

A Usually Daptomycin-Resistant Gram-Positive Dermabacter Hominis: Rare Pathogen

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Abstract

Dermabacter hominis is a gram-positive, catalase-positive, glucose-fermenting rod, which, as it grows forms small greyish-white colonies with a characteristic pungent odor. Previously known as coryneform Centers for Disease Control and Prevention groups 3 and 5, it was catalogued as D. hominis in 1994. Various strains isolated in blood cultures, abscesses, or wounds in the 1970s were retrospectively characterized in referral centers as D. hominis. In this report we describe two patients with severe underlying pathology who developed bacteremias by D. hominis within the context of their clinical pictures.

INTRODUCTION

A 29-year-old woman was human immunodeficiency virus positive in stage B3 of AIDS. She was admitted to our hospital with left hemiparesia. A cerebral computer tomography scan showed a hypodense lesion in the left parietal lobe compatible with progressive multifocal leukoencephalopathy. A peripheral venous catheter was placed. While in the hospital the patient suffered a marked deterioration of her neurological situation, together with severe worsening of her general state, with alteration of pulmonary function, upper digestive tract hemorrhage, and fever peaks. Various antimicrobial therapies were administered, including clindamycin, ciprofloxacin, and the combination of fluconazole, meropenem, and sulfamethoxazole, in spite of the fact that no pathogenic microorganism was isolated in the various cultures requested, including the catheter culture. The patient's situation worsened and reached terminal stage, which led to the decision to suspend all medication and to maintain only sedation. Dermabacter hominis and Candida albicans were isolated from two blood cultures taken 48 h earlier. Treatment with fluconazole and vancomycin was initiated, but the patient died 24 h later.

A 65-year-old male patient had a personal history of cardiopathy and broncho-obstructive pneumopathy. He was brought to our Emergency Service with thoracic pain, for which he was admitted to the Cardiology Department. While in the hospital he suffered a cardiorespiratory arrest, from which he recovered after cardiopulmonary reanimation although he was left with irreversible neurological sequelae. During the following days he suffered episodes of fever peaks for which antibiotic treatment with cloxacillin plus gentamicin was administered. After 4 days without fever and without antibiotic treatment he presented with a febrile peak of more than 38°C. At this point two blood cultures were taken and the catheter was removed for culture. The patient's condition deteriorated, and he died suddenly 48 h later. That same day D. hominis grew in the two blood cultures. The catheter culture was negative.

In both patients small gram-positive coccobacilli with a coryneform appearance were detected in two blood cultures per patient, processed with the VITAL system (bioMerieux, Marcy l'Etoile, France). These grew, after 24 h of incubation in an atmosphere enriched with 5% CO₂, in the subcultures

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degree of wound contamination, and it is estimated that 50% of wounds contaminated by bacteria become clinically infected (6). These bacteria can seriously delay wound healing process by disrupting the normal clotting mechanisms and promoting disordered leukocyte function and poor quality granulate on tissue formation, reduce tensile strength of connective tissue, and impair epithelization.

Ola subscorpioidea.oliv is a shrub or tree which belongs to the family of Olacaceae. It is widely distributed in West African countries such as Nigeria, Zaire and Senegal (7). The Shrub or small tree is between 9 m and 10 cm in diameter, and is fairly common in the semi-deciduous forests and gallery forests. The branches are flexible and angular with elliptex leaves; lanceolate in its entire margin. The Leaf apex has a characteristic point (mucronate); petiole 2- 4 mm; blade about 10 x 3 cm, with about 7 pairs of looped laterals. *Ola subscorpioidea* Oliv. fruit is small globular, about 1.5 cm in diameter, and yellowish but turning red when ripe. It has a slash smell like garlic. Two other *Ola* species are possibly present as shrubs or small trees: *O. mannii* (smooth twigs) and *O. gambecola* (pustulate twigs). Their leaves slightly resemble those of *Celtis* because of the strong, ascending basal nerves (tri-nerved).



