Susceptibility profile of methicillin-resistant Staphylococcus aureus (MRSA) isolates to antibiotics and methanolic extracts of Parkia biglobosa (Jacq) Benth

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ABSTRACT:

Aims: To study the susceptibility profile of methicillin-resistant *Staphylococcus aureus* (MRSA) isolates from orthopaedic patients to antibiotics and methanolic extracts of *Parkia biglobosa*.

Background: Antimicrobial resistance in *Staphylococcus aureus* has attained alarming proportions worldwide, with methicillin resistant *Staphylococcus aureus* (MRSA) becoming a major pathogen of public health importance associated with community and hospital acquired infections. Wound infections in orthopaedic patients with multidrug resistant pathogens significantly delay or prevent the union of fractured bones. The increasing prevalence of multidrug resistance in *Staphylococcus aureus* isolates calls for the search for alternative anti-staphylococcal agents.

Methodology: Suspected staphylococcal isolates from wound, skin and bed swab samples from orthopaedic patients in a tertiary hospital in Zaria, Nigeria were characterized by established microbiological procedures and their antibiotic susceptibility pattern determined by the Kirby-Bauer-CLSI modified disc agar diffusion (DAD) technique. The activity of crude methanolic extract of the root, stem bark and leaf of *Parkia biglobosa* on the isolates determined.

Results: A total of 179 isolates were confirmed *S. aureus*: wounds (24.6%), skin (39.1%) and bed (36.3%). The isolation rates for MRSA from the various sites was: wound (75%), skin (51.4%) and bed (73.8%). Antibiotic susceptibility testing revealed that the isolates were generally resistant to ampicillin (100% for all sites); ceftriazone (wound 69.7%, skin 72.2%, bed 70.8%); gentamicin (wound 54.5%, skin 52.8%, bed 37.5%) and ciprofloxacin (wound 51.5%, skin 47.2%, bed 35.4%). The phytochemical screening of the methanolic extract of the leaf, root and stem bark of *Parkia biglobosa* showed the presence of saponin, tannin, flavonoids and cardiac glycosides. The stem bark of *Parkia biglobosa* showed the greatest activity against all the multidrug resistant MRSA isolates at the 10mg/ml-25mg/ml concentration range used.

Conclusion: There was high prevalence of multidrug resistant *Staphylococcus aureus* isolates from the clinical and surveillance samples from the orthopaedic patients. In the search for alternative antistaphylococcal agents from natural sources, *Parkia biglobosa* will be a possible candidate for further investigation.

Keywords: Methicillin resistant Staphylococcus aureus (MRSA), orthopaedic

1. INTRODUCTION

The emergence of multi-drug resistant (MDR) strains of S. aureus, especially the methicillin-resistant staphylococcus aureus (MRSA), has made the chemotherapy of staphylococcal infections in community and hospital settings increasingly challenging. Increased prevalence of these MDR strains of S. aureus in different, special patient groups has resulted in poor prognosis of infections. Methicillin resistant *S. aureus* (MRSA) was first discovered in 1961, they are isolates of *S. aureus* which have acquired genes encoding antibiotic resistance to all penicillins including methicillin and other narrow spectrum β lactamase resistant penicillin antibiotics. Since then hospitals worldwide have reported varying proportion of MRSA among *S. aureus* isolates (Foster, 1996). Wound infections in orthopaedic patients with such MDR strains significantly delay or prevent the union of fractured bones. Reports of development of resistance to a wide range of anti-staphylococcal drugs like the glycopeptides; vancomycin and teicoplanin, linezolid and strengraminquinupristin/dalfopristin mixture necessitates the search for new anti-staphylococcal agents of plant origin.

Parkia biglobosa is a multipurpose fodder tree that belongs to the family MIMOSACEAE (Sabiti and Cobbina, 1992). Popularly called the "African locust bean tree", they are known to occur in a diversity of agroecological zones from tropical rainforest where the rain is high to the arid zone where it is low. The height ranges from 7 - 30 m. It is crown large and spreads wide with low branches, the leaves are alternate, dark green, bipinnate and about 8 – 30 mm x 1.5 – 8 mm in size with about 13 - 60 pairs of leaflets of distinct venation on along rachis. The pods are pink brown to dark brown when matured, they are up to 45 cm long and 2 cm wide. Each pod contains up to 30 seeds embedded in a yellow pericarp.

