In vitro phenotypic antibiotic resistance in bacterial flora of some indigenous orally consumed herbal medications in Nigeria

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Abstract

Due to massive problems facing the primary health care system in Nigeria, the consumer preference for indigenous herbal medications is on the increase. Since it is possibility that herbal medications may harbour pathogenic bacteria of clinical significance, this study determined the phenotypic antibiotic pattern of associated bacterial isolates using modified agar disc-diffusion and agar well-diffusion methods. All isolated bacterial species exhibited mono and/or multiple *in vitro* phenotypic antibiotic resistance to the test antibiotic (discs). High resistance patterns were also observed towards paediatric antibiotic suspensions. This study confirmed that most indigenous orally consumed herbal medications in Nigeria harbour bacterial flora that exhibit multiple resisu7tance to routinely used antibiotics.

Keywords: antibiotics, bacteria flora, herbal medication, indigenous, resistance.

Introduction

Herbal medicine, in which dried plants or plant extracts are used as therapeutic substances, is one of a number of increasingly popular practices encompassed by the term "complementary and alternative medicine" or CAM (Drew and Myers 1997). This broad use of traditional medicine is attributable to its accessibility and affordability (Steenkamp 2003) and the World Health Organization estimates that 65%-80% of the world's population use traditional medicine as their primary form of health care. The use of herbal medicine, the dominant form of medical treatment in developing countries, has been increasing in developed countries in recent years (Drew and. Myers, 1997) Assessment of the safety and efficacy of these medicines is an important concern of health professions who often regard them with suspicion because of either a lack of proof of safety and efficacy or a lack of knowledge of existing evidence-based data.

Past changes to Canadian government regulations for example, allow registration of traditional herbal remedies as over-the-counter drugs if they are backed by scientific evidence indicating efficacy against minor self-limiting ailments. They also need to show standardization for dosage, formulation, indications and contraindications, and proof of adherence to Good Manufacturing Practices and quality control (Marles 1997). In Nigeria, there is unparalleled advertising of herbal medicine products by herbal practitioners despite research confirming that confirmed that most indigenous orally consumed herbal medications in Nigeria harbour bacterial flora that pose a significant clinical concern (Ogunshe et al. 2006c).

The control of microorganisms is critical for the prevention and treatment of diseases, however, the increasing number and variety of drug-resistant pathogens is a serious public health problem (Prescott et al. 2005). When microbes began resisting penicillin, medical researchers fought back with chemical cousins, such as methicillin and oxacillin. By 1953, the antibiotic armamentarium included chloramphenicol, neomycin, terramycin, tetracycline, and cephalosporins. But today, researchers fear that we may be nearing an end to the seemingly endless flow of antimicrobial drugs. Resistance to antimicrobial agents has resulted in morbidity and mortality from treatment failures and increased health care costs. Although defining the precise public health risk and estimating the increase in costs is not a simple undertaking, there is little doubt that emergent antibiotic resistance is a serious global problem (Lalitha 2004).

The main objective of this research study therefore, is to further determine the antibiotic resistance pattern of such implicated bacterial flora isolated from the indigenous herbal medications

Materials and methods

Antibiotic susceptibility determination of bacterial strains - Agar disc diffusion-method

Three hundred and eighteen bacterial isolates from Nigerian indigenous herbal medicines were tested against 12 antibiotics, in the form of antibiotic discs (ampicillin AMP 25 µg, cotrimoxazole COT 25 µg, gentamicin GEN 10 µg, nalidixic acid NAL 30 µg, nitrofurantoin NIT 300 µg, colistin COL 10 µg, streptomycin STR 10 µg, tetracycline TET 30 µg, chloramphenicol CHL 25 µg, cloxacillin CXC 5 µg, erythromycin ERY 25 µg and penicillin PEN 15 µg), by the Kirby-Bauer methods (Bauer et al. 1966; Lalitha 2004).

