











## Antibacterial Activities of Herbal Extracts and Conventional Antibiotics on Methicillin Resistant *Staphylococcus aureus* Isolated from Wound

Veronica Felicitia Nwode<sup>1</sup> , Kayode Akindele<sup>1</sup> , Christiana Inuaesiet Edemekong<sup>2</sup> ,  
Ugwu Nnenna Ugwu<sup>1</sup> , Farotimi Chinenye Asumpta<sup>1</sup> , Ikechukwu Michael Obya<sup>1</sup> ,  
Augustina Nebechi Nwatu<sup>1</sup> , Henrietta Onu<sup>1</sup> , Ikemesit Udeme Peter<sup>3, 4\*</sup> ,  
Ifeanyichukwu Romanus Iroha<sup>5</sup> 

<sup>1</sup>Department of Microbiology, Faculty of Biological Science, Alex Ekwueme Federal University Ndufu-Alike, P.M.B. 1010, Ikwo, Ebonyi State, Nigeria

<sup>2</sup>Department of Biotechnology, Faculty of Pure and Applied Sciences, Federal University of Allied Health Sciences, Trans-Ekulu, P.M.B. 01473, Enugu, Nigeria

<sup>3</sup>Department of Medical Microbiology, Faculty of Basic Medical Sciences, Federal University of Allied Health Sciences, Trans-Ekulu, P.M.B. 01473, Enugu, Nigeria

<sup>4</sup>Department of Public Health, Faculty of Health Technology and Engineering, Federal University of Allied Health Sciences, Trans-Ekulu, P.M.B. 01473, Enugu, Nigeria

<sup>5</sup>Department of Applied Microbiology, Faculty of Science, Ebonyi State University, Abakaliki, P.M.B. 53, Nigeria

### ABSTRACT

**Background:** Methicillin-resistant *Staphylococcus aureus* (MRSA) strains continue to be a leading cause of infections with high morbidity and mortality in wound patients, but little is known about the prevalence, characterization, and evaluation of the antibacterial activity of methanol leaf and stem bark extracts of *Newbouldia laevis*, *Parkia biglobosa* and conventional available antibiotics on MRSA from various wound sources in Abakaliki. This study aimed to investigate the prevalence of MRSA and its associated risk factors among wound patients in a Tertiary Hospital. **Methods:** A total of 200 samples from post-surgical wounds, burn wounds, decubitus ulcer, and diabetic foot ulcer patients were collected from March 2021 to February 2022 using a sterile swab stick on the affected site. The collected specimens were immediately subjected to bacteriological analysis. MRSA was screened for Antibiotic Susceptibility Testing (AST) and results were analyzed using the Clinical Laboratory Standard Institute (CLSI) zone diameter breakpoints. The antibacterial activity of methanol leaf and stem-bark extract of *Newbouldia laevis* and *Parkia biglobosa* against the MRSA isolates was determined using the agar well diffusion method. **Results:** The prevalence of MRSA accounted for 55.5%. Antibiogram result shows that the MRSA isolates were 87.8% and 100% susceptible to imipenem and amikacin but 78.9% and 100% resistant to erythromycin, penicillin, lincomycin, vancomycin, trimethoprim-sulfamethoxazole, tetracycline respectively. At 25mg/ml, 12.5mg/ml, and 6.25mg/ml, *Parkia biglobosa* showed high inhibitory activity against MRSA at the range of 9mm – 19mm compared to *Newbouldia laevis* having inhibitory activity against MRSA at the range of 0 – 15mm at the same concentrations. **Conclusion:** Our study reports the prevalence of MRSA with multi-resistant phenotype in wound samples and also advocates for the judicious use of imipenem and amikacin as drugs of choice against the test isolate. These findings validate the use of methanol leaves and stem-bark extract of *P. biglobosa* as an alternative medicine for treating MRSA-associated wound infection and recommend further complementary studies in ethno-pharmacological use.

**Keywords:** methicillin-resistant *Staphylococcus aureus*, *Parkia biglobosa*, *Newbouldia laevis*, wound

---

\* Corresponding Author

## INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) encodes the *mecA* gene that mediates resistance to penicillin-derived methicillin and another beta-lactam antibiotic (Peter *et al.*, 2022a). The presence of an altered penicillin-binding protein (PBP2a) encoded by the *MecA* strain has been recognized as a factor essential for methicillin resistance (Peter *et al.*, 2022a). This housekeeper gene is found as an integral portion of the *Staphylococcal* cassette chromosome *mec* (SCC *mec*) (Peter *et al.*, 2022b). Methicillin-resistant *Staphylococcus aureus* is considered a priority pathogen in the community and hospital-acquired infection of which their potential risk is also a matter of concern (Peter *et al.*, 2022a; Peter *et al.*, 2022b).

MRSA has a high global burden in immunocompromised patients particularly wound patients causing significant morbidity and mortality (Ghaznavi-Rad & Ekrami, 2018; Garoy *et al.*, 2018). In humans, MRSA remains a medically important bacteria that colonizes bone and wound infections (Peter *et al.*, 2022c). The expression of virulence by MRSA in osteomyelitis and postoperative wound infections in orthopedic medicine are the leading cause of delayed bone/wound healing and non-union which may necessitate the amputation of such bones (Peter *et al.*, 2022a; Peter *et al.*, 2022c). Prior studies have reported a high risk of MRSA infection in fracture and post-surgical wound patients (Peter *et al.*, 2022a; Peter *et al.*, 2022c). MRSA is often multi-resistant eroding the effectiveness of most conventional antibiotics with limited treatment options. Another key element that contributes to treatment failure in wound patients is the creation of various virulence factors. For example, MRSA strains that produce Pantone-Valentine Leukocidin (PVL) have higher lethality than PVL-negative bacteria (Holmes *et al.*, 2005) as observed in soft tissue infection, surgical site infection (SSI) and burn wounds (Chen *et al.*, 2018). Although MRSA is a substantial health issue, there is a lack of research on the frequency of MRSA in wounds in Nigeria. In most hospital departments/wards with wound patients, ampicillin, ampicillin, gentamicin, and ciprofloxacin are the most widely prescribed antibiotics. The majority of recently utilized medications in most hospitals are not novel in therapeutic use, but rather refinements of existing classes of antibacterial treatments that can only provide temporary relief to wound patients. It is, nonetheless, difficult to imagine a world without antibiotics. However, this is rapidly approaching because diseases and disease-causing agents that were formerly thought to have been conquered and beaten by antibacterial medicines are emerging in a new pattern of resistance to therapeutic drugs.

Researchers are currently looking for compounds from plants that can operate as cutting-edge antimicrobials, with a broader spectrum of activity against bacteria of both Gram-positive and Gram-negative origin and no major side effects (Ude *et al.*, 2021; Peter *et al.*, 2022d; Agbo *et al.*, 2024). The ability of plants to produce an endless number of secondary metabolites that serve as the foundation for plant-derived antibacterial agents.

Smooth *Newbouldia* or boundary tree is the popular name for *Newbouldia laevis* (Bignoniaceae) (Akerle *et al.*, 2011). It can reach a height of 7 – 15 meters, but it is more commonly a shrub of 2-3 meters with multiple stems producing clumps of gnarled branches (Akerle *et al.*, 2011). Most tribes in Nigeria refer to it as Eto Adanha in Ibibio-Efik, Akoko in Yoruba "Ogirisi" in Igbo, "Ikhimi" in Edo, and "Aduruku" in Hausa (Akerle *et al.*, 2011). *Newbouldia laevis* is commonly used in folk medicine in South Eastern and Midwestern Nigeria for the treatment of infected wounds and eye issues (Akerle *et al.*, 2011). Scientific studies on the plant's phytochemical contents indicated the presence of phenylpropanoids and alkaloids in the root (Germann *et al.*, 2006) tannins, and flavonoids in the leaves (Usman & Osuji, 2007). Also, *Parkia biglobosa* (African locust bean tree) is native to Africa (Jauro *et al.*, 2018) and is commonly referred to as Orgili in the Igbo tribe of Nigeria. *Parkia biglobosa* is a plant that has demonstrated a promising source of phytomedicinal compounds (Millogo-Kone *et al.*, 2007), as well as other ethnobotanical and folkloric applications. The World Health Organization (WHO) has stated that medicinal plants may be the finest source of a wide range

of medications (Agbo *et al.*, 2024). As a result, there has been a global renaissance in the use of herbal remedies in illness management across all continents, with most developing countries now incorporating phytomedicine into their healthcare systems. Diseases are treated in Nigeria's healthcare system utilizing medicinal plants alongside with contemporary medicine. As the looming threat of clinical antibacterial tolerance or resistance persists, susceptibility tests remain essential for the screening and selection of medicinal and conventional antibacterial agents. As a result, this study looks into the screening of available antibiotics and the antibacterial ability of *Parkia biglobosa* and *Newbouldia laevis* leaf and stem bark as a panacea for the treatment of MRSA wound infection.