Figure: 1.4: *Ola subscorpioidea* Oliv

Medicinal plants play a significant role in human health as they make an awesome substitute for orthodox medicine. *Ola subscorpioidea* Oliv. is one of such medicinal plants that is used in folk medicine mostly for giving relief for several ailments.. *Ola subscorpioidea* Oliv leaves is traditionally used in the treatment of diabetic mellitus. The leaves either form part of traditional recipe or is singly used in diabetic conditions. Research has shown that the plant leaf has potential to alleviate postprandial hyperglycaemia by inhibiting α -amylase and α -glucosidase (8). *Ola subscorpioidea* (Olacaceae) is a major component of a recipe used traditionally in the management of Obesity. This plant (especially the root) has shown to have hypolipidemic property

and probably is capable of reducing weight in human (9). *Ola subscorpioidea* is a plants used in Ivorian traditional medicine in the treatment of many diseases including jaundice and hepatitis. The plant is used in mixture with palm wine locally called “bandji” for their traditional therapeutic utilization (10). Researchers have proved that *Ola subscorpioidea* Oliv. has antidepressant effect on effect and this justified its use in the treatment of mental disorders (11).

MATERIALS AND METHODS

Collection of Plant Material

Ola subscorpioidea Oliv. roots were collected across farms in Akungba community in Ondo State. The plant part was authenticated at the herbarium of the Department of plant science and biotechnology Adekunle Ajasin University, Akungba Akoko, Ondo State. Nigeria.

Preparation of Extract

Ola subscorpioidea Oliv. Root was washed with sterile water and air dried for 10days, and then chopped into small pieces to increase surface area. 100 g of the root was soaked in 400 mL of ethanol for 7 days. After which it was filtered with whatman No. 1 filter paper. The solvent was recovered and the crude extract obtained using rotary evaporator. The crude extract was thus kept in refrigerator at 4oC for further screening (12).

Specimen Collection

Specimens were collected hospitals across Akoko region of Ondo state, Nigeria using standard collection techniques (Cheesebrough, 2006). Sterile cotton-wool swabs were used to collect samples from the wound of surgical patients with evident infection. The swabs were introduced gently into the wound sites and rotating the swab tips in the wound, taking care to avoid contamination of specimen with commensals from the skin, and then immersed immediately in a MacCartney bottle containing peptone water. The sample bottles were then transported to the Microbiology laboratory of Adekunle Ajasin University, Akungba-Akoko, Ondo state(12).

Isolation of Bacteria

At the laboratory, the inoculated peptone water was serially diluted to 10⁷ and the bacteria isolated using pour plate method. Aliquot of 1 ml from 10⁴ and 10⁷ were dispensed into sterile petri dishes, molten agar was then added, swirled and allowed to solidify. The media used were MacConkey agar, Nutrient agar, Eosin Methylene Blue agar and Mannitol salt agar and were each prepared according to the instruction of the manufacturer. The agar plates were then incubated at 37oC for 24 hours.

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Isolation of Pure Isolates

After 24 hours of incubation, the colonies were subcultured by streaking each colony on the surface of freshly prepared agar plate. The plates were then incubated at 37°C for 24 hours. Distinct colonies were then picked into nutrient agar slant and stored in the refrigerator for further use.

Biochemical Test

Microbact (24E) kit identification

This kit was used for biochemical tests with the range of simple, standardized system, for the rapid identification of Gram-negative bacteria. Preparation of 18-24 hours old pure culture of the organism to be identified, oxidase test was performed which must be negative or positive for 24E kit, selected isolate colony was emulsified in saline, test strip was placed in holding tray and the back seal was peeled and addition of 4 drops of bacterial suspension to each well, addition of 2 drops of mineral oil (MB1093A) to the black wells, the seal was replaced and incubated at 35°C + 20°C for 18-24 hours, the tray was removed from incubator and appropriate reagents was added. The results was then recorded and interpreted using the Microbact™ identification software package (Balows et al., 1991; Oxoid limited). A purity check was performed by inoculating a purity plate with 1 drop of bacterial suspension and incubated at 35°C + 20°C for 24 hours, Well 13 was read at 24-48 hours for Enterobacteriaceae and at 48 hours for MGNB, Well 24 was interpreted differently at 24 hours and 48 hours, A nitrate reduction test was done in well 7 after reading the ONPG reaction, Performance was monitored by testing appropriate control strains (Oxoid Limited)(13).

