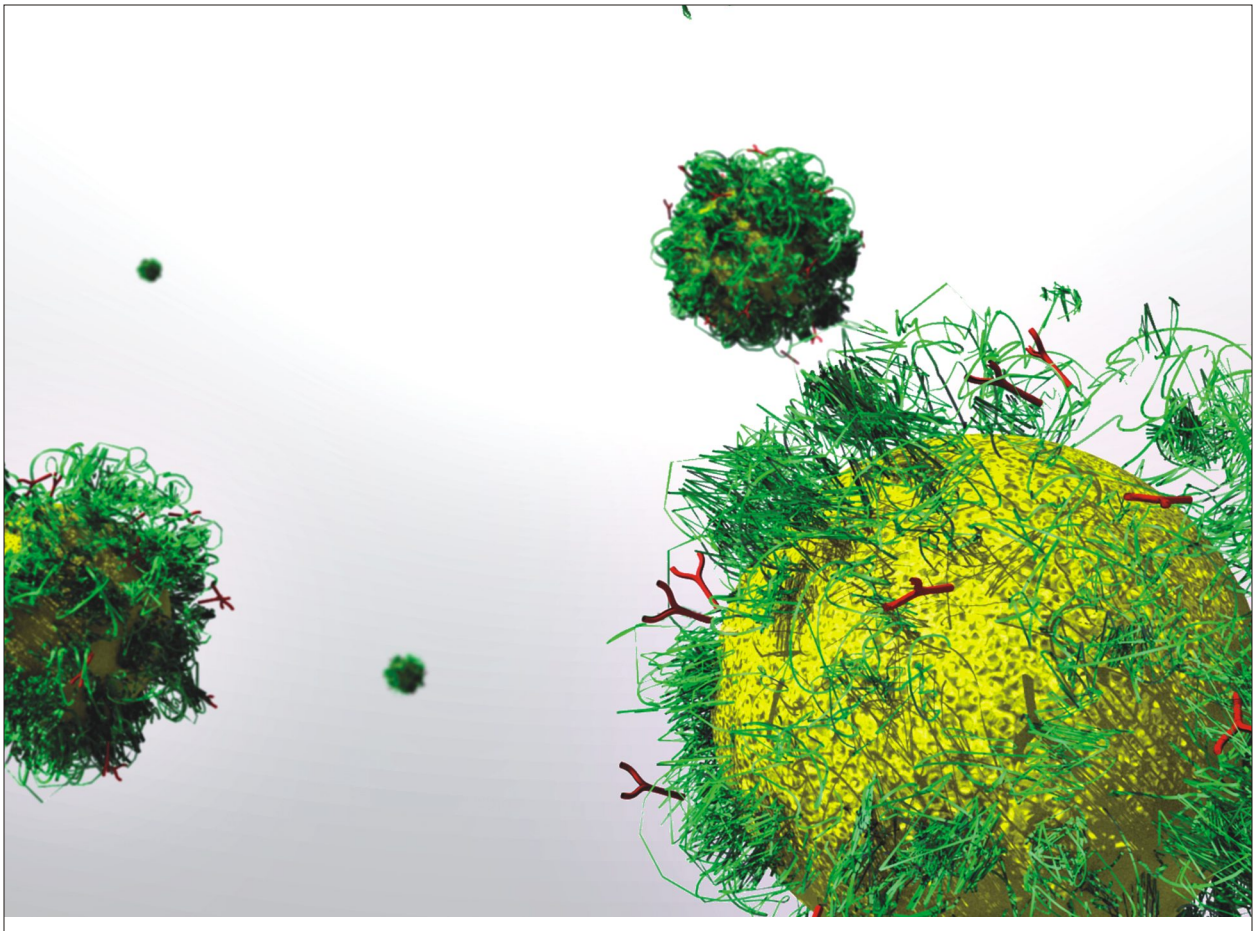

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Antimicrobial Effect of *Diodia scandens* and *Phyllanthus amarus* on Multi-drug Resistance Pattern of *Staphylococci* from Clinical Sources of Surgical Unit of a Tertiary Hospital