## METHODS

### Inclusion and Exclusion Criteria

The wound samples were collected from patients at Alex Ekwueme Federal Teaching Hospital Abakaliki, Ebonyi State Abakaliki, located at latitude 6.3231° N and longitude 8.1121° E (Ilang *et al.*, 2023). Ebonyi State is located 64 kilometers southeast of Enugu in southeastern Nigeria. The study comprised consenting patients with current post-surgical wounds, burn wounds, decubitus ulcer, and diabetic foot ulcers occurring within 30 days or one year if an orthopedic implant is in place. Patients with community-acquired pyogenic infections such as furuncle, abscess, and carbuncle; patients with episiotomy infection; and patients with open fractures were excluded from the study.

### Demographic Data and Sample Collection

Demographic and clinical data were acquired from consenting patients utilizing a standardized validated questionnaire and patient files. These included age, gender, previous antibiotic intake, beginning of the lesion/length of hospital stay, type, and associated medical condition. A total of 200 samples (Fifty [50] each) were collected within the period of 12 months from post-surgical wounds, burn wounds, decubitus ulcers and diabetic foot ulcers patients were collected by rotating a sterile swab on the affected site. The collected specimens were taken within one hour of collection via moist Amie's transport medium to the microbiology research unit of the Ebonyi State University Abakaliki, Nigeria for bacteriological investigation.

### Isolation and Identification of Test Organisms

The wound swab specimens were suspended in a sterilized nutrition broth (Oxoid, UK) and incubated for 24 hrs at 37°C. The turbid bacterial growth was plated onto MP1571 Denim Blue Chromogenic MRSA Screening Agar (Oxoid, UK), and incubated for 24 hrs at 37°C. Bacterial colonies on MP1571 Denim Blue Chromogenic MRSA Screening Agar (Oxoid, UK) with atypical MRSA features of denim blue colonies were aseptically purified by subculturing onto nutrient agar (Oxoid, UK) and incubated at 24 hrs at 37°C. The pure culture strains were subsequently identified via and PBP2 latex agglutination tests (DR0900A) and Vitex 2 compact 60 next-generation automated system (BIOMERIEUX, U.S.A).

### Antibiotic Susceptibility Testing

According to the Clinical and Laboratory Standards Institute (CLSI) criteria (CLSI, 2019), a standardized Kirby-Bauer disk diffusion method with the Mueller-Hinton agar (MHA) (Oxoid, Basingstoke, Hampshire, UK) plate technique was performed. A suspension of bacterial cells (i.e., the equivalent of  $1 \times 10^6$  colony forming unit per milliliter (CFU/ml) was swabbed onto each Petri dish containing solidified Mueller-Hinton agar and adjusted to 0.5 MacFarland turbidity standard. The inoculated organisms were allowed for 15 minutes for pre-

diffuse of bacteria suspension. Commercially available antibiotic discs comprising the following antibiotics: Vancomycin (30 µg), Amikacin (10µg), Ceftazidime (30 µg), Cefotaxime (30 µg), Erythromycin (5µg), lincomycin (15µg), Imipenem (30 µg), Trimethoprim-Sulfamethoxazole (30 µg), Penicillin (10 µg), Tetracycline (15µg) (Oxoid) were aseptically applied with sterile forceps onto the surfaces of the solidified Mueller-Hinton agar (Oxoid, Basingstoke, Hampshire, UK) plates and gently pressed to achieve uniform contact. The plates were incubated for 24 hrs at 37° C. The inhibition zones were interpreted in millimeters (mm) according to CLSI guidelines for antibiotics sensitivity (CLSI, 2019; Oke *et al.*, 2024).

#### ***Collection and Authentication of Parkia biglobosa and Newbouldia laevis***

*Parkia biglobosa* and *Newbouldia laevis* leaf and stem bark were collected at Ndufe-Alike community (located at latitude 6° 7' 54''N and longitude 8° 8' 29''E) in Ikwo L. G. A in Ebonyi State. The plant materials were examined and validated with voucher no: HP/344/67R by Dr. C. D Udechukwu, a taxonomist in the Department of Biology at Alex Ekwueme Federal University Ndufe-Alike in Ikwo L. G. A in Ebonyi State, Nigeria.

#### ***Extraction of Stem-bark and Leaf of Parkia biglobosa and Newbouldia laevis***

This was performed following a modification in the methods of Osuntokun *et al.* (2018) as stated below.

**Modification:** The work of Osuntokun *et al.* (2018) air-dried the plant material for 10 days and the plant materials were not cut into pieces before maceration. The filtrate was dried using a rotary evaporator. *Parkia biglobosa* and *Newbouldia laevis* leaf and stem bark were sun-dried for 5 days after being washed with sterile water. The plant material was cut into pieces and then pulverized with a sterile mortar and pestle to reduce into powdered particles (Jauro *et al.*, 2018; Osuntokun *et al.*, 2018). Before extraction, exactly 200 grams of *Parkia biglobosa* and *Newbouldia laevis* in each leaf and stem bark were soaked in 400 ml volume of methanol (Labpak Chemicals Ltd Coventry, England) and allowed to stand overnight at room temperature. It was then filtered with a muslin cloth (Peter *et al.*, 2022d). The filtrate was evaporated to dryness at <40°C using a rotary evaporator and stored in the desiccator until needed.

#### ***Preparation of Different Concentration of the Extracts***

Exactly, one gram (1g) of each plant part was reconstituted separately in 10 ml of 70% Dimethylsulphoxide (Tianjin kermel Chemical Reagent Co., Ltd, China) as diluent to obtain a concentration of 100 mg/ml. Exactly, 10<sup>-5</sup> serial dilution was carried out from the stock solution to obtain extract concentrations of 50 mg/ml, 25 mg/ml, 12.5 mg/ml and, 6.25 mg/ml.

#### ***Antibacterial Activity of Methanol stem-bark and leaf extract of Parkia biglobosa and Newbouldia laevis***

The antibacterial activity of plant extracts was determined using the agar well diffusion method as described by earlier researchers (Nwankwo *et al.*, 2023). A suspension of bacterial cells (i.e., the equivalent of 1x10<sup>6</sup> colony forming unit per milliliter (CFU/ml) was seeded onto each petri dish containing solidified Mueller-Hinton agar (Oxoid, Basingstoke, Hampshire, UK) and adjusted to 0.5 MacFarland turbidity standard. This was allowed to stand for 15 minutes to enable the inoculated organisms to pre-diffuse. The wells were then aseptically bored on seeded agar plates with an 11 mm sterile Cork borer (supertek®, U. S. A). The wells were filled with 1 ml of each extract concentration. The inoculated agar plates were incubated for 24 hours at 37°C. After incubation, clear inhibition zones were measured using a metric rule and interpreted in millimeters (mm).

## RESULTS

### Prevalence of MRSA from Wound Samples

MRSA from isolated *Staphylococcus aureus* recovered from post-surgical wound, burn wounds, diabetic foot ulcers, and decubitus ulcer samples showed prevalence rates of 87.2, 78.5, 51.7, and 62.9 respectively. The total prevalence rate was 55.5 % as shown in Table 1.

### Demographic and Relevant Clinical Information of Wound Patients with MRSA

MRSA among wound patients was predominant amongst 35-49 years recording 21.5%, followed by 50 – 69 years recording 18.5%, and 20 – 34 years having 15.5%. Gender distribution of MRSA was observed in females than males with MRSA occurrence rates of 33.5% and 22.0% respectively. Duration of hospitalization within 2 months displayed a high prevalence of MRSA 18.5%. Duration of 4 months and above showed 10.5%, greater than 1 week 10.0%, 7 days showed 7.5%, duration of 3 months showed 5.0% and the least prevalence of MRSA was found within 1 month duration as shown in Table 2.

### Antibiotic Susceptibility Profile of MRSA

The results of the antibiotic susceptibility profile of MRSA from post-surgical wound swabs in Table 2 showed 100%, 87.8%, and 51.2% susceptibility to imipenem, amikacin, and cefotaxime respectively. The isolates also showed the highest (100%) resistance to penicillin and tetracycline, followed by 97.5%, 95.1% and 87.8% resistance to lincomycin, trimethoprim-sulfamethoxazole, and vancomycin.