Seeded Mueller-Hinton agar plates were prepared by transferring 500-1000 µl culture broth onto the agar plates followed by surface streaking of the entire agar surface with a sterile wire loop. The seeded agar plates were left for about 15 min before the antibiotics discs were aseptically placed onto the agar surfaces. The plates were then incubated at 35^{o} C for 18-24 h. Zones of inhibition were measured and recorded in millimeter diameter according to the methods of Bauer et al. (1966), NCCLS (1999, 2003) and the modified method of Ogunshe (2004).

Agar well diffusion- method

Antibiotic susceptibility determination of the herbal bacterial isolates to 10 groups of 28 oral paediatric antibiotic suspensions- (ampicillin/ampicillin-cloxacillin, cotrimoxazole, metronidazole, chloramphenicol, cephalexin and erythromycin) was carried out using the modified agar disc-diffusion and agar well-diffusion methods. Seeded Mueller-Hinton agar plates were prepared by transferring 500-1000 µl culture broth unto the agar plates. The seeded agar plates were then left for about 15 min before aseptically dispensing the paediatric antibiotic suspensions into the agar wells already bored in the agar plates. The plates were then incubated at 35^{o} C for 18-24 h. Zones of inhibition were measured and recorded in millimeter diameter according to the methods of Bauer et al. (1966), NCCLS (1999, 2003) and the modified method of Ogunshe (2004).

Interpretation of zones of inhibition

Each zone size was interpreted by reference to the Performance Standards for Antimicrobial Susceptibility Testing: Twelfth Informational Supplement as susceptible, intermediate and resistant Lalitha (2004).

Results

The isolated bacteria from the Nigerian indigenous orally consumed herbal medications sampled were of the genera *Bacillus*, *Citrobacter*, *Clostridium*, *Enterobacter*, *Escherichia*, *Klebsiella*, *Micrococcus*, *Morganella*, *Proteus*, *Pseudomonas*, *Salmonella*, *Shigella*, *Staphylococcus*, *Streptococcus*, *Vibrio* and *Yersisnia*.

Resistance to antibiotics ranged from 2.56 % to 100 % among the bacterial isolates from the indigenous orally consumed herbal medications. The antibiotic resistance patterns recorded among the Gram-negative bacterial isolates using the agar disc-diffusion method were between 2.56 % in nitrofurantoin and 71.8 % in ampicillin (Table 1). The phenotypic resistance profile of each Gram-negative bacterial strain indicate that only *Proteus mirabilis* OCHM11X, OCH9MCC and *Proteus vulgaris* OCHM3 had 100 % resistance recorded against all the test antibiotics. *Shigella flexneri* OCHM92, *Morganella morganii* OCHM162, *Escherichia coli* OCHM331, *Pseudomonas aeruginosa* OCHM432 had antibiotic resistance of 62.5% to all the test antibiotics, while *Enterobacter aerogenes* OCHM11, OCHM7, *Klebsiella pneumoniae* OCHM26, OCHM263, *Escherichia coli* OCHM29 and *Shigella dysenteriae* OCHM39 had the lowest antibiotic resistance of 12.5%.

About 92% of the Gram-positive bacterial isolates were phenotypically resistant to ampicillin; cloxacillin and penicillin while only 8.9% of the isolates were resistant to chloramphenicol, gentamicin, streptomycin and tetracycline. Resistance to other antibiotic (discs) were erythromycin (66.7%); nalidixic acid (58.5%); cotrimoxazole (51.7%) nitrofurantoin (35.7%) and colistin (36.1%). Three of the Gram-positive bacterial isolates *Staphylococcus aureus* OCHM32 *B. licheniformis* and *B. cereus* OCHM351 had 50.0% phenotypic antibiotic resistance while *S. aureus* OCHM463 had the lowest resistance of 37.5%.