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ABSTRACT

Multiple antibiotic resistance *S. aureus* and coagulase negative *Staphylococci* (CoNS) constitute a major healthcare problem but with the resuscitation of traditional and complementary alternative medicine, these infectious diseases have been reduced drastically. Two hundred swab samples were obtained from various clinical sites of patients in surgical unit. Grounded plants were soxhlet extracted using ethanol and n-hexane and purified. Primary characterization, antibiotic sensitivity testing of commercial antibiotics and impregnated disk of plant extracts were analyzed according to standards. Fifty three isolates were characterized as *S. aureus* (47) and CoNS (6) 15 isolates were β-lactamase producing and 39 were DNase positive. Multiple antibiotic resistances were observed against the isolates with 17 *S. aureus* and 4 CoNS strains demonstrating multiple resistance to all the 9 antibiotics tested. High resistance pattern were seen in rifampicin (76.6), streptomycin (70.2), ampiclox (68.1), erythromycin (63.8) and the fluoroquinolones (51.1-61.7). The multiple antibiotic resistance index ranges between 0.11-1.0. These results therefore call for an urgent attention in addressing drugs misuse, specific clinical diagnosis and treatment to the emerging staphylococcal infection especially the CoNS and incorporating herbal drugs prescription in orthodox medicine.

Key words: Antibiotics, multiple resistance, β-lactamase, *Staphylococci*, medicinal plants

INTRODUCTION

Staphylococci have again emerged as the predominant organisms causing infections in the hospital setting and are the leading cause of nosocomial infections and community-acquired infections including impetigo, folliculitis, furuncles, sycosis barbe, cellulitis, abscesses, osteomyelitis and bacteraemia (Al-Hamdani and Hamad, 2012; Gad *et al*., 2010; Yameen *et al*., 2010; Bashir *et al*., 2007). Most developed countries have reported an increase in colonization and infection in hospitalized patients by coagulase negative *Staphylococci* (CoNS) while there are scanty data in developing countries.

Since the introduction of antimicrobials, bacteria have developed mechanism for resisting the effects of antibiotics and the levels of antibiotic resistant infections in the developing world have increased steadily in the last few decades as a result of combination of microbial characteristics and the selective pressure of antimicrobial use (Bashir *et al*., 2007; Blondeau and Tillotson, 2000).

Several mechanisms by microorganisms in overcoming the activities of antimicrobial agents include the production of structure-altering or inactivating enzymes (e.g., beta-lactamase or aminoglycoside-modifying enzymes), alteration of penicillin-binding proteins or other cell wall target sites, altered DNA gyrase targets, permeability mutations, active efflux systems and ribosomal modification (Akinjogunla and Enabulele, 2010; Gad *et al*., 2010). Multi-drug resistance bacteria in both the hospital and community environment are of importance to the clinician, as it is the major cause of failure in the treatment of infectious diseases, increased morbidity and mortality and the evolution of new pathogens (Akinjogunla and Enabulele, 2010).

However, multi-drug resistant strains of *Staphylococci* have been reported with increasing prevalence worldwide among macrolides, aminoglycosides, fluoroquinolones or combination of these antibiotics. Thus, making infection caused by *Staphylococci* practically difficult to treat especially among the catheter in-dwelling patients and patients with prolonged hospital stay.

Recently, the discovery and development of medicinal plants as drugs (especially from China, India and Nigeria and some African countries) has proven effective in the treatment of multi-drug resistance pattern among clinical and environmental isolates, which has plagued the healthcare system especially in developing and underdeveloped countries. The primary benefits of using plant-derived medicines are that they are safer than synthetic alternatives, offering profound therapeutic benefits and more affordable treatment (Ojo *et al*., 2013a). They are effective with minimal or no side effects in the treatment of infectious diseases, while simultaneously mitigating many of the side effects that are often associated with synthetic drugs (Ojo *et al*., 2013b). This study, however tends to present the effectiveness of *Diodia scandens* and *Phyllanthus amarus* on the multi-drug resistant *Staphylococci* from surgical unit in Delta State Tertiary Hospitals, Nigeria.