MRSA isolated from burn wound patients were 100% resistant to penicillin, tetracycline, trimethoprim-sulfamethoxazole, and vancomycin while 78.9%, 69.7%, and 57.6% resistant was observed against erythromycin, lincomycin and ceftazidime respectively. The MRSA isolated was 100% susceptible to amikacin, imipenem, and cefotaxime followed by 42.2%, 30.3% and 21.2% recorded against ceftazidime, lincomycin, and erythromycin respectively. While MRSA isolates from burn wound patients were 100 % susceptible to amikacin, cefotaxime and imipenem while ceftazidime, erythromycin, and lincomycin recorded 42.4 %, 21.2 % and 30.3 % respectively as shown the Table 2.

MRSA isolated from decubitus ulcer patients were susceptible to aminoglycoside (amikacin 90.9 %), cephalosporin (cefotaxime 100 %) and carbapenem (imipenem 100%) but less susceptible against erythromycin and ceftazidime recording 13.6 %, and 27.3 % respectively. MRSA isolated from decubitus ulcer patients exhibits a high level of resistance recording 100 % to trimethoprim-sulfamethoxazole, vancomycin, tetracycline, penicillin, and lincomycin while 86.4 %, 72.7 %, and 9.1% resistance rates was recorded against erythromycin, ceftazidime and amikacin respectively as presented in Table 3. Also, MRSA recovered from diabetic foot ulcer patients were 100 % resistant to lincomycin, penicillin, tetracycline, trimethoprim-sulfamethoxazole, and vancomycin. Amikacin showed resistance of 9.1 % against 90.9 % susceptible. Then, ceftazidime and erythromycin recorded 72.7 % and 86.4 % resistance as presented in Table 3.

### *Antibacterial activity of methanol crude extract of Parkia biglobosa against MRSA isolates from post-surgical wound*

Methanol leaf extract of *Parkia biglobosa* had an inhibition zone diameter within the range of 11-15 mm at 100 mg/ml concentration while stem-bark extract had inhibition zone diameters within the range of 19-26 mm at 100 mg/ml concentration against MRSA isolates from a post-surgical wound in Table 4.

### *Antibacterial activity of methanol crude extract of Parkia biglobosa against MRSA isolates from Burn wound*

Methanol leaf extract of *Parkia biglobosa* showed no inhibitory effect against MRSA isolates from burn wounds while stem-bark extract showed antibacterial activity at 100mg/ml concentration within the range of 15-25mm against the test isolates (Table 5). Stem-bark extract demonstrated antibacterial activity within the range of 13-21mm and 9-17mm at 50mg/ml and 25mg/ml concentration against MRSA isolates from burn wounds.

***Antibacterial activity of methanol crude extract of Parkia biglobosa against MRSA isolates from Burn wound***

MRSA isolates from Diabetic foot ulcers were less sensitive to methanol leaf extract of *Parkia biglobosa* at 12.5 mg/ml and 6.25 mg/ml concentration with no inhibitory effect while high inhibitory zone diameter of 24mm and 21mm was observed at 100mg/ml and 50 mg/ml concentration of stem-bark extract as presented in Table 6.

***Antibacterial activity of methanol crude extract of Parkia biglobosa against MRSA isolates from Decubitus ulcer***

Stem-bark extract of *Parkia biglobosa* was active at 6.25 mg/ml concentration recording 9-11mm against MRSA isolates from Decubitus ulcer. The stem bark showed active resistance at a concentration of 100 mg/ml recording 14-25mm and 9-21mm at the concentration of 50mg/ml as shown in Table 7.

***Antibacterial activity of methanol crude extract of Newbouldia laevis against MRSA isolates from Post-surgical wound***

Methanol crude extract of *Newbouldia laevis* showed no inhibitory activity at 12.5 mg/ml and 6.25 mg/ml concentration against MRSA isolates from post-surgical wounds. The leaf extract at 100mg/ml showed the highest activity measuring 18mm but the stem bark recorded high activity measuring 20mm as shown in Table 8.

***Antibacterial activity of methanol crude extract of Newbouldia laevis against MRSA isolates from Burn wound***

Leaf extract of *Newbouldia laevis* demonstrated high antibacterial activity with inhibitory zone diameter of 15mm and 12mm at 100mg/ml and 50 mg/ml concentration respectively while *Newbouldia laevis* stem-bark extract showed no inhibitory activity at 12.5 mg/ml and 6.25 mg/ml concentration against MRSA isolates from burn wounds shown in Table 9.

***Antibacterial activity of methanol crude extract of Newbouldia laevis against MRSA isolates from Diabetic foot ulcers***

*Newbouldia laevis* leaf and stem bark extract against MRSA isolates from diabetic foot ulcers revealed inhibitory zone diameters (20mm, 17mm), and (15mm, 15mm) at 100mg/ml and 50 mg/ml concentrations respectively as shown in Table 10.

***Antibacterial activity of methanol crude extract of Newbouldia laevis against MRSA isolates from Decubitus ulcer***

Methanol leaf extract of *Newbouldia laevis* demonstrated an inhibitory effect against MRSA isolates from decubitus ulcer while Stem-bark extract revealed high inhibitory zone diameter of 21mm and 17mm at 100mg/ml and 50mg/ml concentrations respectively (Table 11).

**Table 1: Prevalence of MRSA from wound samples.**

Clinical Sample	No. of sample	MRSA positive (%)
Post-surgical wound	67	41(20.5)
Burn wound	51	33(16.5)
Diabetic foot ulcers	35	15(17.5)
Decubitus ulcer	47	22(11.0)
<b>Total</b>	<b>200</b>	<b>111(55.5)</b>

**Table 2: Demographic and Relevant Clinical information of wound patients with MRSA**

Characteristic		No. sampled	MRSA (%)
Age group	20-34 years	68	31(15.5)
	35-49 years	84	43(21.5)
	50-69 years	48	37(18.5)
Gender	Male	96	67(33.5)
	Female	104	44(22.0)
Duration of hospitalization	7 days	26	15(7.5)
	> 1 week	39	20(10.0)
	1 month	27	8(4.0)
	2 months	53	37(18.5)
	3 months	24	10(5.0)
	4 months & above	31	21(10.5)
Medical Condition	Post-surgical wound	67	41(87.2)
	Burn wound	51	33(78.5)
	Decubitus ulcer	47	22(62.9)
	Diabetic foot ulcers	35	15(51.7)
Previous Antibiotic intake	Ampicillin	56	29(14.5)
	Ampiclox	67	47(23.5)
	Ciprofloxacin	43	15(7.5)
	Gentamicin	34	20(10.0)

**Table 3: Antibiotic susceptibility profile of MRSA**

Antibiotic	Disc potency (µg)	Post-surgical wound <i>n</i> =41		Burn wound <i>n</i> =33		Decubitus ulcer <i>n</i> =22		Diabetic foot ulcers <i>n</i> =15	
		R (%)	S (%)	R (%)	S (%)	R (%)	S (%)	R (%)	S (%)
Amikacin	20	5(12.2)	36(87.8)	0(0.0)	33(100)	2(9.1)	20(90.9)	0(0.0)	15(100)
Cefotaxime	30	20(48.9)	21(51.2)	0(0.0)	33(100)	0(0.0)	22(100)	7(46.7)	8(53.3)
Ceftazidime	30	24(58.5)	17(41.5)	19(57.6)	14(42.4)	16(72.7)	6(27.3)	12(80)	3(20)
Erythromycin	15	21(51.2)	20(48.9)	26(78.9)	7(21.2)	19(86.4)	3(13.6)	15(100)	0(0.0)
Imipenem	30	0(0.0)	41(100)	0(0.0)	33(100)	0(0.0)	22(100)	0(0.0)	15(100)
Lincomycin	15	40(97.5)	1(2.4)	23(69.7)	10(30.3)	22(100)	0(0.0)	15(100)	0(0.0)
Penicillin	10	41(100)	0(0.0)	33(100)	0(0.0)	22(100)	0(0.0)	15(100)	0(0.0)
Tetracycline	30	41(100)	0(0.0)	33(100)	0(0.0)	22(100)	0(0.0)	10(66.7)	5(33.3)
Trimethoprim-Sulfamethoxazole	30	39(95.1)	2(4.9)	33(100)	0(0.0)	22(100)	0(0.0)	15(100)	0(0.0)
Vancomycin	30	36(87.8)	5(12.2)	33(100)	0(0.0)	22(100)	0(0.0)	15(100)	0(0.0)

Key: R-Resistance, S- Susceptible, *n*-number of MRSA

**Table 4: Antibacterial activity of methanol crude extract of *Parkia biglobosa* against MRSA isolates from post-surgical wound**