The seeds are relatively large with an average weight of 0.26 g and have a hard testa. (Agroforestree Database, 2008). The bark is used as a mouthwash, vapour inhalant for toothache, or for ear complaints. It is macerated in baths for leprosy and used for bronchitis, pneumonia, skin infectious, sores, ulcers bilharzias, washes for fever, malaria, diarrhea, violent colic and vomiting, sterility venereal diseases, guinea worm, oedema and tickets and as a poison antidote. Leaves are used in lotions for sore eyes, burns, haemorhoids and toothache. Seed is taken for tension and pulp for fevers, as a diuretic and as a mild purgative. Roots are used in a lotion for sore eyes. (Irvine 1961)

This paper reports the susceptibility profile of methicillin-resistant *Staphylococcus aureus* (MRSA)isolates to antibiotics and methanolic extracts of *Parkia biglobosa*.

2. MATERIAL AND METHODS

Bacteriology

Suspected staphylococcal isolates from clinical (wound swab) and surveillance (skin, bed) samples from orthopaedic patients in a tertiary referral hospital in Zaria, Nigeria over a three-month period were characterized by established microbiological procedures. Isolates that were Gram-positive cocci, catalase positive and coagulase positive were considered *S. aureus* in this study.

Detection of Methicillin Resistance

This was carried out according to Clinical Laboratory Standards Institute (CLSI, 2006) guidelines using oxacillin in agar screen test whereby all phenotypic MRSA isolates were spot - inoculated onto Mueller-Hinton agar supplemented with 6µg/ml oxacillin and 4%

sodium chloride, from a 0.5 McFarland standard suspension. The plates were incubated at 35^{0}C for 24hours and the isolates that had growth (more than one colony) were considered methicillin-resistant.

Antibiotic Sensitivity Tests

Kirby Bauer – NCCLS (now CLSI) modified disc agar diffusion technique was used (Cheesbrough, 2002). Discreet colonies of isolates on nutrient agar plates were emulsified in 3 ml of phosphate buffered solution (PBS) and the turbidity adjusted to 0.5 McFarland standard. Using sterile swab sticks, the surface of Mueller Hinton agar (MHA) in a 90 mm diameter plate was inoculated with the bacterial suspension by streaking the surface of agar in three directions, rotating the plate approximately 60^0 to ensure even distribution. The inoculated plates were allowed to dry for 10 minutes before the antibiotic discs were applied asceptically to the surface of the agar. After 30 minutes of applying the discs the plates were inverted, and incubated at 35^{0}C. Similar treatment was extended to standard *S. aureus* (ATCC) 25923 which was used as control.

Collection and Authentication of *Parkia biglobosa*

The plant materials namely leaves, roots and stem bark were collected from Samaru-Zaria in Kaduna State, Nigeria. They were authenticated in the herbarium section of the Biological Science Department of Ahmadu Bello Univeristy, Zaria with the herbarium number 2846.

Preparation and Extraction of Plant Samples

Each plant sample was air dried for five days and ground into powder in a mortar, prior to extraction with methanol using soxhlet apparatus (Oboh *et al*., 2007). The solvent was thereafter removed and the methanolic extract yielded was stored in the desiccator until needed.

Phytochemical Screening

The methanolic extract was subjected to phytochemical screening to test for the presence of saponins, tannins, flavonoids, carbohydrates, alkaloids and steroids using standard methods as described by Trease and Evans (1989);Harbone, (1991).

Antibacterial Activity of Crude Methanolic Extract of Leaves, Roots and Stem Bark of *Parkia biglobosa* to MRSA

The isolates that were found to be MRSA were used for this test, agar cup diffusion method was used. An overnight broth culture of each isolate was used to seed sterile molten Mueller Hinton agar medium maintained at 45^{0}C. They were allowed to set and wells (6mm in diameter) were made on them using a sterile standard cork borer. Various concentrations of the plant extract (ranging from 10mg/ml to 25mg/ml) were added to each well. The plates were allowed to stand at room temperature for about one hour and thereafter incubated at 37^{0}C for 24hours. The diameter of each zone of inhibition was measured after incubation.