Gram Stain

A loopful of sterile distilled water was dropped on a clean grease free slide by using a sterile inoculating loop after which an inoculum from the culture was mixed with the water on the slide. The smear was air dried and heat fixed by passing it quickly over a Bunsen flame. The smear was flooded with crystal violet solution for 60 seconds (one minute) and rinsed with water. The smear was again flooded with Lugol's iodine for 30 seconds and rinsed with water, 70% alcohol was poured on the slides for 15 seconds until the crystal violet had been completely washed off. It was then counterstained with Safranin for 60 seconds and allowed to dry. The slides were then observed under oil immersion objective lens (x100). Gram positive cells remained purple while Gram negative cells appeared red or pink (14).

Catalase Test

A drop of hydrogen peroxide solution was placed on a clean grease free slide. A flamed inoculating

loop was used to place a loopful of an inoculum on the slide and gently mixed after which it was observed for bubbles or effervescence which is an indication of catalase positive organism (14).

Oxidase Test

A piece of filter paper was placed in petri dish and three (3) drops of freshly prepared oxidase reagent was added. Using a sterile glass rod, a colony of test organisms was removed from a culture plate and it was smeared on the filter paper. Oxidase positive organisms gave blue colour within 5 to 10 seconds, and in oxidase negative organisms, colour did not change (15).

Coagulase Test

A loopful of normal saline solution was placed on each glass slide and was emulsified. Human plasma was added to one of the suspension and was stored properly for 15 minutes while the other was left as control. Coagulase positive was indicated by clumping which did not re-emulsify (15).

Antibiotic Susceptibility Test and Antimicrobial Assay of the Extract

Antibiotic susceptibility tests were performed using Kirby-Bauer's disc diffusion method on Mueller-Hinton agar. The inocula were prepared from the stock cultures which were maintained in nutrient agar slant at 4°C and subculture in nutrient broth using a sterilized wire loop. 1 ml of 10⁻⁴ normal saline dilution of a 24 hours broth culture was mixed with 19 ml of the agar in a sterile universal bottle and poured into sterile petri dish. The agar plate was left to solidify and the antibiotic disc were thus placed on the agar surface at minimum of 22 mm apart and were incubated overnight at 37°C for 24 hours. The diameter of zones of inhibition were measured and recorded in millimeter and the results interpreted according to the Clinical Laboratory Standard Institute (16) guidelines.

The antimicrobial screening of the Olax subscorpioidea Oliv. Root extract against the bacterial isolates was carried out using the agar well diffusion method. A stock concentration of 100 mg/ml was constituted by dissolving 1 g each, of the extracts in 10 ml of Dimethyl sulfoxide (DMSO) diluted with sterile distilled water in ratio 1:3. 50 mg/ml, 25 mg/ml and 12.5 mg/ml concentrations of the extracts were the prepared using dilution formula (C1V1=C2V2). 1 ml of 10⁻⁴ normal saline dilution of a 24 hours broth culture was mixed with 19 ml of the agar in a sterile universal bottle and poured into sterile petri dish. The agar plate was left to solidify and wells were bored on them using 6 mm cork borer. 50 µl of each concentration of the extracts was poured into each well and incubated at 37°C for

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24 hours. The diameter zones of inhibition were measured and recorded in millimeter and the results interpreted according to the Clinical Laboratory Standard Institute (16) guidelines. Piperacillin/tazobactam (0.125 mg/ml) and Dimethyl sulfoxide (DMSO) were used as positive and negative controls respectively.

Minimal Inhibitory Concentration and Minimal Bacteriocidal Concentration

The minimal inhibitory concentration (MIC) was determined using the tube dilution method. Graded concentrations of the extract were prepared using Mueller Hinton broth medium into different test tubes. The concentrations were 100mg/ml, 50mg/ml, 25mg/ml, 12.5mg/ml, 6.25mg/ml and 3.125mg/ml. Standardized inoculum of 24 hours broth culture was inoculated into the test tubes and incubated at 37°C for 24 hours. After incubation, the test tubes were examined for sign of growth (turbidity) and the minimal concentration with no growth was recorded as the MIC (17).