Strain	Plant part	Extract Concentration					
		100mg/ml	50 mg/ml	25 mg/ml	12.5 mg/ml	6.25 mg/ml	
MRSA 2	Leaf	11	10	NI	8	7	
MRSA 5		NI	NI	NI	NI	NI	
MRSA 7		NI	NI	NI	NI	NI	
MRSA 9		15	12	10	7	8	
MRSA10		14	9	9	8	NI	
MRSA 11		14	12	10	9	9	
MRSA 12		13	12	8	NI	NI	
MRSA 14		NI	NI	NI	NI	NI	
MRSA 15		NI	NI	NI	NI	NI	
MRSA 2		Stem-bark	21	20	19	NI	NI
MRSA 5			20	15	NI	NI	NI
MRSA 7			21	17	12	NI	NI
MRSA 9			19	15	11	NI	NI
MRSA 10			24	16	16	15	NI
MRSA 11			26	19	15	12	17
MRSA 12	18		12	10	NI	NI	
MRSA 14	19		14	11	10	NI	
MRSA 15	20		17	15	12	10	

Key: NI-No inhibition, MRSA-Methicillin Resistant *Staphylococcus aureus***Table 5: Antibacterial activity of methanol crude extract of *Parkia biglobosa* against MRSA isolates from Burn wound**

Strain	Plant part	Extract Concentration					
		100mg/ml	50 mg/ml	25 mg/ml	12.5 mg/ml	6.25 mg/ml	
MRSA 20	Leaf	14	9	10	NI	NI	
MRSA 22		15	12	10	8	7	
MRSA 10		12	9	NI	NI	NI	
MRSA 2		NI	NI	NI	NI	NI	
MRSA 7		NI	NI	NI	NI	NI	
MRSA 8		NI	NI	NI	NI	NI	
MRSA 5		NI	NI	NI	NI	NI	
MRSA 6		NI	NI	NI	NI	NI	
MRSA 1		NI	NI	NI	NI	NI	
MRSA 20		Stem-bark	21	18	15	14	NI
MRSA 22			23	21	16	10	NI
MRSA 10			18	15	13	9	9
MRSA 2			16	11	9	NI	NI
MRSA 7			22	20	17	11	NI
MRSA 8			25	19	15	12	9
MRSA 5	15		13	10	NI	NI	
MRSA 6	19		18	11	11	NI	
MRSA 1	20		14	9	NI	NI	

Key: NI-No inhibition, MRSA-Methicillin Resistant *Staphylococcus aureus*



**Table 6: Antibacterial activity of methanol crude extract of *Parkia biglobosa* against MRSA isolates from Burn wound**

Strain	Plant part	Extract Concentration				
		100mg/ml	50 mg/ml	25 mg/ml	12.5 mg/ml	6.25 mg/ml
MRSA 1	Leaf	14	12	NI	NI	NI
MRSA 3		11	NI	NI	NI	NI
MRSA 15		NI	NI	NI	NI	NI
MRSA 4		17	15	11	NI	NI
MRSA 7		19	16	13	NI	NI
MRSA 10		NI	NI	NI	NI	NI
MRSA 12		NI	NI	NI	NI	NI
MRSA 2		NI	NI	NI	NI	NI
MRSA 5		NI	NI	NI	NI	NI
MRSA 1		Stem-bark	23	21	17	15
MRSA 3	18		15	13	13	NI
MRSA 15	18		14	NI	NI	NI
MRSA 4	22		17	15	NI	NI
MRSA 7	16		13	10	NI	NI
MRSA10	16		11	9	NI	NI
MRSA 12	24		19	17	16	11
MRSA 2	21		15	13	12	9
MRSA 5	19		15	12	NI	NI

Key: NI-No inhibition, MRSA-Methicillin Resistant *Staphylococcus aureus*

**Table 7: Antibacterial activity of methanol crude extract of *Parkia biglobosa* against MRSA isolates from Decubitus ulcer**

Strain	Plant part	Extract Concentration				
		100mg/ml	50 mg/ml	25 mg/ml	12.5 mg/ml	6.25 mg/ml
MRSA 15	Leaf	14	11	NI	NI	NI
MRSA 16		NI	NI	NI	NI	NI
MRSA 18		NI	NI	NI	NI	NI
MRSA 3		NI	NI	NI	NI	NI
MRSA 5		17	15	14	11	NI
MRSA 8		19	17	11	10	10
MRSA 9		NI	NI	NI	NI	NI
MRSA 7		15	12	NI	NI	NI
MRSA 22		18	13	NI	NI	NI
MRSA 15		Stem-bark	22	19	17	14
MRSA 16	24		16	12	10	NI
MRSA 18	18		18	14	11	9
MRSA 3	16		11	NI	NI	NI
MRSA 5	14		9	NI	NI	NI
MRSA 8	23		17	15	14	10
MRSA 9	25		21	19	17	11
MRSA 7	19		17	13	9	NI
MRSA 22	15		11	NI	NI	NI

Key: NI-No inhibition, MRSA-Methicillin Resistant *Staphylococcus aureus*

**Table 8: Antibacterial activity of methanol crude extract of *Newbouldia laevis* against MRSA isolates from Post-surgical wound**

Strain	Plant part	Extract Concentration				
		100mg/ml	50 mg/ml	25 mg/ml	12.5 mg/ml	6.25 mg/ml
MRSA 40	Leaf	17	16	15	NI	NI
MRSA 41		12	8	9	NI	NI
MRSA 30		15	8	NI	NI	NI
MRSA 35		NI	NI	NI	NI	NI
MRSA 1		18	11	NI	NI	NI
MRSA 3		NI	NI	NI	NI	NI
MRSA7		NI	NI	NI	NI	NI
MRSA 8		NI	NI	NI	NI	NI
MRSA 10		NI	NI	NI	NI	NI
MRSA40		Stem-bark	20	15	NI	NI
MRSA 41	19		15	13	NI	NI
MRSA 30	NI		NI	NI	NI	NI
MRSA 35	18		11	NI	NI	NI
MRSA 1	15		13	NI	NI	NI
MRSA 3	NI		NI	NI	NI	NI
MRSA 7	NI		NI	NI	NI	NI
MRSA 8	20		12	NI	NI	NI
MRSA 10	NI		NI	NI	NI	NI

Key: NI-No inhibition, MRSA-Methicillin Resistant *Staphylococcus aureus*

**Table 9: Antibacterial activity of methanol crude extract of *Newbouldia laevis* against MRSA isolates from Burn wound**

Strain	Plant part	Extract Concentration				
		100mg/ml	50 mg/ml	25 mg/ml	12.5 mg/ml	6.25 mg/ml
MRSA 23	Leaf	14	12	NI	NI	NI
MRSA 25		12	9	NI	NI	NI
MRSA 2		15	12	8	NI	NI
MRSA 5		9	NI	NI	NI	NI
MRSA 7		NI	NI	NI	NI	NI
MRSA 10		NI	NI	NI	NI	NI
MRSA 6		NI	NI	NI	NI	NI
MRSA 3		14	NI	NI	NI	NI
MRSA 16		11	NI	NI	NI	NI
MRSA 23		Stem-bark	18	15	NI	NI
MRSA 25	20		19	15	NI	NI
MRSA 2	15		9	NI	NI	NI
MRSA 5	NI		NI	NI	NI	NI
MRSA 7	NI		NI	NI	NI	NI
MRSA 10	NI		NI	NI	NI	NI
MRSA 6	NI		NI	NI	NI	NI
MRSA 3	NI		NI	NI	NI	NI
MRSA 16	NI		NI	NI	NI	NI

Key: NI-No inhibition, MRSA-Methicillin Resistant *Staphylococcus aureus*

**Table 10: Antibacterial activity of methanol crude extract of *Newbouldia laevis* against MRSA isolates from Diabetic foot ulcers**

Strain	Plant part	Extract Concentration				
		100mg/ml	50 mg/ml	25 mg/ml	12.5 mg/ml	6.25 mg/ml
MRSA 2	Leaf	NI	NI	NI	NI	NI
MRSA 4		NI	NI	NI	NI	NI
MRSA 8		17	14	12	NI	NI
MRSA 10		12	NI	NI	NI	NI
MRSA 6		NI	NI	NI	NI	NI
MRSA 1		NI	NI	NI	NI	NI
MRSA 14		NI	NI	NI	NI	NI
MRSA 15		15	NI	NI	NI	NI
MRSA 12		20	15	NI	NI	NI
MRSA 2		Stem-bark	NI	NI	NI	NI
MRSA 4	NI		NI	NI	NI	NI
MRSA 8	12		9	NI	NI	NI
MRSA 10	NI		NI	NI	NI	NI
MRSA 6	NI		NI	NI	NI	NI
MRSA 1	17		15	15	8	NI
MRSA 14	NI		NI	NI	NI	NI
MRSA 15	NI		NI	NI	NI	NI
MRSA 12	NI		NI	NI	NI	NI

Key: NI-No inhibition, MRSA-Methicillin Resistant *Staphylococcus aureus***Table 11: Antibacterial activity of methanol crude extract of *Newbouldia laevis* against MRSA isolates from Decubitus ulcer**

