on the MHA plate containing the standardized inoculum streak using a sterile cork borer and *C. violaceum* culture dispensed into each well. Both set up were observed for zones of inhibition following a 24 h incubation period at 37 ºC.

2.3 Statistical Analysis

Sampling and analysis were carried out in duplicates and data analyzed with using Microsoft Excel 2010 package

3. RESULTS

Of the 6 different water samples collected from the 3 different sampling points, the purple pigmented *Chromobacterium violaceum* characteristic colony was obtained from only one of these samples obtained from the Omuihuechi River and its identity confirmed using standard biochemical tests. Eight different isolates were obtained from various sources (Table 1), four of which were Gram positive and 4 Gram negative. *Staphylococcus aureus* was the most commonly represented bacteria (3/8) among the isolates.

Table 1: Identity and source of test isolates

Source	Isolate Identity
Soil	*Achromobacter* sp
Soil	*Bacillus* sp
Soil	*Staphylococcus aureus* (SA_1)
Clinical Environment	*Staphylococcus aureus* (SA_2)
Clinical Patient	*Staphylococcus aureus* (SA_3)
Tap Water	*Escherichia coli*
Laboratory Surface	*Pseudomonas aeruginosa*
Laboratory Surface	*Serratia marcescens*

Antibiotic susceptibility testing of the isolates revealed varying rates of resistance (Table 2). Majority of isolates (8/9, 88.9%) were resistant to amoxicillin. With the exception of the *E. coli* isolate, all other isolates had MAR index values greater than 0.5, with multidrug resistance (resistance to 3 or more drug classes) observed in 66.7% (6/9) of the isolates. Using the spot agar test as a preliminary screen to detect antibacterial activity of *C. violaceum* against the test isolates, no antibacterial activity was detected, with no zone of inhibition observed in all cases

(Table 3). The agar well diffusion test gave a different result. Using this test, antibacterial activity was observed in some cases. This presented as clear zones of inhibition (Figure 1). *C. violaceum* showed antibacterial activity against 4 different isolates representing 3 genera, one of which was the Gram negative *S. marcescens*.

Table 2: Antibiotic resistance pattern of screening and test isolates

Organism	Antibiogram	MAR Index	No of resistant drug classes
C. violaceum	Amoxicillin-Ampicillin-Ceftazidime-Cefuroxime	0.5	2
Achromobacter sp	Amoxicillin-Ampicillin-Ceftazidime-Cefuroxime	0.5	2
Bacillus sp	Amoxicillin-Ceftazidime-Cefuroxime-Cloxacillin-Erythromycin	0.63	3
S. aureus (SA_1)	Amoxicillin-Ceftazidime-Ceftriaxone-Cefuroxime-Cloxacillin-Erythromycin-Gentamicin-Ofloxacin	1	6
S. aureus (SA_2)	Ceftazidime-Ceftriaxone- Cloxacillin-Erythromycin	0.5	3
S. aureus (SA_3)	Amoxicillin-Ceftazidime-Ceftriaxone-Cefuroxime-Cloxacillin-Erythromycin-Gentamicin	0.88	4
E. coli	Amoxicillin-Ampicillin-Cefuroxime	0.38	2
P. aeruginosa	Amoxicillin-Ampicillin-Ceftazidime-Cefuroxime-Gentamicin-Nitrofurantoin	0.75	4
S. marcescens	Amoxicillin-Ampicillin-Cefuroxime-Nitrofurantoin	0.5	3

Table 3: Antibacterial activity of *C. violaceum* against different bacteria

Isolate	Spot test	Agar well diffusion
Achromobacter sp	-	-
Bacillus sp	-	+
Staphylococcus aureus (SA_1)	-	+
Staphylococcus aureus (SA_2)	-	-
Staphylococcus aureus (SA_3)	-	+
Escherichia coli	-	-
Pseudomonas aeruginosa	-	-
Serratia marcescens	-	+

Figure 1: Variations in antibacterial activity of *C. violaceum* against several bacterial isolates

4. DISCUSSION

The antibacterial activity of *C. violaceum* from different locations has been characterized and described widely. This activity has been noted to differ with some reports of violacein active against predominantly Gram positive organisms and other studies noting activity against some Gram negative bacteria also. These strains of *C. violaceum* have also been noted to have low levels of resistance to antibacterial agents. This study reports on the isolation of only a single strain of *C. violaceum* from the different water samples. This is in contrast to other studies which reported an abundance of *C. violaceum* in various tropical water bodies [23], [24], [25]. The point of the new Calabar River which the strain was isolated from is associated with a higher level of human activity. Results of this study reported susceptibility to nitrofurantoin, ofloxacin, gentamicin and ciprofloxacin. The susceptibility to nitrofurantoin was similarly reported by a study which isolated *C. violaceum* from a disused tin mining lake [26]. In general though, the strain of *C. violaceum* in this study which exhibited resistance to 50% of the test isolates was more resistant than strains isolated in other studies. Rajalakshmi and colleagues reported in 2011 the detection of a strain with resistance to 16.7% of test isolates while for the type strain resistance was observed against 41.7% of antibiotics [27]. Similar to previous reports the *C. violaceum* in this study showed no antibacterial activity against most of the Gram negative test isolates. This strain of *C. violaceum* however showed antibacterial activity against *Serratia marascences* unlike previous reports which reported no such activity [3], [28]. Goh and colleagues in particular used the same crude preliminary testing method and reported activity against both *Bacillus* sp and *S. aureus* isolates [26]. Like a previous study [29], this study noted activity of *C. violaceum* against a multidrug resistant strain of *S. aureus*. This result was promising because it indicates a mode of action dissimilar to existing antibiotic classes.

5. CONCLUSION

This study reports on a strain of *C. violaceum* isolated from a water body in the South-South of Nigeria which exhibits antibacterial activity. It might be necessary to further explore this to determine how the data generated from Nigeria compares with the rest of the world and how to properly tap this potential.

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Conflict of Interest

Authors declare that no conflict of interests exists.

Contribution of the Authors

This work was carried out in equal collaboration between all authors. Both authors KO and DAG designed the study, managed the literature searches and wrote the protocol. KO wrote the first draft of the manuscript. DAG was primarily responsible for the lab work. All authors read and approved the final manuscript.

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