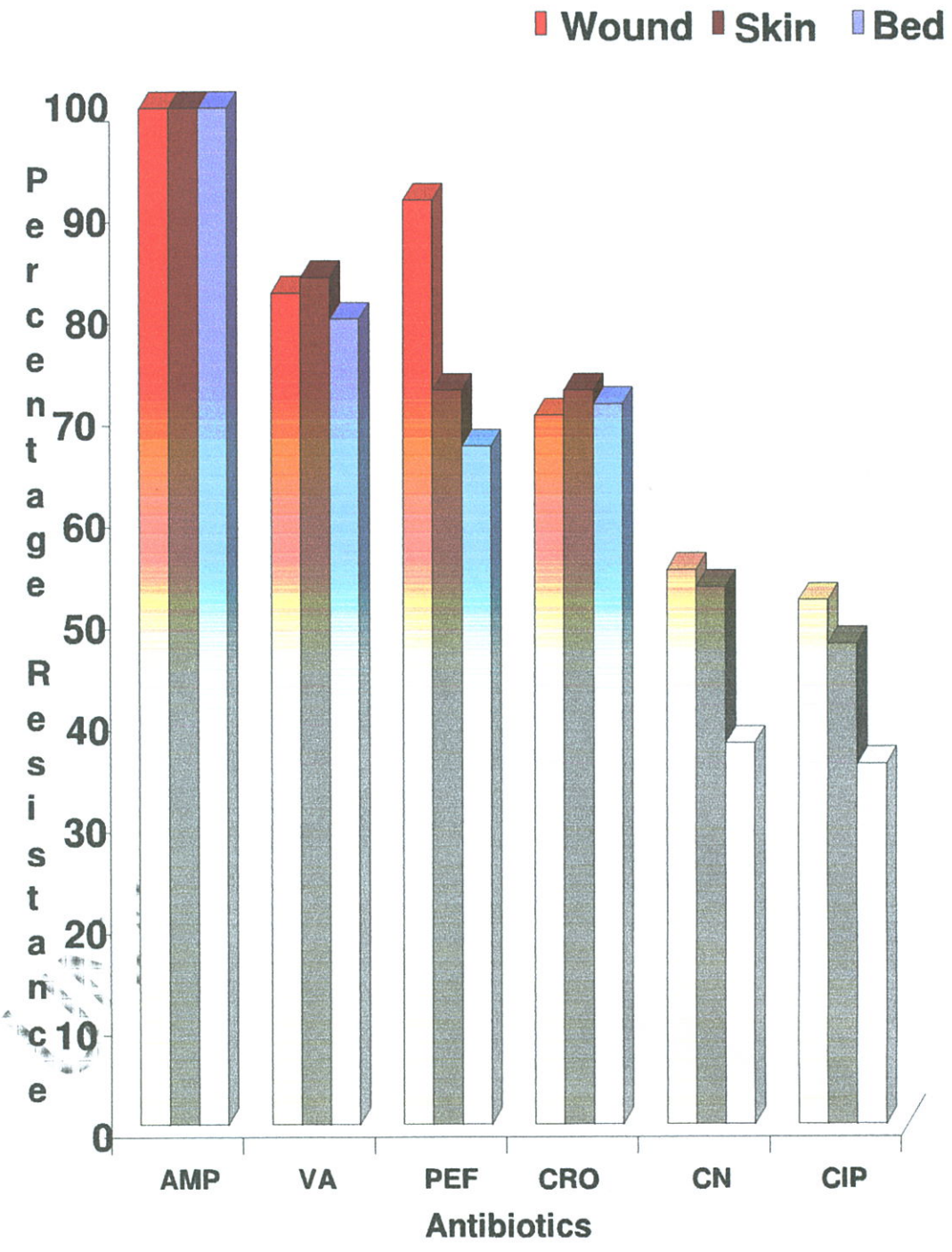
3. RESULTS AND DISCUSSION

Out of the total number of 211 samples collected, 179 confirmed *S. aureus* isolates were recovered from the clinical and surveillance samples and were distributed as wounds (24.6%), skin (39.1%) and bed (36.3%). The isolation rates for MRSA from the various sites was: wound (75%), skin (51.4%) and bed (73.8%) Table 1 shows the distribution of *S. aureus* and MRSA isolates from the various sample sites.

Table 1: Distribution of *S. aureus* and MRSA isolates

Source	No of sample	*S.aureus* No %	MRSA No %
Wound	51	44(86.3)	33(75.0)
Skin	80	70(87.5)	37(51.4)
Bed	80	65(81.3)	48(73.8)
Total	211	179(84.8)	118(65.9)

Antibiotic susceptibility testing revealed that the MRSA isolates were generally resistant to ampicillin (100% for all sites); ceftriazone (wound 69.7%, skin 72.2%, bed 70.8%); gentamicin (wound 54.5%, skin 52.8%, bed 37.5%) and ciprofloxacin (wound 51.5%, skin 47.2%, bed 35.4%), this is shown in Figure 1.