Strain	Plant part	Extract Concentration					
		100mg/ml	50 mg/ml	25 mg/ml	12.5 mg/ml	6.25 mg/ml	
MRSA 13	Leaf	NI	NI	NI	NI	NI	
MRSA 16		NI	NI	NI	NI	NI	
MRSA 2		NI	NI	NI	NI	NI	
MRSA 7		NI	NI	NI	NI	NI	
MRSA 17		NI	NI	NI	NI	NI	
MRSA 21		NI	NI	NI	NI	NI	
MRSA 18		NI	NI	NI	NI	NI	
MRSA 11		NI	NI	NI	NI	NI	
MRSA 13		Stem-bark	18	13	NI	NI	NI
MRSA 16			21	17	14	11	9
MRSA 2	NI		NI	NI	NI	NI	
MRSA 7	NI		NI	NI	NI	NI	
MRSA 17	NI		NI	NI	NI	NI	
MRSA 21	NI		NI	NI	NI	NI	
MRSA 18	17		15	11	9	NI	
MRSA 11	20		NI	NI	NI	NI	

Key: NI-No inhibition, MRSA-Methicillin Resistant *Staphylococcus aureus*

## DISCUSSION

MRSA is assessed by a series of resistant determinants according to different strains. As a result, the chromogenic agar was used to identify MRSA strain in wounds with an occurrence rate of 55.5%. In concordance with this result, high incidence of MRSA has been found in wound infection in Nigeria; Olowe *et al.* (2007) reported 47.7 % in Osogbo, south-western Nigeria, two studies reported 75% in Zaria (Obajuluwa *et al.*, 2013; Udobi *et al.*, 2013), Ikeh (2003) reported an MRSA prevalence of 81% from in-patients in Jos, Nigeria. Nwankwo & Nasiru (2011) reported a prevalence rate of 62% from in-patients in Kano Nigeria. In Enugu, Nigeria, MRSA strain accounted for 86(28.4 %) and 78(25.7 %) of post-surgical wounds and

fracture wounds respectively (Peter *et al.*, 2022a). Taiwo *et al.* (2004) earlier reported a prevalence of 70.6% in the city of Ilorin from in-patients.

However, low and higher values have been reported in wound samples from inter-country studies: 18.8% in Mwanza-Tanzania *et al.* (2011), 25% in Jinja-Uganda (Anguzu & Olila, 2007), 37.4% in Madinah kingdom of Saudi Arabia (Ghanem *et al.*, 2018), 25 (61%) in Iran (Ghaznavi-Rad & Ekrami, 2018), 75% from surgical wounds in Algeria (Rebiahi *et al.*, 2011), 72.0% in Eritrea (Garoy *et al.*, 2018), 80% in Peru (Guzman-Blanco *et al.*, 2009) and in a setting in Colombia 90% (Jimenez *et al.*, 2012). Many published researches have also documented a rise in the prevalence of MRSA, the majority of which was caused by wounds (Ariom *et al.*, 2019; Peter *et al.*, 2022c). Several reasons can explain the intra- and inter-country heterogeneity in MRSA prevalence. These include variances in study design, specimen type, laboratory techniques, and study duration, among other things (Deyno *et al.*, 2017). Some studies, for example, depend completely on phenotypic processes to detect MRSA, while others rely on a DNA-based approach (Multilocus Sequence Typing [MLST]), microarrays, whole genome sequencing, spa-typing, conventional PCR, and among others) to identify *mecA*. Investigations relying on DNA-based technique by PCR tend to reveal a lower incidence of MRSA (Nwankwo & Nasiru, 2011; Peter *et al.*, 2022c).

MRSA was more predominant in post-surgical wounds 20.5% than in another wound sample in the study. This reiterates the prevalence of MRSA 3.9%-35.6% in post-surgical wounds, as documented by other researchers (Kolawole *et al.*, 2013; Garoy *et al.*, 2018). Our findings suggest that post-surgical patients may be prone to toxigenic equipment carriage and antibiotic-resistance clonal strains of *S. aureus*. More research into the pathogenicity potential and characteristics of MRSA populations from post-surgical wound patients could be beneficial. Given the discovery of numerous toxin genes, including the *tst* gene, in hospitalized post-operative patients (Kolawole *et al.*, 2013). Post-surgical wound patients in this study may likely be at risk of postsurgical toxic shock syndrome which results in delayed wound healing and prolonged hospitalization.

In this study, male patients harbor MRSA than females with MRSA occurrence rates of 33.5% and 22.0% respectively, and is consistent with earlier research: males 55.9% vs females 44.1% (Garoy *et al.*, 2018), males 71% vs female 25% (Ghanem *et al.*, 2018). This is also consistent with previous studies from other parts of the world. In one such study from Madinah, it was reported that MRSA strains were three times more prevalent in male patients in all studied cases (Ali *et al.*, 2013; Shirah *et al.*, 2017). A study from Makkah found a similar pattern of males having higher MRSA isolation rates (Haseeb *et al.*, 2016). This trend could be linked to various factors, including males being more susceptible to bacterial sepsis; being less compliant and so inclined to greater infection rates (Magliano *et al.*, 2012). According to research, the female hormone (estrogen) has a negative effect on the development of virulence factors in pathogens (Neuman *et al.*, 2015; Ekuma *et al.*, 2023). Furthermore, the patient's hygiene habits, socioeconomic status, occupation, and lifestyle all play essential roles in determining gender-infection correlations, which must be investigated further. It is generally known that women make up a larger proportion of the workforce in Abakaliki, so it is not surprising that samples taken from female patients were more numerous, resulting in a low MRSA isolation rate.

Age group assessment of MRSA among wound patients was more predominant amongst 35-49 years recording 21.5%. Another study has reported a high rate of MRSA with 22(37.3%) in the 19-40 year-old age group (Garoy *et al.*, 2018). Low prevalence in this study was observed amongst those aged 50-69 years and has been found elsewhere (Seni *et al.*, 2013; Garoy *et al.*, 2018). This low isolation rate amongst ages 50-69 years may be linked to the sample size recruited in the study.

Previous antibiotic intake assessment among wound patients showed 23.5% occurrence rate of MRSA amongst ampiclox users followed by ampicillin users 14.5%, while least occurrence rate 7.5% was recorded against ciprofloxacin users. Ampiclox and ampicillin is the most commonly prescribed antibiotics medications to wound patients in Nigeria. MRSA strain is known to possess low affinity to  $\beta$ -lactam antibiotics. Therefore, exposure to sublethal dose of this  $\beta$ -lactam antibiotics may result in observed increase of MRSA colonization in those users. The finding in this study that MRSA is connected with key risk variables such as age, gender, antibiotic overuse, and prolonged stay in ICU has also been documented in other studies (Garoy *et al.*, 2018; Peter *et al.*, 2022a; Orji *et al.*, 2024). Despite considerable efforts to manage antibiotic resistance bacteria through rigorous infection control strategies, antibiotic-resistant *Staphylococci*, particularly MRSA, have become the most common bacteria encountered in bone and wound infections in orthopedic treatment (Peter *et al.*, 2022a; Peter *et al.*, 2022c).

MRSA is a big concern worldwide (Peter *et al.*, 2022a), with a high significant morbidity and mortality rate in immunocompromised patients, particularly burn wound patients (Ghaznavi-Rad *et al.*, 2015; Peter *et al.*, 2022a).

This study shows that MRSA exhibits resistance to macrolides (that induce *erm* expression) and lincosamide. These isolates demonstrate 51%-100% resistance to lincomycin and erythromycin and it's similar to another study (Seni *et al.*, 2013; Ghaznavi-Rad & Ekrami, 2018; Garoy *et al.*, 2018; Peter *et al.*, 2022a). This means that both lincomycin and erythromycin are ineffective in these patients.

The majority of our MRSA showed 100% resistance to penicillin and tetracycline as per the pattern reported in Abakaliki and Jiangxi province (Chen *et al.*, 2018 Ariom *et al.*, 2019). This could be due to early exposure of these isolates to this antibiotic, which may have accelerated resistance development. In our country, there is a significant degree of antibiotic abuse due to self-medication, which is frequently connected with inappropriate dosage and failure to comply with treatment, as well as the accessibility of antimicrobial agents for patients over the counter without or with a prescription.

Like other studies conducted in Nigeria (Taiwo *et al.*, 2004; Onolitola *et al.*, 2007; Obajuluwa *et al.*, 2013; Peter *et al.*, 2022a). This study confirms the presence of vancomycin-resistant MRSA among wound patients in Abakaliki. Low vancomycin-resistant MRSA from wounds 11.0% in Asmara, Eritrea (Garoy *et al.*, 2018) have been published. In contrast, several studies found that MRSA is 50-100% susceptible to vancomycin (Omuse *et al.*, 2012; Seni *et al.*, 2013; Ghanem *et al.*, 2018; Abdullahi & Iregbu, 2019). According to Falagas *et al.* (2013), the sensitivity of MRSA identified in Africa to VRSA ranges between 82-100%. These estimates and findings contradict an earlier report that VRSA strains are rare, and there is a limited indication of rising prevalence (Kong *et al.*, 2016).