MATERIALS AND METHODS

Collection of samples: Two hundred (200) clinical swab samples were obtained from septic wounds and burns patients undergoing injury dressing on various body sites at the General Hospital, Ekpan, Warri, Delta State, Nigeria. Wound exudates were obtained from the infected sites of each patient with a sterile cotton swab and applied to freshly prepared slant of nutrient agar and mannitol salt agar (oxoid) and were incubated at 37°C for 24 h.

Isolation and identification: Colonies growing on slants were streaked on top of freshly prepared plates of mannitol salt agar and incubated again at 35°C. Primary characterization of isolates was based on Gram stain, morphological and cultural characteristics, growth on nutrient agar and DNase agar and fermentation on mannitol salt agar, catalase and coagulase tests. β-lactamase assay was performed using the method as described by Ako-Nai *et al*. (2005).

Antibiotic sensitivity testing: The isolates were subjected to antibiotic screening by disk diffusion method using CLSI (2008) standard with a reference *S. aureus* strain type 25923. The inocula were prepared by diluting overnight cultures in sterile sodium chloride (0.9%) suspension and then matched with the McFarland turbidity index. Bacterial suspensions were then plated onto Mueller Hinton agar and the commercially available antibiotic disc were placed on the lawn of culture and inoculated overnight at 35°C. The following disks were used: Ciprofloxacin (10 μg), Levofloxacin (5 μg), Norfloxacin (20 μg), Gentamycin (10 μg), Ampiclox (30 μg), Streptomycin (30 μg), Erythromycin (10 μg), Rifampicin (20 μg) and Chloramphenicol (25 μg).

Determination of multiple antibiotic resistance index (MAR): This was determined using the equation:

$$MAR = \frac{x}{y}$$

where, x was the number of antibiotics to which test isolate displayed resistance and y is the total number of antibiotics to which the test organism has been evaluated for sensitivity.

Determination of antimicrobial effect of plant extracts on multi-drug resistant *Staphylococci*: The multi-drug resistant *Staphylococci* obtained were cultured on Mueller Hinton Agar and incubated at 35°C for pure isolates. The extracts were prepared as earlier described by Ojo *et al.* (2013a). The antimicrobial susceptibility profiles of the isolates were determined using agar diffusion method as described by the Clinical Laboratory Standards Institute CLSI (2008) Wayne, PA, USA. Paper disks of 6 mm diameter in size were impregnated for 6 h in various concentrations of ethanolic and n-hexane extracts of *D. scandens* and *P. amarus*. Reference type strains of *S. aureus* ATCC 25923 were included for quality control.

RESULTS

Various sample sources in this study reveal the isolation rate of *Staphylococci* with the highest from lap (23.4), hand (17.0) and leg and feet (14.9) respectively for *S. aureus* while for CoNS, the leg (33.3) showed higher isolation rate (Table 1). Table 2 showed the biochemical analysis of the clinically isolated *Staphylococci* from various sites of the body, thus indicating presence of β-lactamase producing and DNase positive *Staphylococci*. The antibiotic sensitivity pattern on *S. aureus* and CoNS revealed high resistance pattern among the rifampicin (76.6), streptomycin (70.2), ampiclox (68.1), erythromycin (63.8), gentamycin (59.6) and the fluoroquinolones (51.1-61.7) (Table 3). Seventeen isolates of the *S. aureus* and 4 CoNS isolates showed multiple antibiotic resistance on the 9 antibiotics tested with at least 1 isolate showing multiple antibiotic resistance on 2 antibiotics. Thus, indicating high level of multiple antibiotic resistance with the multiple antibiotic index ranging from 0.11-1.0 in the *Staphylococci* isolates (Table 4, 5). Antimicrobial effect of *D. scandens* and *P. amarus* were demonstrated on the multi-drug resistance

Table 1: Sample source composition of *Staphylococci* from wounds and burns patients

