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Prevalence and the effect of plant extracts on community associated methicillin resistant *Staphylococcus aureus* in Owerri, Imo State, Nigeria

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ABSTRACT:

The prevalence of Methicillin resistant *Staphylococcus aureus* (MRSA) among apparently healthy inhabitants of Eziobodo Community and Students of Federal University of Technology Owerri (FUTO), Imo State, Nigeria was studied. The work further ascertained the antibacterial activities of medicinal plants including *Azadirachta indica*, *Pterocarpus mildbraedii, Garcinia kola, Phyllanthus amarus and Vernonia amygdalina* against the MRSA isolates. A total of two hundred nasal swab specimens were randomly collected from the participants. The Kirby-Bauer technique was used to determine the susceptibility pattern of the isolates to Vancomycin (5µg), Ciprofloxacin (5µg), Ceftriaxone (30µg), Oxacillin (5µg), Methicillin (10µg) and Erythromycin (15µg). The antibacterial properties of the ethanolic plant extracts were determined using the agar well diffusion technique. A total of 181 (90.5%) and 141 (70.5%) of the nasal swab samples, yielded *Staphylococcus* species and *Staphylococcus aureus* respectively. The antibiotic sensitivity screening revealed that 38 (27%) of the *S. aureus* isolates were methicillin resistant. The MRSA isolates also exhibited the highest resistance to vancomycin and the least to ceftriaxone. Furthermore, the result showed that crude ethanolic extracts of all tested plant extracts except *Pterocarpus mildbraedii* exhibited antibacterial activities against the MRSA isolates. Phytochemical components such as Alkaloids, Tannins, Glycosides, Saponins, Flavonoids, Terpenoids, Phlobatannins, Steroids and Anthraquinones were detected in the plant materials in varying proportions. This study unveils a relatively high occurrence of MRSA among the study population which could be a risk factor for infection with MRSA. These plant extracts could also serve as potential sources of therapy for the treatment of MRSA infections.

Keywords:
Antibiotic resistance, *Staphylococcus aureus*, Methicillin, Plant extracts, Isolates.

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INTRODUCTION

Staphylococcus aureus is a coagulase positive, gram positive cocci, which apart from being a normal flora of the anterior nares, skin and large intestine, is also capable of causing a wide range of diseases varying from minor skin infections to life threatening septicemia, pneumonia, endocarditis, deep-seated abscess among others (Willey *et al*., 2008; Lowy 2003; Kuehnert *et al*., 2006; Tenover and Gaynes, 2000; Holmes *et al*., 2005; Nester *et al.,* 2007). Penicillin and later methicillin were very efficacious in the management of Staphylococcus infections in the early 1960s. However, over the years, most strains have acquired resistance to these drugs due to acquisition of gene encoding the enzyme penicillinase. In recent times, strains of *S. aureus* have emerged that not only produce penicillinase, but also have Penicillin binding proteins (PBPs) with low affinity for all β-lactam drugs. These strains referred to as methicillin resistant *Staphylococcus aureus* (MRSA) are resistant to methicillin and other β -lactam drugs (Nester *et al.,* 2007; Willey *et al*., 2008). Nearly all MRSA have additional genetic material known as *mec* A gene not found in methicillin sensitive strains, which encodes PBP 2a, a cell wall transpeptidase, having reduced affinity for β -lactam antibiotics. The *mec* A gene is found as a part of a mobile genetic element found in MRSA strains known as Staphylococcal cassette chromosome *mec* (SCC mec) (Jeshina and Surekha, 2009; Pinho *et al*., 2001).

In addition to β lactam drugs, MRSA isolates have become resistant to a number of antimicrobial agents such as, fluoroquinolones, aminoglycosides and macrolides (Shittu *et al*., 2009). MRSA could be categorized as either hospital acquired (HA-MRSA) or community acquired (CA-MRSA), depending on the source of acquisition. While the former occur in individuals who are/have recently been in a hospital or other healthcare facility, the latter are acquired by persons not recently hospitalized. According to David and Daum (2010), all infections occurring among outpatient or among inpatients with an MRSA obtained earlier than 48 hours after hospitalization could be regarded as CA-MRSA. In addition, livestock associated MRSA (LA-MRSA) have been reported to pose a challenge particularly in countries with low level of MRSA (Stefani *et al*., 2012). Morris *et al*., (2012) reported the potential for pet animals to harbour MRSA when residing with human MRSA patients.