The observed heterogeneity could be explained by changes in antibiotic prescription patterns between countries. Additionally, the *vanA* gene complex, which encodes a high degree of glycopeptide resistance in enterococci, was detected in the vancomycin-resistant *Staphylococcus aureus* (VRSA) isolates (Peter *et al.*, 2022a). To date, all VRSA isolates found in the United States are methicillin-resistant and carry the *mecA* gene. The *vanA* gene complex appears to confer alterations in the cell wall comparable to those seen in vancomycin-resistant enterococci in *S. aureus*, and *mecA* and *vanA* appear to function independently (Peter *et al.*, 2022a).

MRSA from wounds in this study develops resistance to oxacillin, cephalosporins, macrolides, lincosamides, glycopeptide, and trimethoprim-Sulfamethoxazole. MRSA evolution has been linked to the acquisition of the exogenous gene (*mecA*), which is part of the staphylococcal cassette chromosome *mec* (SCC*mec*) (types I-VII) and is controlled by MecI (a repressor) and MecR1 (a transducer) and represents the regulatory/signaling proteins of the

blaZ system (Peter *et al.*, 2022a; Peter *et al.*, 2022d) The *mecA* gene encodes an extra penicillin-binding protein (PBP2a), a peptidoglycan transpeptidase that can give resistance to all -lactam antibiotics and others antibiotic category (Peter *et al.*, 2022a; Orji *et al.*, 2024) as evidence in this study.

Interestingly, all MRSA isolates showed high susceptibility to imipenem 100% while the isolates were 87.8-100% susceptible to Amikacin. In corroboration with earlier literature, MRSA 73.2% and 92.6% susceptibility to imipenem and Amikacin has been reported (Abdullahi & Iregbu, 2019; Rajaduraipandi *et al.*, 2006). The susceptibility of this aminoglycoside and Carbapenem (regarded as last-resort antibiotics for Gram-positive and negative bacterial infections) may result from their relative expensive and unavailability of these drugs for abuse. As such, imipenem and Amikacin could be considered for the treatment of wound infections harboring MRSA.

Considerably, a substantial quantity of the toxin *tst* gene that mediates toxic shock syndrome in wound patients and PVL operon (*lukS-PV* and *lukF-PV* genes) has been reported from wound infection (Shittu *et al.*, 2011; Kolawole *et al.*, 2013). Expression of these virulent genes may delay wound healing which leads to prolonged hospitalization and increased duration of antibiotic administration in wound patients. The profile of multidrug antibiotic resistance reported in wound samples in this study is in line with reports in the same setting (Garoy *et al.*, 2018; Peter *et al.*, 2022a). This implies that the isolates emerged from a high-risk source of contamination, where antibiotics are frequently administered and probably abused as a result of poor infection control and prevention policies. As such, the high multidrug-resistant value demonstrated among MRSA isolates may also be linked to external sources of contamination besides the hospital setting in the studied patients.

Plant-derived antimicrobial compounds are effectively utilized in disease management globally. Keeping the above fact in view, the antibacterial activity of *P. biglobosa* leaf and stem-bark methanol extracts shows concentration dependent pattern on the MRSA isolates. *P. biglobosa* stem-bark produces an inhibition zone diameter of 14mm at 25mg/ml concentration against MRSA isolates from Decubitus ulcer patients which is corroborated with the work of Obajuluwa *et al.* (2013) who reported that twenty-eight (28) MRSA isolates from bedsore had inhibitory zone of 11-14mm at 25mg/ml concentration. Stem-bark and leaf extract exhibit high inhibitory effects of 25mm and 19mm respectively at 100mg/ml against MRSA isolates. This finding revealed that stem-bark extract had higher antibacterial activity than leaf extract. However, variability in the zone of inhibition diameter was observed at different concentrations. The variations were comparable to prior research on *P. biglobosa* methanol leaf extract (Ajaiyeoba, 2002; Udobi *et al.*, 2010; Obajuluwa *et al.*, 2013). By the observed inhibitory activity, *Parkia biglobosa* contains secondary metabolites e.g., tannins, flavonoids, and saponins (Ajaiyeoba, 2002), which possess high antibacterial properties. According to Millogo-Kone *et al.* (2007), the stem bark contains triterpenes, sterols, tannins, anthocyanins, saponosides, coumarins, reducing compounds, and flavonoids, while the leaf contains flavonoids, coumarins, tannins, coumarins, reducing compounds, and anthocyanins (Millogo-Kone *et al.*, 2007). The higher antibacterial activity of *Parkia biglobosa* stem-bark extract found in the present research was attributed to the presence of many constituent groups that may be functioning synergistically against MRSA isolates.

From the results, most MRSA isolates were not susceptible to methanol leaf and stem bark *Newbouldia laevis* extract. It was noted that, for fewer MRSA isolates, the inhibitory effects became more pronounced at 100mg/ml and 50mg/ml as presented in the result. In contrast with these findings, previous literature has reported the antibacterial activity of stem bark *Newbouldia* against *Staphylococcus aureus* from infected wounds and eyes. Our result, although inconsistent with reports from earlier findings (Akerlele *et al.*, 2011), the composition of phytochemicals in the extracts may have been changed directly by changes in the extraction

process, nature, and selection of solvents utilized across investigations. This could explain the observed discrepancy. Comparatively, the general variability observations amongst *P. biglobosa* and *Newbouldia laevis* against MRSA in this study can be attributed to phytomolecules' affinity for solvents. The bioactive compounds found in *P. biglobosa* plant extract are thus more soluble in methanol than those found in *Newbouldia laevis*. The methanol extracts of the stems and barks were more active than the extracts of the leaves. This gap can be explained by differences in a plant's physiological features depending on its environmental microclimate, maturity, and soil type. These variables are critical in the production of chemical principles and thereby influence plant pharmacological action.

### CONCLUSION

The present study reports that MRSA isolates constituted a percentage frequency of 55.5% recovered from various wound samples. *In vitro*, failure of most antibiotics (erythromycin, lincomycin, tetracycline, Trimethoprim-Sulfamethoxazole, Vancomycin, penicillin, ceftazidime) with resistant trend of 50-100% against MRSA in this study justifies the need for more effective antibiotic therapy for wound infections with MRSA. Amikacin and imipenem were the only antimicrobial drugs that demonstrated 87.8-100% sensitivity to the test isolate, making them a better therapeutic option in single or combination regimen treatment. In comparison to *P. biglobosa* leaf and stem extract, *Newbouldia laevis* leaf and stem bark methanol extract had no good inhibitory effect at 25mg/ml, 12.5mg/ml, and 6.25 mg/ml concentrations against MRSA isolate. As a result, the findings of this study have verified folklore medicine's assertions about the use of *P. biglobosa* for the treatment of wound infections. The ample presence of phytochemicals substance in the methanol extracts *P. biglobosa* underscores their antibacterial activity against MRSA isolates in this study. The *Newbouldia laevis* plant's limited or low antibacterial activity *in vitro* needs further testing with different solvents. More research with a wide range of target strains is required to determine the abundant pharmacological value of the *Newbouldia laevis* and *P. biglobosa* plants on a large scale.

### ABBREVIATIONS

MRSA	Methicillin Resistant <i>Staphylococcus aureus</i>
NI	No Inhibition
AST	Antibiotic Susceptibility Testing
CFU	Colony Forming Unit

### FUNDING

There is no source of funding to declare.

### AUTHOR'S CONTRIBUTION

This work was carried out in collaboration among all authors. Authors VFN and KA wrote the protocol. Authors IUP and IRI wrote the first draft of the manuscript. Authors CIE, UNU, FCA, IMO, ANN, and HO managed the characterization and analyses of the study. Author IRI did the supervision. All authors read and approved the final manuscript.

### ACKNOWLEDGEMENTS

The authors sincerely acknowledge the patients and Medical Staff of Alex Ekwueme Federal University, Abakaliki for their support.

### COMPETING INTERESTS

Authors have declared that no competing interests exist.

**DATA AVAILABILITY STATEMENT**

Data supporting these findings are available within the article or upon request.