Overall susceptibility and resistance patterns of the bacterial isolates obtained from the herbal samples to various oral paediatric antibiotic suspensions using the agar well-diffusion method varied between

4.13 % in cephalexin and 56.2 % in chloramphenicol (Table 2). Overall resistance exhibited against oral paediatric suspensions were ampicillin+cloxacillin (2.48 - 4.13 %), erythromycin + ethylsuccinate (4.13 %), erythromycin (6.61 %), ampicillin (6.61 - 13.2 %), cephalexin (4.13 - 9.92 %), amoxicillin (12.3 - 34.7 %) metronidazole (23.1 - 43.8 %), cotrimoxazole (33.0 - 45.4 %), sulfamethoxazole+ trimethoprim (45.5 %) and chloramphenicol (52.9 - 56.2 %). All the bacterial isolates from the herbal samples exhibited mono or multiple antibiotic resistance to all the test paediatric antibiotic suspensions except *Enterobacter aerogenes* OCHM1MCC, *Shigella flexneri* OCHM27X, *Klebsiella pneumoniae* OCHM28X, and *Salmonella enterica* serovar Typhimurium OCHM25X. The antibiotic susceptibility/resistance patterns of the various species of bacterial isolates are as shown in Tables 1 & 2.

Table 1: Antibiotic resistance pattern of the bacterial isolates from indigenous herbal medications using the disc diffusion method. Isolates of less than 21% indicate those that were least resisted.

Antibiotics	Antibiotics lab codes/ conc. (Discs)	% Antibiotic pattern of the bacterial isolates	
		Gram +	Gram -
Chloramphenicol	CHL (25µg)	8.9	na
Erythromycin	ERY (25µg)	66.7	na
Ampicillin	AMP (25µg)	92.0	71.8
Cloxacillin	CXC (5µg)	92.0	na
Cotrimoxazole	COT (25µg)	51.7	46.2
Colistin	COL (10µg)	36.1	28.2
Gentamicin	GEN (10µg)	8.9	20.5
Nalidixic acid	NAL (30µg)	58.5	66.7
Nitrofurantoin	NIT (300µg)	35.7	2.56
Penicillin	PEN (15µg)	92.0	na
Streptomycin	STR (10µg)	8.9	5.12
Tetracycline	TET (30µg)	8.9	20.5

na: not available

Table 2: Antibiotic susceptibility profiles of the bacterial isolates from indigenous herbal medications using the agar well-diffusion method.

Paediatric antibiotics	% Antibiotic pattern of the bacterial isolates (Paediatric suspensions)	
	Susceptible	Resistance
Sulfamethazole + Trimethoprim	54.5	45.5*
Cephalexin	95.87-90.1	4.13 – 9.92
Chloramphenicol	47.1-43.8	52.9 - 56.2*
Erythromycin	93.4	6.61
Erythromycin + Ethylsuccinate	95.9	4.13
Ampicillin + cloxacillin	97.7- 95.9	2.28 - 4.13
Ampicillin	93.4 – 86,8	6.61 - 13.2
Amoxicillin	87.7 – 65.3	12.3 - 34.7^
Cotrimoxazole	67.0 – 54.6	33.0^ - 45.4*
Metronidazole	76.9 – 56.2	23.1^ - 43.8*

* = moderate / high antibiotic profile differences
^ = narrow antibiotic profile differences
_ = least resisted

Discussion

Health Canada defines traditional herbal medicines as “finished products intended for self-medication that contain, as the active principles, herbal ingredients that have received relatively little attention in world scientific literature but for which traditional or folkloric use is well-documented in herbal references” (Marles 1997). Despite the fact that some unscrupulous or uncaring manufacturers are producing herbal supplements with levels of active ingredients that can be as much as 300 times below the dosage established to be effective (Marles 1997), the study of Ogunshe et al. (2006a) also indicated the unwholesome nature of most of the indigenous orally-consumed herbal medications in Nigeria due to the high recovery rates of pathogenic bacterial species from such medications. Although it is widely

