Evalution of Antibactierial Activity of Methanol leaf and stem Bark Extracts of Newboulia laevis, Parkia biglobosa And Conventional Available Antibiotics on Methicillin Resistant Staphylococcus aureus

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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 antibacterial activiy of methanol leaf and stem bark exracts of Newbouldia laevis, Parkia biglobosa and conventional available antibiotics on MRSA from various wound sources in Abakaliki. This study aimed at investigating the prevalence of MRSA and its associated risk factors amongst wound patients in a Tertiary Hospital. Methods: A total of 200 samples from post-surgical wounds, burn wounds, decubitus ulcer and diabetic foot ulcers patient were collected from March 2021 to February 2022 using a sterile swab sticks on the affected site. The collected specimens were immediately subjected to bacteriological analysis. MRSA were screened for Antibiotic Susceptibility Testing (AST) and results were analyzed using the Clinical Laboratory Standard Institute (CLSI) zone diameter breakpoints. Antibacterial activity of methanol leaf and stem-bark extract of Newbouldia laevis and P. biglobosa against the MRSA Isolates were determined using agar well diffusion Method. Results: the prevalence of MRSA accounted 55.5 %. Antibiogram result shows that the MRSA isolate 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 Newbodia leavis having inhibitory activity against MRSA at the range of 0 – 15mm at the same concentrations. Conclusion: Our study report the prevalence of MRSA with multi-resistant in wound samples and also advocate for jurdicious use of imipenem and amikacin as drug of choice against the test isolate. This findings validate the use of methanol leaves and stem-bark extract of P. biglobosa as alternative medicine for the treatment of MRSA-associated wound infection and recommends further complementary studies in ethno pharmacological use.

Keywords: Methicillin-resistant Staphylococcus aureus, Parkia biglobosa, Newbodia leavis, wound.

Introduction

Methicilin Resistant Staphylococcus aureus (MRSA) are strain encoding MecA gene which mediate resistant to penicillin-derived methicillin and other beta-lactam antibiotic [1]. The presence of an altered penicillin binding protein (PBP2a) encoded by MecA strain has been recognized as factor essential for methicillin resistant [2]. This housekeeper gene is found as an integral portion of the Staphylococcal cassette chromosome mec (SCC mec) [2, 3]. Methicillin Resistant Staphylococcus aureus are considered as priority pathogen in community and hospital acquired infection of which their potential risk are also matters of concern [2].

MRSA has a somewhat high global burden in immunocompromised patients particularly wound patients causing significant morbidity and mortality [4]. In human, MRSA remains one of the medically important bacteria that colonizes bone and wound infections. The expression of virulence by MRSA in osteomyelitis and postoperative wound infections orthopedic medicine [6] are the leading cause of delayed bone/wound healing and non-union which may necessitate the amputation of such bones [2, 6]. Prior study has reported high risk of MRSA infection in fracture and post-surgical wound patients [6]. MRSA are often multi-resistant eroding the effective of most conventional antibiotic with limited treatment option. Another key element that contributes to treatment failure in wound patients is the creation of various virulence factors. For example, MRSA strains that produce Panton-Valentine Leukocidin (PVL) have higher lethality than PVL-negative bacteria [6] as observed in soft tissue infection, surgical site infection (SSI) and burn wound [7].