**INSTITUTIONAL REVIEW BOARD STATEMENT**

The ethical committee of Alex Ekwueme Federal University Teaching Hospital in Abakaliki, Ebonyi State, authorized this study conveyed with approval number: AEFUTHA26/11/2020. This was based on a thorough knowledge of the scientific literatures, satisfactory laboratory protocols, and other relevant sources of information guiding this area of research. Every fundamental study was done in line with the World Medical Association (WMA) declaration of Helsinki on the principles for medical research involving human subjects, and identifiable human material or data.

**REFERENCES**

- Abdullahi, N & Iregbu, K. C (2019). Methicillin-Resistant *Staphylococcus aureus* in a Central Nigeria Tertiary Hospital. *Annals of Tropical Pathology*, 9, 6-10.
- Agbo, O. O., Thompson, M. D., Etu, K. E., NseAbasi, P. L., Elom, K. O., Peter, I. U & Iroha, I. R (2024). Antibacterial activity of ethanol & methanol extract of *Vernonia amygdalina*, *Ocimum gratissimum* & *Aloe barbadensis* against *Pseudomonas aeruginosa* & *Staphylococcus aureus* Isolated from Wound Infected Patients. *International Journal of Pharmacognosy & Pharmaceutical Research*, 6(1), 54-60.
- Ajaiyeoba, E. O (2002). Phytochemical & Antibacterial Properties of *Parkia biglobosa* & *Parkia bicolor* leaf extracts. *African Journal of Biomedical Research*, 5(3), 125–129.
- Akerele, J. O., Ayinde, B. A & Ngiagah, J (2011). Phytochemical & Antibacterial Evaluations of the Stem Bark of *Newbouldia laevis* against Isolates from Infected Wounds & Eyes. *Tropical Journal of Pharmaceutical Research*, 10(2), 211-217.
- Ali, S. M., Bayoumi, E. A. M & Alshazly, T. A (2013). Molecular Identification of Methicillin-resistant *Staphylococcus aureus* & Evaluation of Panton-valentine leukocidin Gene as a Sole Marker for CA-MRSA. *Journal of American Science*, 9(8), 108-115.
- Anguzu, J. R & Olila, D (2007). Drug Sensitivity Patterns of Bacterial Isolates from Septic Post-operative Wounds in a Regional Referral Hospital in Uganda. *African Journal of Health Science*, 7, 148–154.
- Ariom, T. O., Iroha, I. R., Moses, I. B., Iroha, C. S., Ude, U. I & Kalu, A. C (2019). Detection & phenotypic characterization of methicillin-resistant *Staphylococcus aureus* from clinical & community samples in Abakaliki, Ebonyi State, Nigeria. *African Journal of Health Science*, 19(2), 2026-2035.
- Chen, K., Lin, S., Li, P., Song, Q., Luo, D., Liu, T., Zeng, L & Zhang, W (2018). Characterization of *Staphylococcus aureus* isolated from patients with burns in a regional burn center, Southeastern China. *BMC Infectious Diseases*, 18, 51.
- Clinical & Laboratory Standards Institute (CLSI). (2019). *Performance standards for antimicrobial susceptibility testing* (28th ed.). Wayne, PA: Clinical & Laboratory Standards Institute.
- Deyno, S., Fekadu, S & Astatkie, A (2017). Resistance of *Staphylococcus aureus* to Antimicrobial Agents in Ethiopia: a Metaanalysis. *Antimicrobial Resistance & Infection Control*, 6(1), 85-17.
- Ekuma, P. U., Ibiam, F. A., Ekuma, M I., Iroha, C. S., Peter, I. U & Iroha, I. R (2023). Evaluating the Bacteria Profile and Drug Susceptibility Patterns of Urinary Tract Infectious Pathogens in Pregnant Women in Abakaliki Metropolis, Nigeria. *International Journal of Pathogen Research*, 12(5), 52-62.



- Falagas, M. E., Karageorgopoulos, D. E., Leptidis, J & Korbila, I. P (2013). MRSA in Africa: Filling the Global Map of Antimicrobial Resistance. *Public Library of Science One*, 8(7), 680-24.
- Garoy, E. Y., Gebreab, Y. B., Achila, O. O., Tekeste, D. G., Robel, R. K., Kiflay, G. R & Tesfu, T. (2018). Methicillin-Resistant *Staphylococcus aureus* (MRSA): Prevalence & Antimicrobial Sensitivity Pattern among Patients—A Multicenter Study in Asmara, Eritrea. *Canadian Journal of Infectious Diseases & Medical Microbiology*, 9, 832-1834.
- Germann, K., Kaloga, M., Ferreira, D., Marais, J. P & Kolodziej, H (2006). Newbouldioside A-C Phenylethanoid Glycosides from the Stem-bark of *Newbouldia leavis*. *Journal of Phytochemistry*, 67(8), 805–811.
- Ghanem, S., Bahashwan, S. A., El Shafey, H. M., Fayed, A. A., Alhhazmi, A & Manzoor, N (2018). Antimicrobial resistance pattern of MRSA strains isolated from patients of a hospital in Madinah, Kingdom of Saudi Arabia. *African Journal of Microbiology Research*, 12(47), 1044-1049.
- Ghaznavi-Rad, E & Ekrami, A (2018). Molecular Characterization of Methicillin-Resistant *Staphylococcus aureus* Isolates, Isolated from a Burn Hospital, in Southwest Iran in 2006 & 2014. *Hindawi International Journal of Microbiology*, 5(23), 123-200.
- Guzman-Blanco, M., Carlos, M & Raul, I (2009). Review Epidemiology of Methicillin-resistant *Staphylococcus aureus* (MRSA) in Latin America. *International Journal of Antimicrobial Agents*, 34(4), 304–308.
- Haseeb, A., Faidah, H. S., Bakhsh, A. R., Malki, W. H., Elrggal, M. E., Saleem, F., Rahman, S. U., Khan, T. M & Hassali, M. A (2016). Antimicrobial Resistance Amongst Pilgrims: A Retrospective Study from Two Hospitals in Makkah, Saudi Arabia. *International Journal of Infectious Diseases*, 47, 92-94.
- Holmes, A., Ganner, M., McGuane, S., Pitt, T. L., Cookson, B. D & Kearns, A. M (2005). *Staphylococcus aureus* isolates carrying Panton-Valentine leucocidin genes in Engl & Wales: frequency, characterization, & association with clinical disease. *Journal of Clinical Microbiology*, 43(5), 2384–90.
- Ikeh, E. C (2003). Methicillin-resistant *Staphylococcus aureus* at Jos University Teaching Hospital. *African Journal of Clinical & Experimental Microbiology*, 4(1), 52–62.
- Ilang D. C., Peter I. U., & Iroha I. R (2023). Antibiotic Resistance Profile of Clinical Importance Biofilm forming Extended Spectrum Beta-lactamase and Carbapemase Phenotype in Gram-negative bacteria isolates. *International Journal of Pharmacognosy and Life Science*, 4(2), 120-127.
- Jauro, S., Abubakar, M. B., Geidam, Y. A., Zanna, M. Y., Kwoji, I. D., Isa-Gulani, A & Ibrahim, I (2018). Phytochemical & Antimicrobial Profile Analysis of *Parkia biglobosa* against Methicillin-Resistant *Staphylococcus aureus*. *Journal of Advanced Veterinary & Animal Research*, 5(2), 173-181.
- Jimenez, J. N., Ocampo, A. M & Vanegas, J. M (2012). CC8 MRSA Strains Harboring SCCmec type IV are Predominant in Colombian Hospitals. *Public Library of Science One*, 7(6), 38-576.
- Kolawole, D. O., Adeyanju, A., Schaumburg, F., Akinyoola, A. L & Lawal, O. O (2013). Characterization of Colonizing *Staphylococcus aureus* Isolated from Surgical Wards' Patients in a Nigerian University Hospital. *Public Library of Science One*, 8(7), 68-721.
- Kong, E. F., Johnson, J. K & Jabra-Rizk, M. A (2016). Community Associated Methicillin-resistant *Staphylococcus aureus*: An Enemy Amidst Us. *Public Library of Science Pathogens*, 12(10), 100-5837.
- Magliano, E., Grazioli, V., Deflorio, L., Leuci, A. I., Mattina, R & Romano, P (2012). Gender & Age-dependent Etiology of community-acquired urinary tract infections. *Scientific World Journal*, 16(12), 123-456.