perceived that "natural" products are safe, the evidence suggests that during growth and storage, crude plant material can become contaminated by pesticide residues, microorganisms, aflatoxins, radioactive substances and heavy metals (Bisset 1994). In ascertaining whether a substance is associated with an adverse effect, the medical literature may be of limited help; there may be no previous report of such an event, as was the case for fatal anaphylaxis, which occurred in an 11-year-old child with asthma after her third exposure to royal jelly (Drew and Myers 1997). Prior to this event, contact dermatitis had been documented with royal jelly (which contains proteins, carbohydrates, amino acids, vitamins, lipids and fatty acids), but the allergen had not been identified. (Bullock et al., 1994). At the time of the child's death, the Adverse Drug Reactions Advisory Committee (ADRAC) of the Commonwealth Department of Health and Family Services had three reports of adverse reactions to royal jelly on file: one of anaphylaxis and two of bronchospasm (Dr Ian Boyd, Adverse Drug Reactions Advisory Committee, Canberra; data, 1972-May 1993; personal communication). Raised awareness of this problem resulted in the TGA advising manufacturers to label royal jelly products to warn of their potential to cause severe allergic reactions in people who suffer from asthma or allergies (Anonymous 1994). This type of adverse reaction therefore should call for alarm and caution in consuming most of the indigenous orally consumed herbal medications that do not usually pass through any official screening tests.

Antibiotics are used to treat bacterial infections. They may be used as a short- or long-term treatment, depending on whether the problem is acute or chronic. Testing bacterial pathogens for their responses to chemotherapeutic agents is common practice in clinical and food microbiology. This study showed that there is also a need to assay bacteria from indigenous herbal medications for antibiotic susceptibility/resistance profiles to reveal significant antibiotic resistance patterns of clinical importance.

It was observed in this study that a relatively high level of resistance was displayed by bacterial pathogens to cloxacillin, cotrimoxazole and nalidixic acid, in accordance with previous findings of Ryan et al. (1987) who had earlier reported a high resistance to same antibiotics by certain gastroenteritic bacterial isolates. Despite the fact that cotrimoxazole was a mixture of trimethroprim and sulfamethoxazole in a 1:5 ratio, developed as an approach to combat resistance to the sulfonamides (Hitching, 1983), a high level resistance (46.2 % - 51.7 %) to cotrimoxazole was still recorded. These relatively high resistance patterns are of serious clinical concern.

The high resistance to nalidixic acid observed in this study contradicts earlier reports of Wolfson and Hooper (1989), Neu (1992) and Dax (1997) in which quinolones such as nalidixic acid were documented as potent broad-spectrum antibacterial agents. However, Dax (1997) also reported that clinically, *Pseudomonas aruginosa*, *Klebsiella pneumoniae*, *Escherichia coli*, *Serratia* and *Staphylococcus* are most likely to be resistant due to changes in permeability of the micro-organisms.

Tetracyclines are closely related bacteriostatic antibiotics and are similar in antibacterial spectrum and toxicity. According to Morin and Gorman (1982), tetracyclines inhibit bacterial protein biosynthesis at the macromolecular level, and this phenomenon is responsible for bacteriostatic effects. Bacterial resistance to one tetracycline indicates likely resistance to others. The result of this study therefore confirms the findings of Morin and Gorman (1982) due to the relatively low antibiotic resistance recorded in the bacterial isolates. Koneman et al. (1992) reported that aminoglycoside antibiotics, such as streptomycin and gentamicin, are bactericidal and tend to be most active against Gram-negative pathogens but further noted that there was a decrease in the usefulness of streptomycin because of wide spread drug resistance. This is contrary to the findings of this present study in which the bacterial pathogens exhibited low resistance towards gentamicin and streptomycin. This may be due to the fact that the isolates were not clinical isolates.

Examples of penicillins according to Dax (1997) are ampicillin; amoxycillin etc. Ampicillin and the ampicillin-like drugs (e.g., amoxicillin) have a spectrum of activity very similar to that of penicillin G. The difference is greater activity against certain Gram-negative bacilli, such as non-penicillinase-producing *Haemophilus influenzae*, some *E. coli*, *Proteus mirabilis*, *Salmonella*, and *Shigella* (Beers and Berkow 2005). Certain reports however highlighted the relatively high antibiotic resistance of bacteria to ampicillin. Oyelese and Oyewo (1995) recorded 77.6 % antibiotic resistance against ampicillin and penicillin by Gram-positive and Gram-negative bacterial isolates of clinical origin. The fact that a similarly higher antibiotic resistance was recorded in ampicillin and penicillin in this study however confirms such earlier findings. Beers and Berkow (2005) also raised an alarm as regards the high-level resistance of pneumococci and enterococci to penicillins as an increasing problem.