The minimal bacteriocidal concentration (MBC) was determined by streaking out samples from the test tubes with no growth on the surface of freshly prepared nutrient agar. The plates were then incubated at 37°C for 24 hours, after which plates were observed for any bacterial growth. Again, the minimal concentration with no growth was taken as the MBC (17).

Secondary Metabolite (Phytochemical) Screening of *Oxalobacter formicosa* Oliv. root

Qualitative Method of Analyses

Preliminary test / Preparation test

Plant filtrates were prepared by boiling 20 g of the fresh plant in distilled water. The solution was filtered through a vacuum pump. The filtrates were used for the phytochemical screening for flavonoids, tannins, saponins, alkaloids, reducing sugars, anthraquinones and anthocyanosides.

Test for Alkaloids

About 0.2 gram was warmed with 2% of H₂SO₄ for two minutes, it was filtered and few drops of Dragendoff's reagent were added. Orange red precipitate indicates the presence of Alkaloids (18).

Test for Tannins

One milliliter of the filtrate was mixed with 2 ml of FeCl₃. A dark green colour indicated a positive test for the tannins (18,19).

Test for Saponins

One milliliter of the plant filtrate was diluted with 2 ml of distilled water; the mixture was vigorously shaken and left to stand for 10 min during which time, the development of foam on the surface of the mixture lasting for more than 10 mm, indicates

the presence of saponins (20).

Test for Anthraquinones

One milliliter of the plant filtrate was shaken with 10 ml of benzene; the mixture was filtered and 5 ml of 10 % (v/v) ammonia were added, then shaken and observed. A pinkish solution indicates a positive test (20).

Test for Anthocyanosides

One milliliter of the plant filtrate was mixed with 5 ml of dilute HCl; a pale pink colour indicates the positive test (21).

Test for Flavonoids

One milliliter of plant filtrate was mixed with 2 ml of 10% lead acetate; a brownish precipitate indicated a positive test for the phenolic flavonoids. While for flavonoids, 1 ml of the plant filtrate was mixed with 2 ml of dilute NaOH; a golden yellow colour indicated the presence of flavonoids (21,22).

Test for Reducing Sugars

One milliliter of the plant filtrate was mixed with Fehling A and Fehling B separately; a brown colour with Fehling B and a green colour with Fehling A indicate the presence of reducing sugars (23).

Test for Cyanogenic glucosides

This was carried out by subjecting 0.5 g of the extract to 10 ml sterile water filtering and adding sodium picrate to the filtrate and heated to boil (24).

Test for Cardiac glucosides

Legal test and the Keller-Kiliani was adopted, 0.5 g of the extract were added to 2 ml of acetic anhydride plus H₂SO₄ (25).

Quantitative Method of Analyses

Saponins

About 20 grams each of dried plant samples were ground and, put into a conical flask after which 100 ml of 20 % aqueous ethanol were added. The mixture was heated using a hot water bath. At about 55°C, for 4 hours with continuous stirring, after which the mixture was filtered and the residue re-extracted with a further 200 ml of 20% ethanol. The combined extracts were reduced to 40 ml over a water bath at about 90°C. The concentrate was transferred into a 250 ml separatory funnel and 20 ml of diethyl ether were added and then shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated three times. 60 ml of n-butanol were added. The combined n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation, the samples were dried in the oven to a constant weight; the saponin content was calculated as percentage of the starting material (25,26).

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Flavonoids

About 10 g of the plant sample were extracted repeatedly with 100 ml of 80% aqueous methanol, at room temperature. The whole solutions were filtered through Whatman filter paper No 42. The filtrate were later transferred into a crucible and evaporated into dryness over a water bath; the dry content was weighed to a constant weigh (27).

Cardiac glucosides

Legal test and the killer-kiliani wwas adopted, 0.5g of the extract were added to 2ml of acetic anhy- drate plus H2S04(28).

Tannins

About 500 mg of the plant sample were weighed into a 50 ml plastic bottle. 50 ml of dis- tilled water was added and shaken for 1 hour on a mechanical shaker. This was filtered into a 50 ml volumetric flask and made up to the marked level. Then, 5 ml of the filtrate was transferred into a test tube and mixed with 2 ml of 0.1 M Fecl in 0.1 M Hcl and 0.008 M potassium ferrocyanide. The absorb- ance was measured at 120 nm within 10 minutes. The tannins content was calculated using a standard curve of extract (29).