Despite the fact that MRSA is a substantial health issue, there is a lack

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of research on the frequency of MRSA in wounds in Nigeria. In most hospital department/ward with wound patients, Ampicillin, ampiclox, gentamicin, and ciprofloxacin are the most widely prescribed antibiotics. The majority of recently utilized medications in most hospital 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. 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 [8, 9]. 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) [10]. It can reach a height of 7 - 15meters, but it is more commonly a shrub of 2-3 meters with multiple stems producing clumps of gnarled branches [10]. 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 [10, 11]. Newbouldia laevis is commonly used in folk medicine in South Eastern and Midwestern Nigeria for the treatment of infected wounds and eye issues [10]. Scientific studies on the plant's phytochemical contents indicated the presence of phenylpropanoids and alkaloids in the root [12] tannins and flavonoids in the leaves [13]. Also, Parkia biglobosa (African locust bean tree) is native to Africa [14] and is commonly referred to as Orgili in the Igbo tribe of Nigeria. Parkia biglobosa is a plant that has demonstrated promising source of phytomedicinal compound [15], 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 [16]. 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 health care 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 antibiotic and 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 study comprised consenting patients with current post-surgical wound, burn wound, decubitus ulcer, and diabetic foot ulcers occurring within 30 days or within 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, and 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 col-lected within the period of 12 months (March 2021 to February 2022) from post-surgical wound, burn wound, decubitus ulcer and diabetic foot ulcers patients were collected by rotating a sterile swabs 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 37oC. The turbid bacterial growth was plated onto MP1571 Denim Blue Chromogenic MRSA Screening Agar (Oxoid, UK), and incubated for 24 hrs at 37oC. 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 37oC. The pure culture strain 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., equivalent of $1x10^6$ 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 was allowed for 15 minutes for pre-diffuse of bacteria suspension. Antibiotic discs that are commercially available and 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 was aseptically applied with sterile forceps onto the surfaces of the solidified Mueller-Hinton plates and gently pressed to achieve uniform contact. The plates was incubated for 24 hrs at 37° C. The Inhibition zones were interpreted in millimeter (mm) according to CLSI guideline for antibiotics sensitivity [2, 16].

Collection and Authentication of Parkia biglobosa and Newbouldia laevis

Parkia biglobosa and Newbouldia laevis leaf and stem bark were collected at Ndufe-Alike community in Ikwo L. G. A in Ebonyi State. The plants 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.

3.1.25 Extraction of Stem-bark and Leaf of Parkia biglobosa and Newbouldia laevis

This was performed following a modification in methods of Osuntokun et al. [17] as stated below.

Modification: The work of Osuntokun et al. [17] air-dried the plant material for 10days 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 were cut into pieces and then pulverize with a sterile mortar and pestle to reduce into a powdered particles [14, 17]. Prior to extraction, exactly 200 grams of Parkia biglobosa and Newbouldia laevis each leaf and stem bark was soaked in 400 ml volume of methanol and allowed to stand for overnight at room temperature. It was then filtered with muslin cloth [8]. The filtrate was evaporated to dryness at <40oC using 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 10ml of 70% Dimethylsulphoxide as diluent to obtain a concentration of 100mg/ml. Exactly, 10-5 serial dilution was carried out from the stock solution to obtain extract concentration 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 [8, 18]. A suspension of bacterial cells (i.e., equivalent of 1x106 colony forming unit per milliliter (cfu/ml) was seeded onto each Petri dish containing solidified Mueller-Hinton agar 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. The wells were filled with 1ml of each extract concentration. The inoculated agar plates were incubated for 24 hours at 37OC. After incubation, clear inhibition zones were measured using a metric rule and interpreted in millimeters (mm).

Result

Prevalence of MRSA from wound samples

MRSA from isolated Staphylococcus aureus recovered from postsurgical wound, burn wound, diabetic foot ulcers and decubitus ulcer samples showed prevalence rate 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 were predominant amongst 35-49years 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 Female than male with MRSA occurrence rate of 33.5% and 22.0% respectively. Duration of hospitalization within 2months displayed 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 duration of 1 month as shown in Table 2.

Antibiotic susceptibility profile of MRSA

The result of antibiotic susceptibility profile of MRSA from post-surgical wound swabs in Table 2 showed 100%, 87.8%, 51.2%susceptibility to Imipenem, Amikacin and Cefotaxime respectively. The isolates also showed highest (100%) resistance to Penicillin and tetracycline, followed by 97.5%, 951% and 87.8% resistance to Lincomycin, trimethaprime-sulfamethoxazole and Vancomycin.