Sample	Source	No. of *S. aureus*	%	No. of CoNS	%
Wound	Head	5	11	1	17
	Hand	8	17	0	0
	Leg	7	15	2	33
	Feet	7	15	0	0
	Eye	2	4	0	0
	Ear	0	0	1	17
	Mouth	1	2	1	17
	Shoulder	2	4	0	0
	Finger	3	6	0	0
	Lap	11	23	1	17
Burns	Finger	1	2	0	0
		47	99	6	101

Table 2: Biochemical analysis of clinically isolated *Staphylococci* from wounds and burns patients

			Coagulase		Mannitol fermentation		DNase		β-lactamase	
Sample/source	Gram +ve	Catalase	+ve	-ve	+ve	-ve	+ve	-ve	+ve	-ve
Head (W)	6	6	5	1	4	2	5	1	1	5
Hand (W)	8	8	8	0	2	6	5	3	4	4
Leg (W)	9	9	7	2	4	5	6	3	2	7
Feet (W)	7	7	7	0	3	4	3	4	1	6
Eye (W)	2	2	2	0	0	2	2	0	2	2
Ear (W)	1	1	0	1	0	1	1	0	0	1
Mouth (W)	2	2	1	1	0	2	1	1	0	2
Shoulder (W)	2	2	2	0	0	2	2	0	1	1
Finger (W)	3	3	3	0	2	1	3	0	2	1
Lap (W)	12	12	11	1	7	5	10	12	4	8
Finger (B)	1	1	1	0	0	1	1	0	0	1
	53	53	47	6	22	31	39	14	15	38

W: Wound, B: Burns, +ve: Positive, -ve: Negative

Table 3: Profile of antibiotic sensitivity testing on *Staphylococci* isolated from wounds and burns patients

		S. aureus (N = 47)			CoNS (N = 6)		
		Sens	Interm	Resist	Sens	Interm	Resist.
Antibiotics	ND	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)
Gentamicin	1	15(31.9)	3(6.4)	28(59.6)	0(0.0)	0(0.0)	6(10.00)
Ampiclox		12(25.5)	3(6.4)	32(68.1)	0(0.0)	1(16.7)	5(83.30)
Ciprofloxacin	3	3(6.4)	14(29.8)	27(57.4)	0(0.0)	0(0.0)	6(100.00)
Streptomycin		8(17.0)	6(12.8)	33(70.2)	0(0.0)	0(0.0)	6(100.00)
Erythromycin	3	0(0.0)	14(29.8)	30(63.8)	0(0.0)	1(16.7)	5(83.30)
Rifampicin	6	4(8.5)	2(4.3)	36(76.6)	0(0.0)	0(0.0)	5(83.30)
Levofloxacin	6	11(23.4)	7(14.9)	24(51.1)	0(0.0)	0(0.0)	5(83.30)
Chloramphenicol	7	4(8.5)	8(17.0)	29(61.7)	1(16.7)	0(0.0)	4(66.70)
Norfloxacin	8	6(12.8)	5(10.6)	29(61.7)	1(16.7)	0(0.0)	4(66.70)

ND: Not determined

Staphylococci using the absolute ethanol and n-hexane extracts of the two plants at different concentrations. However, results showed the zone sizes of inhibition of *P. amarus* ethanolic extracts (7-16 mm), *P. amarus* n-hexane extracts (6-24 mm), *D. scandens* ethanolic extracts (6-20 mm) and *D. scandens* n-hexane extracts (7-13 mm) (Table 6).

DISCUSSION

Staphylococcal infections have become a significant clinical problem in medical practice (Gad *et al*., 2010) posing great health threat both to the immunocompetent and immunosuppressed individuals. The exceedingly increases and emergence of multidrug resistance pathogens (especially among *S. aureus* and CoNS) in the developing countries can be attributed to the indiscriminate use of antibiotics, complex socio-economic, behavioural antecedents and dissemination of drug-resistant pathogens in human medicine (Yang *et al*., 2008). Thus, the multi-drug resistant strains among the *S. aureus* and the more emerging CoNS is complicating the management of wounds and burns infection and increasing the risk for treatment failure.