120
121 **Figure 1: Percentage resistance of MRSA strains from various sites to antibiotics**

Key: AMP: Ampicillin 10µg CRO: Ceftriaxone 30µg VA: Vancomycin 5µg
CN: Gentamicin 10µg PEF: Pefloxacin 5µg CIP: Ciprofloxacin 5µg

There was high prevalence of ~~multidrug resistance~~ *S. aureus* from clinical and surveillance samples. *S. aureus* is among the most common cause of surgical site infection (SSI) in orthopaedic patients (Price *et al.*, 2008). Patients infected with ~~multidrug resistant~~ microorganisms may shed such into the environment, this is an indication that *S. aureus* can cause nosocomial infection. Centre for Disease Control and Prevention (1996) reported that *S. aureus* was the most common cause of nosocomial infections reported in National Nosocomial Surveillance System between 1990 – 1996. Also, Witte *et al.* (1994), Roberts *et al.* (1999) and Narezkina *et al.* (2006) reported that *S. aureus* is one of the most common cause of nosocomial infections. The majority of nosocomial infection is caused by a patient's own endogenous microbial flora present upon admission to the hospital (Arif *et al.*, 2007). The ~~multidrug reistance (MDR)~~ status of the MRSA isolate suggest limited therapeutic options. The MRSA isolates showed resistance to all the antibiotics used including vancomycin. Vancomycin was believed to have retained activity against MRSA but there is recent alarming increasing emergence of vancomycin resistance to *S. aureus* worldwide (Fridkin, 2001), even though there are other reports that showed 100% vancomycin susceptibility (Anupurba *et al.*, 2003; Umolu *et al.*, 2002). Frequent use of commonly available antibiotics provided sufficient selective pressure to promote colonization and/or infection with vancomycin resistance enterococci (Carmeli *et al.*, 2002) and MRSA eventually resulting in the emergence of vancomycin resistant *S. aureus* (Whitener *et al.*, 2004). Wound infections in orthopaedic patients with such MDR pathogens is believed to significantly delay or prevent union of fractured bone, leading to long hospital stays (John and David, 1991)

Plants have long been used as a source of therapeutic agents. Plants are known to synthesize antibacterial natural product following microbial attack, to protect them from invasive and pathogenic microorganisms in their environment. Now, workers in the field of plant medicines research, regard higher plants as living chemical factories that provide a vast number of unusual chemical substances that display a variety of biological actions (Oyi, 2001).

The result of the phytochemical screening of the crude methanolic extract of leaf, root and stem bark of *Parkia biglobosa* showed the presence of secondary metabolites which include: saponins, carbohydrate, tannins flavonoids and cardiac glycosides, this is presented in Table 2. These results are consistent with those obtained by Ajaiyeoba (2002) by studying the phytochemical and antibacterial properties of *Parkia biglobosa* and *Parkia bicolor* leaf extracts, in Nigeria. Also, these results are in agreement with those obtained by Hall *et al.* (1997).
Tables 3 -10 showed the results of the antibacterial activity of the crude extracts of the leaf, root and stem bark of *P. biglobosa*, respectively.

Table 2: Results of Phytochemical Screening

Metabolites	Methanolic extract of leaf	Methanolic extract of root	Methanolic extract of stem bark
Saponnin	+	+	+
Carbohydrate	+	+	+
Alkaloid	-	-	-
Tannins	+	+	+
Flavonoids	+	+	+
Anthraquinones	-	-	-
Cardiac glycosides	+	+	+
Resins	-	-	-

Key: + present

\- absent

The crude methanolic extracts of leaf of *Parkia. biglobosa* at the various concentrations used (10mg/ml to 20 mg/ml)showed no activity against the MRSA isolates from wound but little activity at 25mg/ml against MRSA isolates from skin and bed (Tables 3 and 4).

Table 3: Antibacterial activity of crude extract of leaves of *P. biglobosa* against MRSA isolates from skin

Concentration of extract	Diameter zone of inhibition (% of isolates)		
	No. zone (No activity)	8 – 10mm	11 – 14mm
10mg/ml	36 (100)	-	-
15mg/ml	36 (100)	-	-
20mg/ml	36 (100)	-	-
25mg/ml	27 (75.0)	9 (25.0)	-

Table 4: Antibacterial activity of crude extract of leaves of *P. biglobosa* against MRSA isolates from bed

Concentration of extract	Diameter zone of inhibition (% of isolates)		
	No. zone (No activity)	8 – 10mm	11 – 14mm
10mg/ml	48 (100)	-	-
15mg/ml	48 (100)	-	-
20mg/ml	48 (100)	-	-
25mg/ml	31 (64.6)	17 (35.4)	-

The crude methanolic extract of the root showed no activity against MRSA isolates at 10mg/ml and a very low activity at 15mg/ml however, there was an increased activity at 20mg/ml and 25mg/ml (Tables 5-7).

Table 5: Antibacterial activity of crude extract of roots of *P. biglobosa* against MRSA isolates from wound.