MRSA isolated from burn wound patient were 100% resistant to penicillin, tetracycline, trimethoprim-Sulfamethoxazole, Vancomycin while 78.9%, 69.7% and 57.6% resistant was observed against erythromycin, lincomycin and ceftazidine respectively. The MRSA isolated was 100% susceptible to Amikacin, imipenem and Cefotaxime followed by 42.2%, 30.3% and 21.2% recorded against Ceftazidine, lincomycin and Erythromycin respectively. While Amikacin, cefostaxime and imipenen showed 100% susceptible to the MRSA isolates from burn wound patients, ceftazidine, erythromycin an lincomycin recorded 42.4, 21.2 and 30.3 susceptible respectively against MRSA isolate from burn wound as shown the Table 2.

MRSA recovered from decubitus ulcer patient were susceptible to aminoglycoside (amikacin 90.9%), cephalosporin (cefotaxime 100%) and Cabapenem (imipenem 100%) but less susceptible against erythromycin and ceftazidine recording 13.6% and 27.3% respectively. MRSA isolated from Decubitus ulcer Patient exhibit high level of resistance recording 100% to Trimethoprim-Sulfamethoxazole, Vancomycin, Tetracycline, Penicillin and Lincomycin while 86.4%, 72.7%

and 9.1% resistance rate was recorded against Erythromycin, Ceftazidime and Amikacin respectively as presented in Table 3. Also, MRSA recovered from Diabetic foot ulcers patient were 100% resistance to lincomycin, penicillin, tetracyclin, trimethoprim – sulfamethoxazole and vanconycin. Amikacin showed resistance of 9.1 against 90.9 susceptible. Then, cetrazidine and erythromycin recorded 72.7 and 86.4 resistance as presented in the 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 inhibition zone diameter within the range of 11-15mm at 100mg/ml concentration while stem-bark extract had inhibition zone diameters within the range of 19-26 mm at 100mg/ml concentration against MRSA isolates from 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 wound 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 wound.

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. While the stem barkshowed active resistance at concentration 100mg/ml recording 14-25mm and 9-21mm at the concentration 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 wound .The leaf extract at 100mg/ml showed highest activity measuring18mm 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 wound 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 diameter 20mm, 17mm and 15mm and 15mm at 100mg/ml and 50 mg/ml concentration 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 Stembark extract revealed high inhibitory zone diameter of 21mm and

17mm at 100mg/ml and 50 mg/ml concentration respectively (Table 11).

Discussion

MRSA is assessed by a series of resistant determinant according to different strains. As a result, the chromogenic agar was used to identify MRSA strain in wound with 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. [19] reported 47.7 % in Osogbo, south-western Nigeria [19], two studies reported 75% in Zaria [1, 20], Ikeh. [21] reported an MRSA prevalence of 81% from in-patients in Jos, Nigeria. Nwankwo and Nasiru [22] reported 62% prevalence rate from in-patients in kano Nigeria. In Enugu, Nigeria, MRSA strain accounted 86(28.4 %) and 78(25.7 %) from post-surgical wound and fracture wound respectively [2]. Taiwo et al. [23], 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 [24], 25% in Jinja-Uganda [25], 37.4% in Madinah kingdom of Saudi Arabia

[26], 25 (61%) in Iran [4], 75% from surgical wounds in Algeria [27], 72.0% in Eritrea [5], 80% in Peru [28] and in a setting in Colombia 90% [29]. Many published research have also documented a rise in the prevalence of MRSA, the majority of which was caused by wounds [30, 31]. 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 [32]. Some studies, for example, depend completely on phenotypic processes to detect MRSA, whilst others rely on DNA-based approach (Multilocus Sequence Typing [MLST]), microarrays, whole genome sequencing, spa-typing, conventional PCR and, among others) to identify mecA. Apparently, investigations relying on DNA-based technique by PCR tends to reveal a lower incidence of MRSA [22, 31].