Alkaloids

Five grams of the plant sample were weighed into a 250 ml beaker and 200ml of 10% acetic acid in ethanol was then be added, the reaction mixture were covered and allowed to stand for 4 hour. These were filtered and the extract will be concentrat- ed on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop-wise to the extract until the precipita- tion is complete. The whole solution were allowed to settle and the precipitate was collected, washed with dilute ammonium hydroxide and then filtered; the residue being the alkaloid, which was dried and weighed to a constant mass (30,31,32).

Phlobatannins

About 0.5grams of each plant extracts were dissolved in distilled water and filtered. The filtrates were boiled in 2% Hcl, red precipitate show the present of phlobatannins (31).

RESULTS

Table 3.1 shows the result of preliminary tests carried out to characterize the twenty six (26) bac- terial isolates recovered, six (23.1%) were Gram positive and twenty (76.9%) were Gram negative. Also, from a single surgical site more than two bac- teria were isolated. Twenty two (22) of the isolates were rod shaped, three (3) are tiny rods and One (1) is cocci shaped. They are predominantly Gram neg- ative rods except six (6) which are Gram positive. Eighteen (18) of the isolates are oxidase positive

while eight (8) of the isolates are oxidase negative. Nineteen (19) of the isolates are catalase positive while seven (7) of the isolates are catalase negative.

Table 3.2 shows the identity of the eight (8) Gram negative bacterial isolates as revealed by Mi- crobact 24E identification kit. Fifty percent 50% were oxidase-negative with Escherichia coli most prevalent (25%). Other organisms include Klebsiel- la pneumoniae, Enterobacter agglomerans, Pseu- domonas aeruginosa, Pseudomonas fluorescens, Burkholderia pseudomallei and Mannheimia haemolytical. Most of the SSI-associated bacte- ria were comprised of Staphylococcus aureus and Pseudomonas aeruginosa.

Table 3.1: Gram stain, microscopic examination and preliminary biochemical tests carried on the re- covered isolates

ISOLATE	GRAM STAIN	SHAPE	OXIDASE	CATA- LASE
MAC 1a	+	Rods	+	-
MAC 1b	-	Tiny rods	+	+
MAC 2	-	Tiny rods	+	+
MAC 4	+	Cocci	+	+
MAC 6	-	Rods	-	-
MAC 8	-	Tiny rods	-	-
MAC 9	-	Rods	+	+
MAC 12	+	Rods	+	+
MAC 14	-	Rods	+	+
MAC 16	-	Rods	+	+
MAC 17	-	Rods	+	+
MAC 18	-	Rods	+	+
NA 1	-	Rods	+	+
NA 4	-	Rods	-	+
NA 5	-	Rods	-	-
NA 10	-	Rods	+	+
NA 13	-	Rods	-	+
NA 14	-	Rods	+	+
NA 18	-	Rods	+	+
EMB 2	-	Rods	+	-
EMB 13	-	Rods	+	-
EMB 11	-	Rods	+	+
EMB 18	-	Rods	+	+
MAN 5	+	Rods	-	+
MAN 10	+	Rods	-	-
MAN 18	+	Rods	-	+

Table 3.2: Identification of the Gram negative bac- teria using Microbact 24E identification kit

ISOLATE	IDENTIFICATION CODE	PROBABLE ORGANISM
MAC 12	507041776	Mannheimia haemolytica
EMB 2	46610776	E. coli
MAC 18	707145776	Burkholderia pseudomallei
EMB 11	46710776	E. coli
NA 10	703134001	Pseudomonas aeruginosa
NA 13	43471776	Klebsiella pneumonia
MAC 14b	703134100	Pseudomonas fluorescens
MAC 6	07061776	Enterobacter agglomerans