Having a record of relatively lower resistance to nitrofurantoin and gentamicin in this study indicates that the nitrofurans such as nitrofurantoin are medium-spectrum antibacterial agents, which are potent against a variety of Gram-positive and Gram-negative bacteria assuming that sufficient concentrations are achieved at the site of infection and that resistance is not a problem with the use of these agents. Similarly, Brooks et al. (1998) supported the potency of gentamicin to a large percentage of bacterial isolates. In this study nitrofurantoin was found to be more potent against Gram-negative than Gram-positive bacterial isolates.

Chloramphenicol is primarily bacteriostatic. It binds to the 50S subunit of the ribosome and inhibits bacterial protein synthesis. It has a wide spectrum of activity against Gram-positive and Gram-negative cocci and bacilli (including anaerobes). Chloramphenicol is effective in H. influenzae, meningococcal, and pneumococcal meningitis caused by susceptible organisms, but is relatively ineffective in meningitis caused by *E. coli* and other Enterobacteriaceae (Dax 1997; Beers and Berkow 2005). This probably confirms the extremely low resistance pattern observed in chloramphenicol in this study.

According to Beers and Berkow (2005), erythromycin is active against Gram-positive cocci (including anaerobes), with the exception of enterococci; but many *S. aureus* strains are now found to be resistant and caution has been raised as regards its usage in serious *S. aureus* infection. The result of this study however is contrary to the earlier finding of Beers and Berkow (2005) since about 67.0 % phenotypic resistance was recorded against erythromycin by the Gram-positive-bacterial isolates including the cocci. The frequency of resistance to erythromycin and other macrolides as well as that to lincomycin has also been documented by Soriano et al. (1998) to be high.

There were no documented reports on the antibiotic susceptibility patterns of bacterial isolates using paediatric oral suspensions except that of Ogunshe (2004), which indicated that infantile gastroenteritic bacterial isolates were more resistant to the paediatric oral suspensions than the antibiotic-incorporated discs. The findings indicate that the resistance patterns among the tested bacterial isolates were somehow slightly higher due to the fact that cotrimoxazole (30.6 – 70%), metronidazole (77.1 – 86.1%), erythromycin (100%) and chloramphenicol (100%) were the most resisted paediatric antibiotics while the results obtained in this study indicate quite lower antibiotic resistance among the test antibiotics except chloramphenicol, sulfamethoxazole+trimethroprim, cotrimoxazole and metronidazole. The least resisted paediatric antibiotics in the earlier work of Ogunshe (2004) were ampicillin+cloxacillin, erythromycin, cephalexin and ampicillin, while the least resisted in this present work were ampicillin+cloxacillin, erythromycin, cephalexin and erythromycin+ethylsuccinate. The slight variations in the antibiotic susceptibility profiles obtained in the results of Ogunshe (2004) and that obtained in this study may be due to the fact that the bacterial species assayed for by Ogunshe (2004) were clinical bacterial isolates while those assayed in this study were food-borne bacterial isolates from herbal medications.

The similarity in antibiotic resistance patterns of the bacterial isolates to cotrimoxazole and sulfamethoxazole+trimethoprim antibiotics may be due to the fact that cotrimoxazole is a mixture of trimethroprim and sulfamethoxazole in a 1:5 ratio, developed as an approach to combat resistance to the sulfonamides (Hitching 1983). The antibiotic susceptibility patterns of each bacterial isolate to both antibiotics were also similar. Although percentage resistance of the ampicillin (6.6 - 13.2 %), ampicillin-cloxacillin (2.48 - 4.13 %) and cephalexin (4.13 - 9.92 %) oral paediatric suspensions were relatively lower as compared to other classes of the paediatric suspensions, having found widespread use particularly as children suspensions, the low percentage resistance of 46.2 - 52.5 % recorded against the ampicillin paediatric suspensions is still higher than expected in paediatric chemotherapy. Recording as high as 43.8 - 56.2 % resistance in other classes of paediatric antibiotics also shows the great danger these commonly prescribed and consumed antibiotics may pose in paediatric infectious conditions, especially since it has already been reported that antibiotic resistance is a worldwide problem.