MRSA was more predominance in Post-surgical wound 20.5% than other wound sample in the study. This reiterate with the prevalence of MRSA 3.9%-35.6% in Post-surgical wound documented by other researchers [5, 33]. 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 [33]. Post-surgical wound patients in this study may likely be at risk of postsurgical toxic shock syndrome which result in delay wound healing and prolong hospitalization.

In this study, male patients harbor MRSA than female with MRSA occurrence rate of 33.5% and 22.0% respectively and is consistent with earlier research: male 55.9% vs female 44.1% [5], male 71% vs female 25% [26]. 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 prevalence from male patients in all studied cases [34, 35]. A study from Makkah found a similar pattern of males having higher MRSA isolation rates [36]. This trend could be linked to a variety of factors, including males being more susceptible to bacterial sepsis; being less compliant and so inclined to greater infection rates [37]. According to research, the female hormone (estrogen) has a negative effect on the development of virulence factors in pathogens [38]. Furthermore, the patients' personal 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 were more predominant amongst 35-49years recording 21.5%. Another study has reported high rate of MRSA with 22(37.3%) in 19–40year age group [5]. Low prevalence in this study was observed amongst age

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)
Total	200	111(55.5)

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

Characteristic		No. sampled	MRSA (%)
	20-34years	68	31(15.5)
Age group	35-49years	84	43(21.5)
	50-69 years	48	37(18.5)
Gender	Male	96	67(33.5)
	Female	104	44(22.0)
	7 days	26	15(7.5)
	> 1week	39	20(10.0)
Duration of hospitalization	1 month	27	8(4.0)
	2months	53	37(18.5)
	3months	24	10(5.0)
	4months & 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 n=41		Burn wound n=33		Decubitus ulcer n=22		Diabetic foot ulcers n=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	NILL	8	7
MRSA 5		NILL	NILL	NILL	NILL	NILL
MRSA 7		NILL	NILL	NILL	NILL	NILL
MRSA 9		15	12	10	7	8
MRSA 10		14	9	9	8	NILL
MRSA 11		14	12	10	9	9
MRSA 12		13	12	8	NILL	NILL
MRSA 14		NILL	NILL	NILL	NILL	NILL
MRSA 15		NILL	NILL	NILL	NILL	NILL
MRSA 2	Stem-bark	21	20	19	NILL	NILL
MRSA 5		20	15	NILL	NILL	NILL
MRSA 7		21	17	12	NILL	NILL
MRSA 9		19	15	11	NILL	NILL
MRSA 10		24	16	16	15	NILL
MRSA 11		26	19	15	12	17
MRSA 12		18	12	10	NILL	NILL
MRSA 14		19	14	11	10	NILL
MRSA 15		20	17	15	12	10

Key: *NILL-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	NILL	NILL
MRSA 22		15	12	10	8	7
MRSA 10		12	9	NILL	NILL	NILL
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	11
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
MRSA 10		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 aureu.*

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	11
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

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	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

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	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

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	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

Table 11: *Antibacterial activity of methanol crude extract of Newouldia 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

50-69 years and has been found elsewhere [5, 39]. This low isolation rate amongst age 50-69 years may be linked to 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 are known to possess low affinity to β-lactam antibiotics. Therefore, exposure to sublethal dose of this β-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 [2, 5, 33, 36, 39]. Despite considerable efforts to manage antibiotic resistance bacteria by rigorous infection control strategies, antibiotic resistant Staphylococci, particularly MRSA, have become the most common bacteria encountered in bone and wound infections in orthopaedic treatment [1, 31].

MRSA is a big concern worldwide [2], with a high significant morbidity and mortality rate in immunocompromised patients, particularly burn wound patients [4].

This study shows that MRSA exhibit resistant to macrolides (that induce erm expression) and lincosamide. This isolates demonstrate 51%-100% resistant to lincomycin and erythromycin and it's similar to another study [2, 4, 5, 7, 39]. This means that both lincomycin and erythromycin are ineffective in these patients.