Table 3.3 reveals the antibiotic susceptibility profile of the bacterial isolates. Ciprofloxacin (10µg) was active against 50% of the isolates with diameter of zones of inhibition ranging from 15mm to 30 mm. pefloxacin, ofloxacin and streptomycin were also active against 3 isolates with lesser diameter of zones of inhibition (11mm-17mm). The most susceptible isolates were *Pseudomonas aeruginosa*, *Enterobacter agglomerans* and *Pseudomonas fluorescens*. About 70% of the isolates were resistant to all the antibiotics tested except ciprofloxacin. Ciprofloxacin was effective against 50% of the isolates. Ciprofloxacin was the most effective antibiotic against Gram negative bacteria with exception to *Staphylococcus aureus*. High sensitivity was observed among *Listeria monocytogens*, *Pseudomonas aeruginosa*, *Enterobacter agglomerans* and *Pseudomonas fluorescens*. However, placid effects of septrin were observed against *Pseudomonas aeruginosa*, *Enterobacter agglomerans* and *Pseudomonas fluorescens*.

Table 3.4 shows the antimicrobial activity of the *Olax subscorpioidea* Oliv. root extract against the isolates. The extract inhibited the growth of all the bacterial isolates with diameter of zones of inhibition ranging from 6mm to 25mm. At 100mg/ml the diameter of zones of inhibition ranges from 6mm to 9mm, at 50mg/ml the diameter of zones of inhibition ranges from 6mm to 10mm, at 25mg/ml the diameter of zones of inhibition ranges from 7mm to 9mm, at 12.5mg/ml the diameter of zones of inhibition ranges from 7mm to 9mm while the control (Tazobactam antibiotics) ranges from 7mm to 25mm. This table is well explained in Fig. 3.1 to 3.4 below in which the zones of inhibition of the organisms were represented by bar chart.

ORGANISM	SXT (30 µg)	CH (30 µg)	SP (10 µg)	CPX (10 µg)	AM (30 µg)	AU (30 µg)	CN (10 µg)	PEF (10 µg)	OFX (30 µg)	S (30 µg)
<i>E. coli</i>	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Mannheimia haemolytica</i>	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>E. coli</i>	0.0	0.0	0.0	30.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Listeria monocytogens</i>	0.0	0.0	0.0	25.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Pseudomonas aeruginosa</i>	0.0	0.0	0.0	15.0	0.0	0.0	0.0	15.0	12.0	12.0
<i>Enterobacter agglomerans</i>	0.0	0.0	0.0	15.0	0.0	0.0	11.0	12.0	12.0	12.0
<i>Pseudomonas fluorescens</i>	13.0	12.0	0.0	15.0	0.0	0.0	9.0	11.0	14.0	17.0
<i>Klebsiella pneumonia</i>	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Burkholderia pseudomallei</i>	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Staphylococcus aureus</i>	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

KEY: SXT- Septrin, CH- Chloramphenicol, SP- Sparfloxacin, CPX- Ciprofloxacin, AM- Amoxicillin, AU- Augmentin, CN- Gentamycin, PEF- Pefloxacin, OFX- Ofloxacin and S- Streptomycin

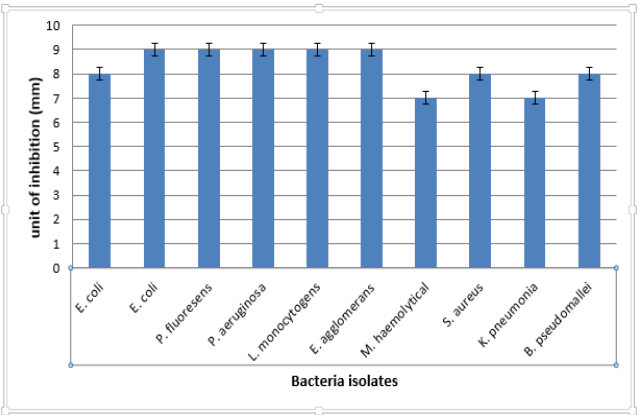


Fig 3.1:Antimicrobial screening of ethanol extract of *Olax subscorpioidea* Oliv. Root at 100mg/ml.