The fact that MRSAs are becoming more prevalent worldwide and also resistant to a wide range of antibiotic groups, underlines the need for alternate strategies to stem the immense public health challenge posed by these organisms. Natural products from local medicinal plants are increasingly being used in the treatment of many hard to treat diseases and the search for more potential compounds from plants has continued (Lai *et al*., 2010; Newman and Cragg, 2007). According to WHO, 65-80% of the world population rely on traditional medication for their ailments (Gurinder and Daljit, 2009). A number of works has highlighted the efficacy of local indigenous plants against a wide range of pathogens (Ugbogu *et al*., 2010; Lai *et al*., 2010; Aliyu *et al*., 2008; Aliyu *et al*., 2011; Ajibade *et al*., 2010). The present study was aimed at ascertaining the occurrence of MRSA among apparently healthy Eziobodo community inhabitants and FUTO students as well as their susceptibility to different antibiotic groups. It also determined the antibacterial effects of some local plant extracts on the MRSA isolates.

MATERIALS AND METHODS

Collection of nasal specimens

Two hundred (200) nasal specimens were collected, 100 each from the anterior nares of apparently healthy individuals of Eziobodo community (one of the communities hosting FUTO) and students of Federal University of Technology (FUTO), all in Owerri West LGA, Imo State, Nigeria. They were aseptically

collected using sterile swab sticks between August and November 2010.

Cultivation and isolation of *Staphylococcus aureus*

The respective nasal specimens were cultivated within one hour of collection in Mannitol salt agar and nutrient agar using standard techniques to obtain discreet colonies. The plates were incubated at 37°C for 24 hours. The axenic cultures of the isolates were subsequently identified using colony morphology, microscopy and biochemical tests including catalase and coagulase tests (Cheesbrough, 2002).

Antibiotic susceptibility test

The antibiotic susceptibility screening of the *S. aureus* isolates was conducted using the Kirby-Bauer disc diffusion method (Cheesbrough, 2002). Standard inoculum, equivalent of 0.5 McFarland standards of the isolates was evenly spread on Mueller Hinton agar plates. Antibiotic discs including Vancomycin (5µg), Ciprofloxacin (5µg), Ceftriaxone (30µg), Oxacillin (5µg), Methicillin (10µg) and Erythromycin (15µg) (Oxoid) were aseptically placed on the plates. The plates were then incubated at 37°C for 24 hours and the inhibition zones recorded in millimeters using a meter rule.

Subsequently, all the isolates identified as Methicillin resistant *Staphylococcus aureus* (MRSA) were subjected to antibiotic screening test using the same disc diffusion technique as above. The following antibiotics were used; Vancomycin (5µg), Ciprofloxacin (5µg), Ceftriaxone (30µg) and Erythromycin (15µg) (Oxoid).

Collection of plant materials

The leaf and bark of *Pterocarpus mildbraedii, Azadirachta indica*, leaves of *Vernonia amygdalina*, and whole plant of *Phyllanthus amarus* were obtained from the premises of FUTO. The seeds of *Garcinia kola* however, were purchased from Ekeukwu Owerri market, Imo State. The plant materials were subsequently authenticated by a taxonomist.

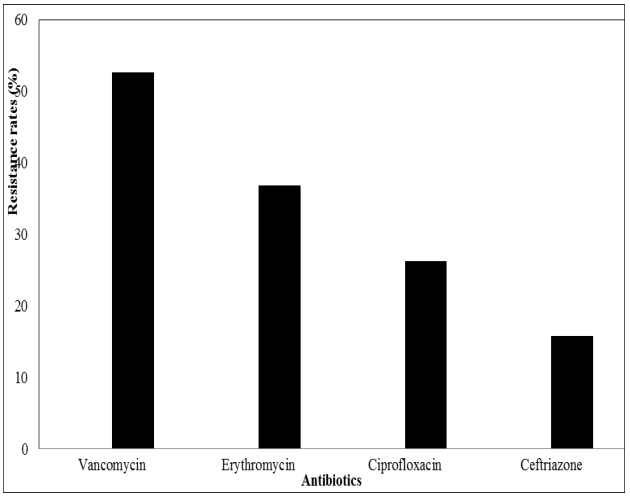

Figure 1: Antimicrobial resistance rates (%) of MRSA isolates to different antibiotics

Preparation of plant extracts

The leaves, barks and seeds of the plants were washed and dried at room temperature and later pulverized. 20gm of each plant powder was separately mixed with 250ml of ethanol and the extraction was done using the soxhlet extraction procedure.