- Mawalla, B., Mshana, S. E., Chalya, P. L., Imirzalioglu, C & Mahalu, W (2011). Predictors of Surgical Site Infections Among Patients Undergoing Major Surgery at Bugando Medical Centre in Northwestern Tanzania. *BioMedical Complement of Surgical Infection*, 11, 21-25.
- Millogo-Kone, H., Guissou, I. P., Nacoulma, O & Traore, A. S (2007). Antimicrobial effects of the stem Bark extracts of *Parkia biglobosa* on *Shigellae*. *African Journal of Traditional, Complementary & Alternative Medicine*, 4, 392–396.
- Neuman, H., Debelius, J. W., Knight, R & Koren, O (2015). Microbial Endocrinology: The Interplay between the Microbiota & The Endocrine System. *Federation of European Medical Societies of Microbiology Reviews*, 39(4), 509–521.
- Nwankwo, C., Okolo, I., Mba, A., Uzoeto, H., Udenweze, E., Okoli, F., Thompson, M., Chukwu, E., Basse, N., Ngwu, J & Peter, I (2023). *In Vitro* Assessment of Conventional & Plant-derived Antifungal Agents against *Candida* Species Prevalence among Pregnant Women in Southeastern Nigeria. *International Journal of Pharmaceutical & Phytopharmacological Research*, 13(1), 13-8.
- Nwankwo, E. O & Nasiru, M. S (2011). Antibiotic Sensitivity Patterns of *Staphylococcus aureus* from Clinical Isolates in a Tertiary Health Institution in Kano, Northwestern Nigeria. *Pan African Medical Journal*, 8(4), 123-131.
- Obajuluwa, A. F., Onaolapo, J. A., Oyi, A. R & Olayinka, B. O (2013). Susceptibility Profile of Methicillin-Resistant *Staphylococcus aureus* (MRSA) Isolates to Antibiotics & Methanolic Extracts of *Parkia biglobosa* (Jacq) Benth. *British Journal of Pharmaceutical Research*, 3(4), 587-596.
- Oke, B., Nomeh, O. L., Egwu, I. H., Edemekong, C. I., Nwiboko, M. C., Peter, I. U and Iroha, I. R (2024). High prevalence of *Salmonella* Typhi from well water harboring *CTX-M* and *tetA* resistance gene. *World Journal of advanced Research and Reviews*, 23(01), 2848-2857.
- Olowe, O. A., Eniola, K. I. T., Olowe, R. A & Olayemi, A. B (2007). Antimicrobial Susceptibility & Beta-lactamase detection of MRSA in Osogbo, SW Nigeria. *Nature & Science*, 5(3), 44-48.
- Omuse, G., Kariuki, S & Revathi, G (2012). Unexpected Absence of Methicillin-resistant *Staphylococcus aureus* Nasal Carriage by Healthcare Workers in a Tertiary Hospital in Kenya. *Journal of Hospital Infection*, 80(1), 71–73.
- Onolitola, O. S., Olayinka, B. O., Salawu, M. J & Yakubu, S. E (2007). Nasal carriage of methicillin resistant *Staphylococcus aureus* with reduced vancomycin susceptibility (MRSA-RVS) by healthy adults in Zaria. *Nigeria Journal of Tropical Microbiology & Biotechnology*, 3, 19-22.
- Orji, C.O., Ogba, R.C., Emeruwa, A.P., Peter, I.U., Uzoeto, H.O & Agumah BN (2024). Prevalence of *Staphylococcus aureus* Nasal Carriage that have Developed Resistance to Second & Third Generation Cephalosporins. *Journal of Advances in Microbiology Research*, 5(1), 130-135.
- Osuntokun, O. T., Jemilaiye, T. A & Akinrodoye, A. R (2018). Comparative Study between the Effect of *Parkia Biglobosa* (JACQ) benth & Conventional Antibiotics against Multiple Antibiotic Resistant Uropathogenic Bacteria (MARUB). *Journal of Bioequivalence & Bioavailability*, 5(4), 200–212.
- Peter I. U., Emelda, N. C., Chukwu, E. B., Ngwu, J. N., Uzoeto, H. O., Moneth, E. C., Stella, A. O., Edemekong, C. I., Uzoamaka, E. P., Nwuzo, A. C & Iroha, I. R (2022c). Molecular detection of bone sialoprotein-binding protein (*bbp*) genes among clinical isolates of Methicillin Resistant *Staphylococcus aureus* from hospitalized Orthopedic Wound Patients. *Asian Journal of Orthopaedic Research*, 8(3), 1-9.

- Peter, I.U., Edemekong, C., Balogun, O.M., Ikusika, B.A., Ngwu, J.N., Bassey, N.U., Mohammed, I.D., et al. (2022d). Phytochemical Screening & Antibacterial Activity of Aqueous Extract of *Costus afer* Plant on Selected Multidrug-Resistant Bacteria. *IOSR J Pharmacy & Biol Sciences*, 17(1), 39-42.
- Peter, I.U., Ngwu, J.N., Edemekong, C.I., Ugwueke, I.V., Uzoeto, H.O., Joseph, O.V., Mohammed, I.D., Mbong, E.O., Nomeh, O.L., Ikusika, B.A., Ubom, I.J., Inyogu, J.C., Ntekpe, M.E., Obodoechi, I.F., NseAbasi, P.L., Ogbonna, I.P., Didiugwu, C.M., Akpu, P.O., Alagba, E.E., Ogba, R.C., & Iroha, I.R. (2022b). First report prevalence of Livestock Acquired Methicillin Resistant *Staphylococcus aureus* (LA-MRSA) strain in South Eastern, Nigeria. *IOSR J Nurs Health Sci.*, 11(1), 50-56.
- Peter, I.U., Okolo, I.O., Uzoeto, H.O., Edemekong, C.I., Thompson, M.D., Chukwu, E.B., Mohammed, I.D., Ubom, I.J., Joseph, O.V., Nwuzo, A.C., Akpu, P.O., & Iroha, I.R. (2022a). Identification & antibiotic resistance profile of biofilm-forming Methicillin Resistant *Staphylococcus aureus* (MRSA) causing infection among orthopedic wound patients. *Asian J Res Med Pharm Sci.*, 11(4), 45-55.
- Rajadurai pandi, K., Mani, K. R., Panneerselvam, K., Mani, M., Bhaskar, M & Manikandan, P (2006). Prevalence & Antimicrobial Susceptibility Pattern of Methicillin Resistant *Staphylococcus aureus*: A Multicentre Study. *Indian Journal of Medical Microbiology*, 24, 34-38.
- Rebiahi, S. A., Abdelouahid, D. E., Rahmoun, M., Abdelali, S., & Azzaoui, H. (2011). Emergence of vancomycin-resistant *Staphylococcus aureus* Identified in the Tlemcen University Hospital (North-West Algeria). *M`edecine et Maladies Infectieuses*, 41(12), 646–651.
- Seni, J., Bwanga, F., Najjuka, C. F., Makobore, P & Okee, M (2013). Molecular Characterization of *Staphylococcus aureus* from Patients with Surgical Site Infections at Mulago Hospital in Kampala, Uganda. *Public Library of Science One*, 8(6), 66-153.
- Shirah, B. H., Zafar, S. H., Alferaidi, O. A & Sabir, A. M. M (2017). Mass Gathering Medicine (Hajj pilgrimage in Saudi Arabia): The Clinical Pattern of Pneumonia Amongst Pilgrims During Hajj. *Journal of Infection & Public Health*, 10(3), 277-286.
- Shittu, A. O., Okon, K., Adesida, S., Oyedara, O & Witte, W (2011). Antibiotic Resistance & Molecular Epidemiology of *Staphylococcus aureus* in Nigeria. *BioMedical Complement of Microbiology*, 11, 92-100.
- Taiwo, S. S., Onile, B. A & Akanbi, A. A (2004). Methicillin resistant *Staphylococcus aureus* (MRSA) Isolates in Ilorin, Nigeria. *African Journal of Clinical & Experimental Microbiology*, 5(2), 189–197.
- Ude, I. U., Moses, I. B., Okoronkwo, C., Ovia, K., Okafor, C., Chukwunwejim, C. R., & Iroha, I. R. (2021). Phytochemical properties and antimicrobial activity of *Buchholzia coriacea* and *Psychotria microphylla* leaf extracts on bacterial pathogens isolated from aquatic environments in Nigeria. *Journal of Medicinal Plants Research*, 15(6), 232-240.
- Udobi, C. E., Obajuluwa, A. F & Onaolapo, J. A (2013). Prevalence & Antibiotic Resistance Pattern of Methicillin-Resistant *Staphylococcus aureus* from an Orthopaedic Hospital in Nigeria. *Hindawi Publishing Corporation BioMedical Research International*, 12(4), 467-860.
- Udobi, C. E., Onaolapo, J A & Agunu, A (2010). Antibacterial Potentials of the Methanolic Extract & Aqueous Fraction of the Stem Bark of the African Locust Bean (*Parkia Biglobosa*). *European Journal of Scientific Research*, 43(4), 590–597.
- Usman, H & Osuji, J. C (2007). Phytochemical & *In vitro* Antimicrobial Assay of the Leaf Extract of *Newbouldia laevis*. *African Journal of Traditional, Complementary & Alternative Medicine*, 4(4), 476-480.