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Incidence, Multi-Drug Resistance and Antimicrobial activity of extract from *Terminalia catappa* on*Streptococcus pyogenes*

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ABSTRACT

The incidence and trend of multidrug resistance among *S. pyogenes* in the Federal Polytechnic Ado-Ekiti community was investigated. Sample of sputum were collected from individuals that complained of throat irritation, sore throat and cough. Out of the sixty (60) samples analyzed, only 27(47%) of the samples were positive for *S. pyogenes* while 33(55%) were negative. Out of the twenty seven (27) positive isolates, 17(63%) isolates were resistant to erythromycin, 11(41%) isolate were resistant to Gentimycin, levofloxacin and chloramphenicol. This showed that the rate of resistance of *S. pyogenes* to conventional antibiotics is very high and could be ascribed to numerous factors described in this work. However, of the resistant isolates; 27 (20%) from cough patients which were multidrug- resistant were tested against 3.0mg/ml of aqueous extract from *Terminalia catappa* and 16 (62.0%) of these were susceptible to the extract. The antimicrobial activities of the extract were of interest since the crude extract was effective at concentration of 3.0mg/ml to multiple resistant isolates of *Strep pyogenes*. The effectiveness of phytotherapy was emphasized.

KEY WORDS: Incidence, Multidrug resistance, *Streptococcus pyogenes*, *Terminalia catappa,*

INTRODUCTION

Group A streptococci (*Streptococcus pyogenes*) are strictly human pathogens that normally colonize the throat or skin without causing disease. Members of this species are differentiated into > 100 types on the basis of immunogenic differences in their surface M proteins and polymorphisms in the *emm*gene [4]. In most cases,this bacterium causes tonsillitis, pharyngitis (sore throat), or skin infections such as impetigo/pyoderma. *S. pyogenes* causes about 60 – 80% of sore throat.

The bacterium can cause disease in the form of post-infection without associating with pus production i.e., 'non pyogenic'. However, infections due to certain strains of *S. pyogenes* can be associated with the release of bacterial toxins. Auto immune-mediated complications include rheumatic fever and acute post infection glomerulonephritis both conditions appear several weeks following the initial streptococcal infection. Studies suggested that about 28% to 55% of cases of sore throat may be attributed to *S. pyogenes*[5, 7].Certain strains of *S. pyogenes* have developed resistance to macrolides, tetracycline and Clindamycin but some remain acutely sensitive to penicillin[12]. Resistance to these antibiotics appears to have resulted from a mutation identified in the *recP* gene that coincides with acquisition of multidrug resistance [14].

Terminalia catappa is a large tropical tree in the leadwood tree family, Combretaceae that is native to the tropical regions of Asia, Africa, and Australia. It is known by the common names Bengal almond, Singapore almond, *ketapang* (Indonesian) and in Benin as *ebelebo*. The leaves contain several flavonoids (such as kaempferol or quercetin), several tannins (such as punicalin, punicalagin or tercatin), saponines and phytosterols. Due to this chemical richness, the leaves (and the bark) are used in different herbal medicines for various purposes. For instance in Taiwan, fallen leaves are used as an herb to treat liver diseases. In Suriname, a tisane made from the leaves is prescribed against dysentery and diarrhea. The leaves may contain agents for prevention of cancers (although they have no demonstrated anticarcinogenic properties) and antioxidants, as well as anticlastogenic characteristics. Extracts of *T. catappa* have shown activity against *Plasmodium falciparum*chloroquine (CQ)-resistant (FcB1) and CQ-sensitive (HB3) strains[7].

The need therefore to research natural products that could give potent and quick therapy at low or no cost at all is necessary. The healthcare delivery of the larger proportion of the rural communities in Nigeria and most part of Africa today hinged to a large extent on medicinal plants based on traditional health care delivery system. Even today, according to the World Health Organization (WHO), as many as 80% of the world's population depend on traditional medicines for their primary health care delivery and needs [18]. Plants have occupied a very important position in health care delivery in various regions of the world; this is even more prominent among rural parts of Nigeria. However, with plants gaining more recognition and relevance in health care delivery in humans, various agencies of government and nongovernmental organizations are supporting the development of traditional medicine [2].

The aims of this study are to determine the incidence and trend of multidrug resistance, to ascertain possible existence of difference in the resistivity as it relates to ten antimicrobial agents, to determine if the symptoms pose some

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difference in resistance and to determine the antibacterial activity of hot water extract of *Terminalia catappa* on *S.pyogenes* isolated from the Federal Polytechnic, Ado - Ekiti community

MATERIALS AND METHODS

Culture of samples and identification of isolates

The samples were cultured on the blood agar by streaking using a sterile inoculating loop. The Petri dishes were incubated anaerobically at 37^{o}C for 24 hours in an incubator. Colonies showing characteristic greenish turbidity of beta-hemolysis in the plates were sub-cultured into a nutrient agar slant as stock in a refrigerator until ready for use. Isolates were identified as *S. pyogenes* on the basis of β- hemolysis, gram staining, negative catalase test result, positive pyrroledonyl arylamidase test result, and agglutination with Lancefield group A antiserum [6].