Majority of our MRSA showed 100% resistant to penicillin and tetracycline and is in accordance with the pattern in Abakaliki and Jiangxi province [7, 30]. 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 the Nigeria [2, 20, 23, 40] this study confirms the presence of vancomycin-resistant MRSA amongst wound patient in Abakaliki. Low vancomycin-resistant MRSA from wound 11.0% in Asmara, Eritrea [5] has been published. In contrast, several studies found that MRSA is 50-100% susceptible to vancomycin. [26, 39, 41, 42]. According to Falagas et al. [43], the sensitivity of MRSA identified in Africa to VRSA ranges between 82-100% [43]. These estimates and findings contradict an earlier report that VRSA strains are rare and there is limited indication of rising prevalence [44].

The observed heterogeneity could be explained by changes in antibiotic prescription patterns between countries. Additionally, the vanA gene complex, which encodes high degree glycopeptide resistance in enterococci, was detected in the vancomycin resistant Staphyloccus. aureus (VRSA) isolates [2]. 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 [2].

MRSA from wound 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 (SCCmec) (types I-VII) and is controlled by MecI (a repressor) and MecR1 (a transducer) and represents the regulatory/signalling proteins of the blaZ system [2, 8] 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 [2, 8] as evidence in this study.

Interestingly, all MRSA our 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 [41, 45]. The susceptibility of this aminoglycoside and Carbapenem (regarded as last-resort antibiotic for Gram positive and negative bacterial infections) may result from their relative expensive and unavailability of this drugs for abuse. As such, imipenem and Amikacin could be considered for treatment of wound infection harboring MRSA.

Considerably, a substantial quantity of the toxin tst gene that mediate toxic shock syndrome in wound patients and PVL operon (lukS-PV and lukF-PV genes) has been reported from wound infection [33, 46]. Expression of this virulent genes may increase delay wound healing which lead to prolong hospitalization and increase duration of antibiotic administration in wound patient. The profile of multidrug antibiotic resistance reported in wound samples in this study is in line with report in the same setting [1, 5]. 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 isolate may also be linked to external source of contamination beside 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 show concentration dependent pattern on the MRSA isolates. P. biglobosa stem-bark produce inhibition zone diameter of 14mm at 25mg/ml concentration against MRSA isolates from Decubitus ulcer patients which is corroborate with the work of Obajuluwa et al. [20] 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 effect of 25mm and 19mm respectively at 100mg/ml against MRSA isolates. This finding revealed that stem-bark extract had high antibacterial activity than leaf extract. Although variability in zone of inhibition diameter was observed at different concentration. The variations were comparable to prior research on P. biglobosa methanol leaf extract [20, 47, 48]. In accordance with the observed inhibitory activity, Parkia biglobosa has been found to be contain secondary metabolites e.g., tannins, flavonoids, and saponins [47], which possesses high antibacterial properties. According to Millogo-Kone et al. [15], the stem bark contains triterpenes, sterols, tannins, anthocyanins, saponosides, coumarins, reducing compounds and flavonoids, whilst the leaf contains flavonoids, coumarins, tannins, coumarins, reducing compounds and anthocyanins [15]. 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 isolate.

From the results, most MRSA isolate were not susceptible to methanol leaf and stem bark Newbouldia laevis extract. It was noted that, for fewer MRSA isolate, the inhibitory effects became more pronounced at 100mg/ml and 50mg/ml as presented in the result. In contrast with this findings, previous literature has reported antibacterial activity of stem bark Newbouldia against Staphylococcus aureus from infected wound and eyes. Our result, although inconsistent with reports from previous study [10], 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. Com-

paratively, 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 report that MRSA isolate constituted a percentage frequency of 55.5% recovered from various wound samples. In vitro failure of most antibiotic (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 infections such as wounds. The ample presence of phytochemicals substance in the methanol extracts P. biglobosa underscore their antibacterial activity against MRSA isolates in this study. The Newbouldia laevis plant's limited or low antibacterial activity in vitro need 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 plant on a large scale.

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