From the overall results of the antibiotic resistance profile obtained in this study, it is therefore an established fact that the onset of drug resistance threatens virtually all classes of antibacterial agents. This type of prophylactic and therapeutic chemotherapy is thus becoming quite dangerous. The need therefore, for new antimicrobial drugs has become an obvious scientific challenge since inappropriate use and clinical conditions have favoured selection for strains resistant to an increasing number of antibiotics (Bregendahl et al. 2003). Follath et al. (1987) and Wei et al. (2003) have documented that 3rd generation cephalosporins are not exempted where resistance resulted by selection. Montefiere et al. (1989) concluded that older generations of antibiotics no longer have an indication in the treatment of infection in Nigeria without prior knowledge of the sensitivity of the aetiological agent. Earlier works of Alausa and Montefiere (1978), Eke and Rotimi (1987), Rotimi et al. (1987) and Obaseiki–Beber

(1988) all have all shown a worsening trend in the antibiotic resistance profiles of isolates from hospital patients in Nigeria. This present study has confirmed the predictions of a worsening situation.

None of the Gram-positive bacterial isolates from herbal medications was completely susceptible to all test antibiotic discs except to chloramphenicol, gentamicin, streptomycin and tetracycline while only four of the bacterial isolates from the herbal samples were susceptible all the test paediatric antibiotics. The danger in this phenomenon is that the multi-resistance determinants can be transferred to new bacterial hosts. The situation is made more difficult in developing countries such as Nigeria where antimicrobial drugs are readily available to consumers across the counter with or without prescriptions from medical practitioners. Such a practice has led to misuse of antimicrobial drugs with the associated high prevalence of drug resistance among the staphylococci for example, (Nnochiri 1973; Adekeye 1979; Paul et al. 1982; Chigbu and Ezeronye 2003).

It has already been reported that antibiotic resistance is a worldwide problem (Fey et al. 2001; Gebreyes and Altier 2002). Though the magnitude of the problem may vary from place to place, the problem of antibiotic resistance is probably amplified in tropical developing countries where infectious conditions account for a substantial percentage of hospital consultations (Oyelese and Oyewo 1995). Several workers in this country and elsewhere have highlighted the problem of antibiotic resistance among several bacterial species (Nnochiri 1973; Adekeye 1979; Paul et al. 1982; Chigbu and Ezeronye 2003; Oyelese and Oyewo 1995; Levy 1998; Dunne et al. 2000; Ogunshe 2004, Ogunshe et al. 2006a). During the antibiotic era, an increasing number of food enterococci developed resistance to various therapeutic antibacterial agents including vancomycin, gentamicin (Follath et al. 1987), tetracycline and streptomycin (Levy et al. 2002). Similarly, in this study, most of the bacterial species isolated from the indigenous herbal medications, which can be grouped as food-borne, developed resistance to various therapeutic antibacterial agents.

It is very apparent that herbal therapy is here to stay in Nigeria since it supplements massive primary health care system problems, however, traditionally prepared herbal medications can harbour high microbial loads of bacterial isolates exhibiting significantly high level of mono or multi resistance to most commonly prescribed and routinely used antibiotics. This situation is unwholesome since the strains revealed in this study have the potential to become pathogenic and may participate in undesirable antibiotic resistance gene-transfer cascades *in vivo*. It can therefore be concluded that the high level resistance to antibiotics by the bacterial isolates from herbal medications observed in this study is clinically indicative and may result in the erosion of their effectiveness not only for the treatment of individual patients but also for the community at large, due to excessive use.

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