Detection of genes

The multiplex PCR was used to detect *erm* and *mef* genes [8] and their clonality was investigated by *emm* typing and by pulse-field gel electrophoresis as described in Andersson and Hughes (2010)

Antibacterial Susceptibility Studies

Antimicrobial susceptibility profiles of the test organisms were determined by disk diffusion method as described by the Clinical and Laboratory Standards Institute (CLSI 2010). Disk containing Amoxil (20µg), Streptomycin (30µg), Gentamycin (10µg), Ciprofloxacin (10µg), Rifampicin (20µg), Erythromycin (30µg), Chloramphenicol (30µg), Norfloxacin (10µg), Ampiclox (20µg) and Levofloxacin (20µg) were used for testing on Mueller-Hinton agar (Oxoid,). A bacterial colony was picked with a loop from a stock culture of the isolates suspended in 0.1ml of saline solution and standardized with 5% barium sulphate. All the tests were performed by placing multodisk (Roche Diagnostic Ltd) of the test antibiotics on the Sensitivity Test Agar surface previously inoculated with bacterial suspension of *S. pyogenes*. Plates were incubated at 37^{o}C for 24hrs and observed for inhibition zones. Zones were measured in millimeters using a meter rule. Results of antibiotic assay scored as susceptible ($\geq$ 5.0mm), Intermediate (5.0mm) and resistant ($\leq$ 4.0mm) according to criteria established by the Clinical and Laboratory Standards Institute (CLSI, 2010).

Collection, Identification and Processing of Plant

Terminalia catappa was collected from farmlands at Iworoko Ekiti. The plant samples were identified at the Plant Science Department of the Ekiti State University, Ado Ekiti and a voucher specimen was kept in the laboratory No: Med Plant 2011/098. The method described by Osho *et al.* (2007) was used. Samples were air-dried at room temperature of (26^{o}C±1^{o}C) and milled using a Thomas Willey Milling Machine. 100grams of the milled samples was soaked with 200ml of methanol. The methanol extract was filtered and evaporated to dryness at 20^{o}C using a rotary evaporator. The residues were reconstituted to different concentrations using distilled water.

Antibacterial potency determination

The agar diffusion method described by the clinical and laboratory Standards Institute (2010) was used. Reactivated culture from stock of multidrug resistant isolate of *S. pyogenes*, diluted serially to 10^{3} was used as inoculum. The inocula were spread uniformly on sensitivity agar (Oxoid) using a sterile glass spreader. Pasteur pipette was used to introduce different concentration of 50mg/ml, 100mg/ml, 150mg/ml and 200mg/ml of the extract into the well bored onto the surface of the culture. A control well containing extracting solvent was also incorporated. The plates were incubated at 37^{o}C for 24hrs in an incubator. The diameter of zones of inhibition was measured in millimeter and recorded.

Statistical analysis

Chi-square test was used to determine non-dependence of two factors while Friedman's test was used to determine randomized block design [10].

RESULTS AND DISCUSSION

The results of this research work are shown in the tables below. Table 1 shows the incidence of *S. pyogenes* from tonsillopharyngitis of male and female in the Federal Polytechnic, Ado-Ekiti community. Out of the sixty (60) samples collected 27(45%) were positive while 33(55%) were negative. 30(40%) were positive for males and 30(83%) for females. The gender carriage showed that incidence is higher in female than in males. Certain occupations are more likely to be performed by men, and work-related exposure differences might contribute to the incidence been higher in female than in male. However, similar exposure risks during travel do not necessarily indicate similar rates of disease for persons of both sexes. Differences in health care-seeking behaviour between male and female patients might also play a major role with a chi-square value of 0.0119 which is not significant. Also, genetic composition and physiologic differences as is applied

above with chi-square value of 0.0119 does not affect disease manifestations in men [17]. Further research is needed to substantiate this hypothesis.

A test of homogeneity was considered to see if the data are from the same population, a non significance at 5% significance level, with chi-square value of 0.912 shows the data are from identical populations. A test to see if the existence of positive isolate in a given symptom is dependent on sex resulted in a chi-square value of 0.0119 which is not significant. In view of this result, it can be concluded that individual's sex has nothing to do with the symptom from which a positive isolate is obtained. From the ANOVAs test using the Friedman's non parameter approach, the value of Fr = 9.39 showed that there is no difference on resistivity pattern. It can therefore be concluded that resistance level differ from symptom to symptom.