Phytochemical screening

The phytochemical screening of each plant extract was carried out to determine the presence or absence of Alkaloids, Tannin, Saponins, Glycosides, Anthraquinone, Steroids, Flavonoids, Terpenoids, and phlobatannins (Harbone, 1973; Sofowora, 1993).

Susceptibility screening of MRSA isolate to plant extracts

The antibacterial effects of each plant extract on MRSA were determined using the agar well diffusion technique (Perez *et al*., 1990). Standard inoculum, equivalent of 0.5 McFarland standards of the isolates was evenly spread on Mueller Hinton agar plates. Sterile cork borer was used to make wells on the agar. The reconstituted extracts (25mg/ml, 50mg/ml, 100mg/ml and 200mg/ml) were respectively introduced into wells and labeled accordingly. Following the incubation of the plates at 37°C for 24 hours, the inhibition zone diameters were recorded using meter rule.

Table 1: Prevalence (%) of *Staphylococcus aureus* and MRSA isolates from Eziobodo and FUTO inhabitants

Target population	No of samples	*Staphylococcus* sp.	*Staphylococcus aureus*	MRSA
Eziobodo	100	95(95)	66(66)	20(30.3)
FUTO students	100	86(86)	75(75)	18(24)
Total	200	181(90.5)	141(70.5)	38(26.9)

RESULTS AND DISCUSSION

Out of the total of 200 specimens collected, 181 isolates were identified as *Staphylococcus* species, while 141 isolates were identified as *S. aureus*, representing a prevalence rate of 90.5% and 70.5% respectively (Table 1). Also, the antibiotic resistance screening of the isolates showed that 38 (27%) of the *S. aureus* isolates were MRSA. The MRSA isolates exhibited their highest sensitivity to Ceftriaxone and the least to Vancomycin antibiotic (Table 2 and Figure 1).

The Phytochemical screening of the plant extracts revealed the presence of Phytochemical components such as alkaloids, saponins, flavonoids and others in varying quantities (Table 3).

The antibacterial screening of the ethanolic extracts of the medicinal plants used in this study indicated that all except *Pterocarpus mildbraedii,* exhibited inhibitory activity against MRSA isolate. (Table 4).

The result of this study showed that 90.5% and 70.5% of the isolates from the anterior nares of Eziobodo inhabitants and FUTO students were respectively *Staphylococcus* species and *Staphylococcus aureus*. This is consistent with the findings of Ugbogu *et al*., (2010) and Chigbu and Ezeronye (2003), but higher than the 33.3% prevalence reported from Amassoma community in Niger Delta, Nigeria (Onanuga and Temedie, 2011).This high occurrence in our present work is not unexpected, since *S. aureus* is a normal microflora of the human body, particularly the upper respiratory tract (Willey *et al*., 2008; Cheesbrough 2002).

The Methicillin resistant *Staphylococcus aureus* (MRSA) prevalence rate of 27% among apparently healthy individuals as reported in the present work is considerably low compared to a report by Ugbogu *et al*., (2010) who isolated 83.5% of MRSA from healthy individuals in Abia State, South East Nigeria and Onanuga *et al*., (2005) that recorded 69% isolation from healthy women in Zaria, Nigeria. Similarly, our current finding is also lower than the report of Olowe *et al*., (2007) and Onanuga and Temedie (2011), in which 47.8% and 47.6% MRSA were isolated in South West and Niger Delta regions of Nigeria respectively. The prevalence rate of 47.15% and 43% has also been reported from Ibadan and Jos, Nigeria (Ghebremedhin *et al*., 2009; Ekeh, 2003). However, the current result is consistent with the report of Nwankwo *et al*., (2010) in which 28.6% was recorded. The difference in the prevalence of MRSA obtained in the present study and those of previous works could be attributable to strain variation in different geographical regions and locations (Ikeagwu *et al*., 2008).