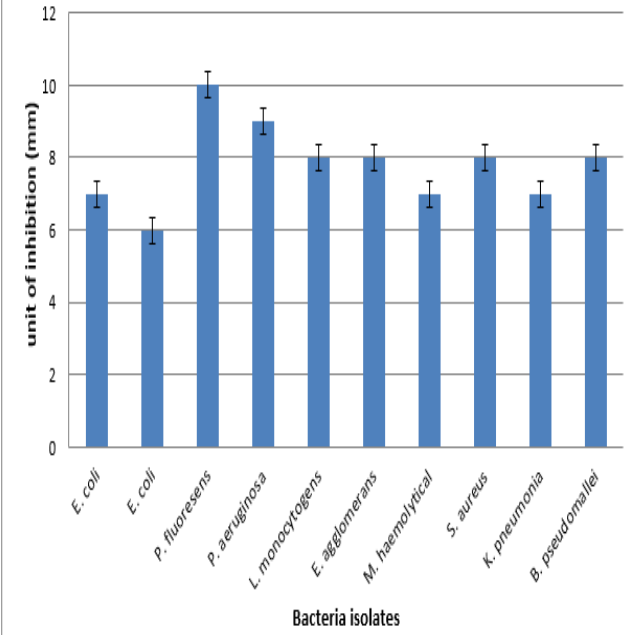


Fig 3.2:Antimicrobial screening of ethanol extract of *Olax subscorpioidea* at 50mg/ml.

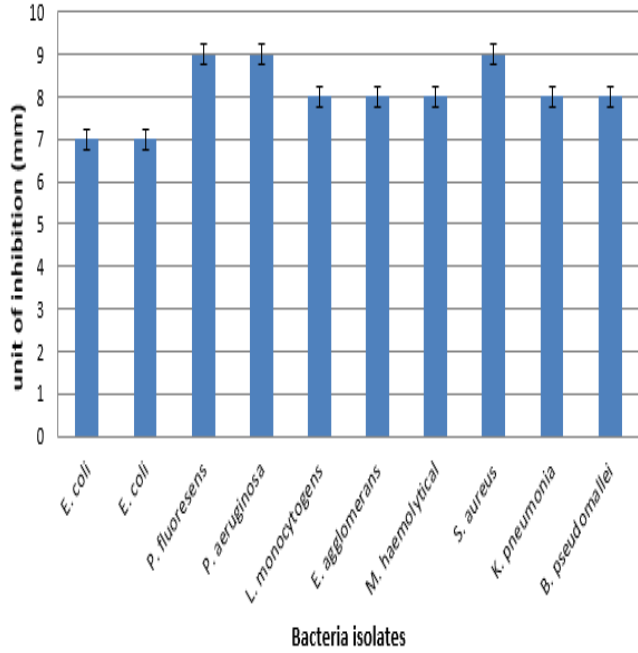


Fig 3.3: Antimicrobial screening of ethanol extract of *Olax subscopioidea* Oliv. rootat 25mg/ml.

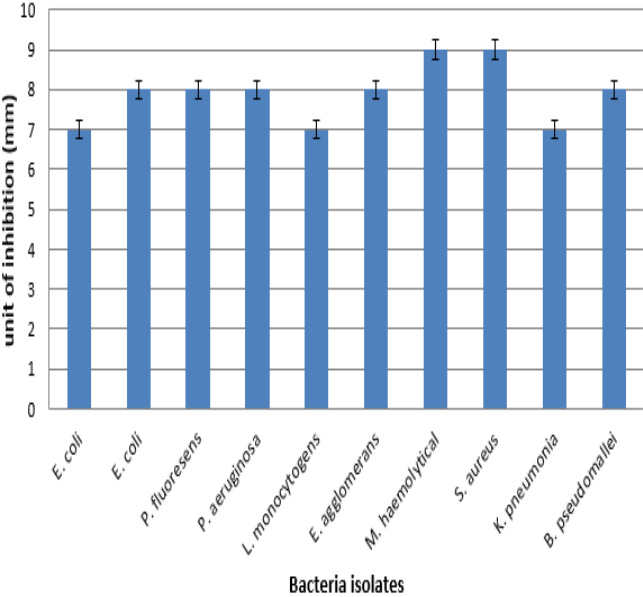


Fig 3.4:Antimicrobial screening of ethanol extract of *Olax subscopioidea* Oliv. rootat 12.5mg/ml.