Concentration of extract	Diameter zone of inhibition (% of isolates)		
	No. zone (No activity)	8 – 10mm	11 – 14mm
10mg/ml	33 (100)	0	-
15mg/ml	29 (87.9)	4 (12.1)	-
20mg/ml	26 (78.8)	6 (18.2)	1 (3.0)
25mg/ml	22 (66.7)	5 (15.2)	6 (18.2)

Table 6: Antibacterial activity of crude extract of roots of *P. biglobosa* against MRSA isolates from skin

Concentration of extract	Diameter zone of inhibition (% of isolates)		
	No. zone (No activity)	8 – 10mm	11 – 14mm
10mg/ml	36 (100)	-	-
15mg/ml	31 (86.1)	3 (8.3)	2 (5.6)
20mg/ml	20 (55.6)	14 (38.9)	2 (5.6)
25mg/ml	14 (38.9)	15 (41.7)	7 (19.4)

Table 7: Antibacterial activity of crude extracts of roots of *P. biglobosa* against MRSA isolates from bed

Concentration of extract	Diameter zone of inhibition (% of isolates)		
	No. zone (No activity)	8 – 10mm	11 – 14mm
10mg/ml	48 (100)	-	-
15mg/ml	36 (75.0)	9 (18.8)	3 (6.3)
20mg/ml	22 (25.8)	21 (43.8)	5 (10.4)
25mg/ml	16 (33.3)	12 (25.0)	20 (41.7)

The crude methanolic extracts of the stem bark was active at the various concentration used (10mg/ml – 25mg/ml) against MRSA isolates from wound, skin and bed. These activities were concentration dependent (Tables 8-10).

Table 8: Antibacterial activity of crude extract of stem bark of *P. biglobosa* against MRSA isolates from wound

Concentration of extract	Diameter zone of inhibition (% of isolates)		
	No. zone (No activity)	8 – 10mm	11 – 14mm
10mg/ml	30 (90.9)	3 (9.1)	-
15mg/ml	26 (78.8)	7 (21.2)	-
20mg/ml	22 (66.7)	5 (15.2)	6 (18.2)
25mg/ml	21 (63.6)	5 (15.2)	7 (21.2)

Table 9: Antibacterial activity of crude extract of stem bark of *P. biglobosa* against MRSA isolates from skin

Concentration of extract	Diameter zone of inhibition (% of isolates)		
	No. zone (No activity)	8 – 10mm	11 – 14mm
10mg/ml	26 (72.2)	10 (27.8)	-
15mg/ml	22 (61.1)	12 (33.3)	2 (5.6)
20mg/ml	9 (25.0)	14 (38.9)	13 (36.1)
25mg/ml	9 (25.0)	11 (30.6)	16 (44.4)

Table 10: Antibacterial activity of crude extract of stem bark of *P. biglobosa* against MRSA isolates from bed

Concentration of extract	Diameter zone of inhibition (% of isolates)		
	No. zone (No activity)	8 – 10mm	11 – 14mm
10mg/ml	28 (58.3)	20 (41.7)	-
15mg/ml	21 (43.8)	20 (21.7)	7 (14.6)
20mg/ml	14(29.2)	13 (27.1)	21 (43.8)
25mg/ml	11 (22.9)	9 (18.8)	28 (58.3)

Comparing the plant parts: leaf, root and stem bark, it was observed in this study that the stem bark showed the greatest activity against the MRSA isolates tested. In accordance with the findings of this study, *Parkia biglobosa* has been reported to be rich in flavonoids, tannins and saponins (Ajaiyeoba, 2002), which are secondary metabolites known to have antibacterial activities. Millogo- Kone *et al*,(2006) reported that the stem bark is rich in sterols, triterpenes, tannins, saponosides, anthocyanins, flavonoids, coumarins and reducing compounds while the leaf is rich in tannins, coumarins, anthocyanins, flavones and reducing compounds. Also, the crude extract of *Parkia biglobosa* root bark contains saponins, glycosides, tannins and a trace of alkaloids (El Mahmood et al,,2007). Since the presence of these metabolites in plants have been linked to the antimicrobial activities of the plants (Lewis and Ausubel, 2006; Cowan, 1999) it can therefore be inferred that these secondary metabolites may be responsible for the observed antibacterial activities.

4. CONCLUSION

There was high prevalence of multidrug resistant *Staphylococcus aureus* isolates from the clinical and surveillance samples from the orthopaedic patients. In the search for alternative anti-staphylococcal agents from natural sources, *Parkiabiglobosa* will be a possible candidate for further investigation. Futher work with this plant could yield single chemical entities (SCES) with better antibacterial activities and greater potential as anti-staphylococcal agent.

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