It is important to note that the recovery of MRSA from apparently healthy community inhabitants in the present study is very significant particularly at this time when infiltrations of Community acquired MRSA (CA-MRSA) to healthcare facilities has been reported in some parts of the world (Stefani *et al*., 2012). According to Creech *et al*., (2005), Farley *et al*., (2008), and Hidron *et al*., (2005) enormous reservoirs of MRSA now exist outside health care settings and this implies that the current methods of MRSA control in health facilities are not likely to succeed. In this regard, preventive measures

Table 2: Frequency (%) of antibiotic resistance *S. aureus* isolates from nasal samples of Eziobodo and FUTO inhabitants

	No (%) of resistant isolates					
	Eziobodo		FUTO			
Antibiotics	No of isolates	No of resistant isolates	No of isolates	No of resistant isolates	Total no of isolates	Total no of resistant isolates
Oxacillin	66	21(31.8)	75	20(26.7)	141	41(29.1)
Methicillin	66	18(27.3)	75	20(26.7)	141	38(27)
Ciprofloxacin	66	16(24.2)	75	10(13.3)	141	36(25.5)
Vancomycin	66	15(22.7)	75	7(9.3)	141	22(15.6)
Erythromycin	66	10(15.1)	75	9(12)	141	19(13.5)
Ceftriaxone	66	3(4.5)	75	0(0)	141	3(2.1)

Table 3: Phytochemical components of plant extracts

	Phytochemical components								
Plant extracts	Alkaloids	Tannins	Glycosides	Saponinss	Flavonoids	Phlobatannins	Steroids	Anthraquinones	Terpenoids
AIL	+	+	-	+	+	-	+	+	-
AIB	-	+	+	+	+	+	-	+	+
PML	+	+	+	+	+	-	-	-	-
PMB	-	+	+	-	-	+	-	+	+
PA	-	+	-	-	-	-	+	+	-
VA	+			+	+	+	+	+	-
GA	-	+	+	+	-	+	-	+	+

Key: AIL – *Azadirachta indica* Leaf, AIB – *Azadirachta indica* Bark, PA – *Phyllanthus amarus*, PML – *Pterocarpus mildbraedii* Leaf, PMB – *Pterocarpus mildbraedii* Bark, VA – *Vernonia amygdalina*, GK – *Garcinia kola.*

to stop the possible transmission in the communities is a viable approach (Charlebois *et al*., 2004; Cooper *et al*., 2004; David *et al*., 2008; Liu *et al*., 2008).

The antibiotic susceptibility test revealed that all the *S. aureus* isolates exhibited the least resistance to ceftriaxone antibiotic in the present study. This finding is consistent with the report of Masood and Aslam (2010) in which 96.1% susceptibility of *S. aureus* isolates to ceftriaxone was highlighted. Ceftriaxone was apparently recommended by these workers as a drug of choice for infections caused by *S. aureus, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pnuemoniae* and *Salmonella typhi*. On the other hand, the *S. aureus* isolates were more resistant to Ciprofloxacin and Erythromycin (Tables 2 and 3). The resistant rates are in line with the reports of Shanhraz *et al*., (2012), and Onanuga and Temedie (2011), but quite low compared to the over 70% resistance recorded by Ojulong *et al*., (2009) in Kampala, Uganda. Azeez-Akande *et al*., (2008), however reported a susceptibility rate of 93.9% of MRSA isolates to ciprofloxacin.