Table 3.5 shows the minimal inhibitory concentration (MIC) and minimal bacteriocidal concentration (MBC) of the extract. MIC ranges from 25mg/ml to 100mg/ml while MBC was between 50mg/ml and 200mg/ml. This table is well explained in Fig. 3.5 to 3.6 below in which the zones of inhibition of the organisms were represented by bar chart.

Table 3.6 shows the qualitative phytochemical screening of the ethanol extract of *Olax subscorpioides* Oliv. root. Flavonoid, tannins, saponins, alkaloids, reducing sugars, steroid, phenol, terpenoid, pyrrolozidine alkaloid, glycoside and cardiac glycoside were present while anthraquinones and volatile oil were not detected.

Table 3.7 present the quantitative phytochemical screening of the ethanol extract of *Olax subscorpioides* Oliv. root. Glycoside and terpenoid were most present with a concentration of 14.01 while saponins were least present with a concentration of 3.21.

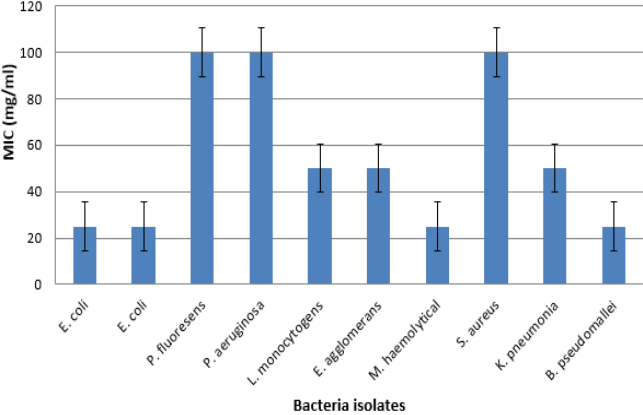


Fig 3. 5: Minimal inhibitory concentration MIC (mg/

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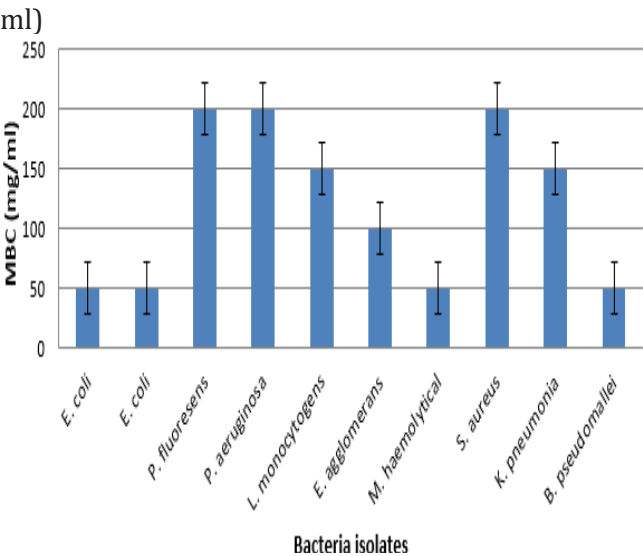


Fig 3.6: Minimal bacteriocidal concentration MBC (mg/ml)

Table 3.6: Qualitative phytochemical (secondary metabolite) screening of ethanol extracts of *Olax subscorpioides* Oliv. root

Constituent	Presence
Alkaloids	+
Glycoside	+
Steroids	+
Anthraquinone	ND
Phenol	+
Tannins	+
Saponin	+
Flavonoids	+
Pyrrolizidine alkaloids	+
Reducing sugar	+
Terpenoid	+
Volatile oil	ND
Cardiac glycosides	+

KEY: + = Present, - = Absent and ND = Not Detected

Table 3. 7: Quantitative phytochemical (secondary metabolite) screening of ethanol extracts of *Olax subscorpioides* Oliv. root

Constituent	Quantity
Alkaloids	13.89
Glycoside	14.01
Steroids	9.71
Anthraquinone	9.75
Phenol	7.51
Tannins	7.60
Saponin	3.21
Flavonoids	4.21
Pyrrolizidine alkaloids	4.00
Reducing sugar	13.89
Terpenoid	14.01
Volatile oil	9.71
Cardiac glycosides	9.75

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