The incidence is highest in purulent sputum and sore throat with 66% of each showing positive isolates. Lowest incidence was also observed in purulent sputum and cough with 25% of each showing positive isolates. These findings were attributed to the lifestyle of the students and their adaptation to changing environment. Some researchers have attributed these irritations to the harvest season of maize which is accompanied by the drop of large numbers of dander to the environment [5, 15]. However, why the isolation of the bacterium was prevalent in purulent sputum and sore throat infections at this time and season was not clear. Table 2 shows the resistance pattern of positive samples. Out of 60 isolates, resistance was highest in throat irritation with 27 (100%) of the isolate resistant to the antibiotics tested with prominence showing in gentamycin, amoxil, norfloxacin, erythromycin and levofloxacin. The percentage resistivity is highest in erythromycin at 0.088% and Gentamycin at 0.083%. This is an indication that the isolates are multi-drug resistance. Since most of the isolates found to be multi-drug resistant harbors *erm*(B)(395[46.5%]) and *mef* (A)(383[45.1%]) genes, their resistance could be ascribed to the possession of these genes [14].

The antimicrobial susceptibility of multidrug-resistant strains to saponin (3.0mg/ml) extracted from *T. catappa* (Table 3) revealed that of the 27 multidrug resistant isolates from cough, 11(45%) were resistant to the extract preparation. It is envisaged that infection control measures and exploitation of plant extract for new drug development will avert the upsurge of emerging infectious diseases and their multi-drug resistant agents. The discovery of higher resistance of isolates of *S. pyogenes* is not unexpected. Given the ambiguity of *S. pyogenes* as human pathogens that normally colonize the throat, resistance among the isolates may be a sensitive indication of distinct therapeutic and non-therapeutic, appropriate and inappropriate use of antimicrobial drugs [16].

Prevalence of resistance is usually positively correlated with prescribed outpatient drug use, consumption of drugs obtained without prescription and use without medical guidance [11]. This is inappropriate because using insufficient dosages or incorrect or unnecessary drugs increases bacterial resistance [13] and the spread of antimicrobial drug resistance [16]. The antimicrobial activities of crude extract of *T. catappa* are of interest since it was effective at concentration of 3.0mg/ml to multiple resistant isolates of *S. pyogenes* from cough patients. The fact that it was active against 62% of these isolates is worthy of note. Further studies on this extract are on-going in our laboratory.

Table 1: Incidence of *Streptococcus pyogenes* isolated from Federal Polytechnic, Ado-Ekiti community.

S/N	Total No. of Samples	Sex		+ve isolates (%)
		M(%)	F(%)	
Throat irritation	15	6(3)	9(5)	8(53)
1	10	5(1)	5(2)	3(30)
2				
Purulent sputum	4	3(1)	1(0)	1(25)
3	6	2(2)	4(2)	4(66)
4	10	5(2)	5(3)	3(30)
Sore throat	3	2(2)	1(0)	2(66)
5	8	5(1)	3(4)	5(62)
6	4	2(0)	2(1)	1(25)
cough				
7				
8				
Total	**60**	**30**	**30**	**27**

M – Male
F – Female
+ve – positive

Table 2: Resistance Table (with Ranks)

Antimicrobial agents (mg)	Throat Irritation	Purulent	Sour Throat	Cough	Total	% Resistance
	Rank A	B	C	D		
CPX	1(1)	1(1)	1(1)	1(1)	4	0.046
NB	2(1)	1(1)	3(3)	2(1)	6	0.069
GN	6(4)	0(1.5)	4(3)	2(1)	10	0.083
AML	1(1)	1(1)	1(1)	1(1)	4	0.056
S	3(2)	0(0)	0(0)	2(3)	5	0.023
RD	1(1)	1(1)	1(1)	1(1)	4	0.046
E	2(3)	1(1)	5(4)	2(3)	12	0.088
CH	1(1)	1(1)	1(1)	1(1)	4	0.056
APX	2(1)	0(1.5)	3(2.5)	2(1)	6	0.065
LEV	2(1)	0(1)	4(2)	1(1)	5	0.069
	27(20)	**8(14)**	**8(21.5)**	**17(16)**	**60**	**0.60**

Cpx-ciproflox, NB-norfloxacin,GN--gentamycin, AML amoxil,S-streptomycin, RD- rifampicin,E- erythromycin.CH-chloramphenicol. APX-ampiclox, LEV- levofloxacin

Table 3: Susceptibility of multiple-antibiotic resistant *Streptococcus pyogenes* to aqueous extract of *Terminalia catappa* (3.0mg/ml)

No (%)	Diameter of Zone on Inhibition (mm)
3 (11)	≥ 9.0
3 (11)	8.0
1 (4)	7.0
6 (25)	6.0
3 (11)	5.0
11 (45)*	≤ 4.0

* Resistant

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