Furthermore, vancomycin has been described as a reliable alternative for the treatment of MRSA infections. Elhamzaoui *et al*., (2009) and Nwankwo and Nasiru (2011) reported 100% sensitivity of *S. aureus* isolates from a University hospital in Rabat Morocco and a tertiary health institution in Kano, Nigeria, to Vancomycin respectively. Nevertheless, this antibiotic, vancomycin, which was initially a drug of choice in the treatment of MRSA infections, is witnessing resistance in recent times (Von-Eiff *et al.,* 2001). In the present work, over 50% of the MRSA isolates were resistant to Vancomycin. This is worrisome because Vancomycin has been described by various workers as very effective

Table 4: Inhibitory activities of plant extracts against MRSA isolates

	Mean zone of inhibition (mm)/ Concentration of plant extracts (mg/ml)			
Plant extract	**25**	**50**	**100**	**200**
AIL	-	-	-	9
AIB	-	7	8	12
PML	-	-	-	-
PMB	-	-	-	-
PA	-	-	-	8
VA	-	-	9	10
GK	-	-	-	11

Key: AIL – *Azadirachta indica* Leaf, AIB – *Azadirachta indica* Bark, PA – *Phyllanthus amarus*, PML – *Pterocarpus mildbraedii* Leaf, PMB – *Pterocarpus mildbraedii* Bark, VA – *Vernonia amygdalina*, GK – *Garcinia kola*.

against MRSA and in fact a drug of choice in the treatment of multidrug resistant *S. aureus* infections (Ojulong *et al*., 2009; Elhamzaoui *et al*., 2009). The vancomycin resistance rate as recorded in the current study is however contrary to the report of Onanuga and Temedie (2011) in Niger Delta Nigeria and Shanhraz *et al*., (2012) in which over 70% susceptibility was recorded. The present finding thus suggests that vancomycin may be inefficient in the treatment of infections caused by MRSA in the near future among our target population. The present study therefore recommends ceftriaxone as a drug of choice for the treatment of MRSA infections in our study area.

The increasing resistance of MRSA to β-lactam and other broad spectrum antibiotics has stimulated recent investigations on plant parts for naturally occurring active compounds as alternatives to treatment of MRSA caused infections. The phytochemical screening of the plant extracts used in this study revealed the presence of alkaloids, Tannins, saponins, flavonoids, terpenoids, anthraquinones, glycosides and steroids (Table 4). The antibacterial screening of the plant extracts showed that all the plant materials used except *Pterocarpus mildbraedii* exhibited inhibitory activity against MRSA. This effect could be attributed to the concomitant effect of the active compounds contained by these plants on MRSA. However, none of the extracts were active against the MRSA isolates at the lowest concentration of 25mg/ml (Table 4).

The inhibitory effect of *Garcinia kola* extract on MRSA as observed in the present study is in agreement with the work of Ugbogu *et al*., (2010) and Adeleke *et al*., (2006), in which *Garcinia kola* extracts exhibited antibacterial activities against MRSA isolates in Nigeria. Also, Taiwo *et al*., (1999) reported that *Garcinia kola* exhibited strong activity against MRSA. Similarly, the inhibitory properties of *Azadirachta indica* and *Vernonia amygdalina* against MRSA as recorded in the present work is consistent with the reports of Skariyachan *et al*., (2011), Aliyu *et al*., (2011) and Aliyu *et al*., (2008). Furthermore, that *Phyllanthus amarus* extract had antibacterial activity against MRSA is in line with the findings of Aliyu *et al*., (2008). Ajibade *et al*., (2010) also highlighted the antimicrobial activity of *Phyllanthus* species against MRSA. Undoubtedly, the findings of this study support the local use of these plant materials in the treatment of most hard to treat infections.

In conclusion, the recovery of CA-MRSA from the external nare of apparently healthy individuals in this study underscores the significance of the nasal region as a reservoir of *S. aureus*, and by implication MRSA. In fact, MRSA colonization of the nares is believed to be a risk factor for a clinically apparent infection with MRSA (Croft *et al*., 2009; Huang *et al*., 2006; Lu *et al*., 2007; Muder *et al*., 1991). It is therefore very imperative that strategies should be designed to halt the further spread of MRSA in communities and most especially to immunodeficient individuals. According to Stefani *et al*., (2012), CA-MRSA clones spreading in the community could also infiltrate healthcare facility in many parts of the world. This certainly would exacerbate the challenges already posed by MRSA. Interestingly however, the therapeutic activities of the plant materials

used in this study could hold a great promise as a potential precursor in the development of therapies for the management of MRSA infections, if properly harnessed.

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