Table 4: Antibiogram pattern of resistant antibiotics

Group	No. of Isolates		Resistant antibiotics	No. of resistant antibiotics
	S. aureus (n = 46)	CoNS (n = 6)		
I	4	0	RD	1
II	2	0	CH	1
III	1	0	S, RD	2
IV	1	0	RD, CH, NB	3
V	1	0	CPX, S, E	3
VI	1	0	APX, S, E	3
VII	1	0	CPX, E,RD, CH	4
VIII	1	0	S,E, CH, NB	4
IX	1	0	CN, APX, RD, LEV	4
X	1	0	CN, APX, CPX, S	4
XI	1	0	CN, APX, S, RD	4
XII	1	0	CN, APX, E, RD	4
XIII	1	0	APX, S, RD, LEV	4
XIV	2	1	CN, APX, CPX, S, E	5
XV	0	1	CN, CPX, S, RD, LEV	5
XVI	1	0	APX, RD, LEV, CH, NB	5
XVII	1	0	CN, CPX, S, E, LEV, NB	6
XVIII	1	0	CPX, S, E, LEV, CH, NB	6
XIX	1	0	APX, S, E, RD, CH, NB	6
XX	1	0	CN, APX, CPX, S, RD, CH, NB	7
XXI	1	0	APX, CPX, S, E, RD, CH, NB	7
XXII	1	0	CN, APX, CPX, E, RD, CH, NB	7
XXIII	1	0	CN, APX, CPX, E, RD, LEV, CH, NB	8
XXIV	1	0	CN, APX, CPX, S, E, RD, CH, NB	8
XXV	1	0	CN, APX, CPX, S, E, RD, LEV, NB	8
XXVI	17	4	CN, APX, CPX, S, E, RD, LEV, CH, NB	9

Table 5: Multiple antibiotic resistance (MAR) index of *Staphylococci* from wounds and burns

No. of isolates	7	3	5	1	6	3	21	3	3
(%)	(13.2)	(5.7)	(9.43)	(1.9)	(11.3)	(5.7)	(39.6)	(5.7)	(5.7)
No. of resistant antibiotics	4	6	5	2	1	7	9	3	8
MAR index	0.44	0.67	0.57	0.22	0.11	0.78	1.0	0.33	0.89

It was obvious from our study and similar studies that *Staphylococci* are becoming more resistance to various antibiotics commonly administered by clinicians or as over-the-counter (OTC) use especially among gentamicin, erythromycin, streptomycin, chloramphenicol and ampiclox. *S. aureus* tested against fluoroquinolones used in this study showed a high resistance pattern as compared with an earlier report (Yameen *et al*., 2010), whereas other studies indicated a low level or no resistance pattern (Akinjogunla and Enabulele, 2010; Al-Hamdani and Hamad, 2012). The result of CoNS resistance to all antibiotics tested especially the fluoroquinolones in this study is alarming, corroborating the reports from Akinkunmi and Lamikanra (2010), Akinjogunla and Enabulele (2010) and Bashir *et al*. (2007). Akinjogunla and Enabulele (2010) reported the multiple antibiotic resistance index to range between 0.25-1.00 while Al-Hamdani and Hamad (2012) reported a range between 0.2-0.75. These earlier reports were in agreement with the result of this study.

Table 6: Zone sizes (mm) on antimicrobial effect of *Diodia scandens* and *Phyllanthus amarus* on clinically isolated *Staphylococci*

Sample ID	PE1	PE2	PE4	DE1	DE2	DE4	PN1	PN2	PN4	DN1	DN2	DN4
W263	15	10	15	12	9	20	9	8	8	9	9	8
W357	10	9	13	8	6	17	10	6	6	6	6	8
W380	13	8	11	9	8	13	9	6	6	8	8	6
W318	6	8	12	6	6	13	6	6	6	6	6	6
W285	8	8	11	8	8	10	9	8	8	8	9	6
W345	6	8	10	6	8	11	6	6	6	6	6	6
W297	10	10	11	10	11	14	10	10	11	11	11	10
W302	8	8	10	8	9	10	8	8	8	8	9	8
W320	8	9	12	6	6	15	6	6	6	6	6	6
W395	11	9	12	6	6	17	6	6	6	6	6	6
W349	6	9	12	8	8	10	6	6	8	8	6	6
W275	8	10	12	8	8	14	10	12	13	8	8	8
W310	10	9	11	9	11	11	9	9	8	8	9	8
W295	13	9	12	6	6	18	6	6	6	6	7	6
B383	10	6	10	11	8	13	6	6	6	8	9	6
W300	10	10	11	10	9	18	9	10	10	8	10	9
W237	7	8	11	6	6	12	6	6	6	7	8	6
W291	10	8	11	6	8	15	6	6	6	6	6	6
W248	8	9	14	6	6	14	6	6	6	6	6	6
W388	6	8	11	6	6	12	6	6	6	8	6	6
W296	16	9	10	11	12	13	10	10	10	10	13	10
W327	6	8	10	6	6	12	6	6	6	6	6	6
W307	9	9	12	8	8	11	8	9	6	8	8	6
W392	8	9	13	9	8	11	9	9	10	8	9	8
W271	15	9	11	6	8	15	6	6	6	6	6	6
W235	11	8	12	9	10	15	10	6	10	6	6	6
W273	6	8	11	6	9	12	8	8	8	9	6	6
W394	11	9	11	8	8	14	22	24	12	6	8	9
W337	12	10	12	12	13	16	9	9	9	10	10	9
W241	16	9	12	9	6	15	6	6	6	6	6	6
W252	6	8	11	6	6	11	6	6	6	6	6	6
W344	6	9	12	6	8	15	6	6	6	6	6	6
S. aureus ATCC 25923	14	18	22	10	10	14	10	12	13	11	12	12

PE1-*P. amarus* ethanolic extract (100 μg mL^{-1}), PE2-*P. amarus* ethanolic extract (200 μg mL^{-1}), *P. amarus* ethanolic extract (400 μg mL^{-1}), DN1-*D. scandens* n-hexane extract (100 μg mL^{-1}), DN2-*D. scandens* n-hexane extract (200 μg mL^{-1}), DN4-*D. scandens* n-hexane extract (400 μg mL^{-1})

Most studies on medicinal plants have shown results only on MIC and MBC determination. However, this study has revealed the antimicrobial effect of the two plants extracts at various concentrations from ethanol and n-hexane using disc diffusion method according to CLSI (2008) standards. The antimicrobial potency of *P. amarus* ethanol extracts was high at the 100 μg mL^{-1} (6-16 mm) and 400 μg mL^{-1} (11-22 mm) than 200 μg mL^{-1} (8-10 mm), which was synonymous with Oluwafemi and Debiri (2008) who reported antimicrobial activity at 100 mg mL^{-1} with zone size of 8.3±0.12 mm while those of *P. amarus* n-hexane extracts showed low potency from the diameter of zone sizes. Conversely, the antimicrobial potency of *D. scandens* ethanol extract had a good antimicrobial effect at the 400 μg mL^{-1} (11-20 mm), 200 μg mL^{-1} (6-13 mm) and 100 μg mL^{-1}

(6-12 mm), respectively as compared with the n-hexane extract which had a low antimicrobial effect on the isolates. This however elucidates the medicinal use of these plants in treating antibiotic resistance *Staphylococci* (MRSA and MRCoNS) and possibly other organisms. It can be deduced from the result that ethanol extraction tends to extract most of the active ingredients than n-hexane, which is reflected in the extraction yield. Hence, n-hexane is not a good solvent for extraction.

CONCLUSION

This study showed the prevalence of multi-drug resistant strains of *S. aureus* and CoNS as a major cause of wound infection among patients in surgical unit of the hospital which can be transferred to both in-patients and out-patients. It is therefore recommended that since *Staphylococci* causes nosocomial infection, overcrowding in healthcare institution should be avoided to reduce the spread of the infection within the hospital and strict policy on OTC use of antibiotics be implemented among the pharmaceutical outlets and patent-medicine dealers vis-à-vis the introduction and prescription of purified traditional medicine in patients' treatment. We know that our result will enhance clinicians' capability to better manage this infection and reduce possible squealer.

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