Research paper

Susceptibility-Resistance Profile of Micro-Organisms Isolated from Herbal Anti-Infectives Products Manufactured and Marketed in South Eastern Nigeria.

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ABSTRACT

Objective: To determine the susceptibility and resistance pattern of bacteria and fungi isolates obtained from herbal anti-infective liquid preparations manufactured and marketed in South-East Nigeria to conventional antibiotics.

Study Design: Experimental

Place and Duration of the study: Pharmaceutical Microbiology and biotechnology Laboratory, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Agulu Campus between October 2011 and March 2012

Methodology: Isolation and characterization of contaminating microorganisms were carried out using standard procedures. A total of forty-nine (49) bacteria and forty (40) fungi isolated from the herbal products were examined for susceptibility to conventional antibiotics using the disc diffusion method. The bacterial isolates were tested against ciprofloxacin, ofloxacin, amoxicillin-clavulanic acid, gentamicin, cefotaxime, ceftazidime, ceftriazone, sulphamethoxazole, tetracycline and ampicillin were employed while fungi isolates were tested against five common antifungal-Griseofulvin, Nystatin, Ketoconazole, Fluconazole and Clotrimazole. The Multiple Antibiotic Resistance Index (MARI) of each of the isolated bacteria was obtained following the standard method.

Result: The antimicrobial susceptibility-resistance profile of the bacteria isolates revealed that most of the bacteria were sensitive to Ciprofloxacin, Ofloxacin, Gentamicin, and Ceftriaxone, On the other hand, a good number of the isolates demonstrated high level of resistance to common antibiotics like Ampicillin, Amoxycillin-Clavulanic Acid, Trimethoprim-Sulphamethoxazole, and moderate level of resistance to Tetracycline, and some of the third generation Cephalosporins -Ceftazidime and Cefotazime. Multiple Antibiotic Resistance Index (MARI) evaluation revealed that most of the isolates were resistance to more than Fifty Percent (50%) of the number of antibiotics used. The fungal isolates were susceptible to Nystatin, Ketoconazole and clotrimazole, resistance to Fluconazole and high resistance recorded against Griseofulvin.

Conclusion: The results of this study revealed that the herbal medications can serve as a trail of spread of antibiotic-resistance genes.

Keywords: {Susceptibility, antibiotic resistance, herbal anti-infectives}

1. INTRODUCTION

The use of herbal medicine has always been part of human culture, as some plants possess important therapeutic properties, which can be used to cure human and other animal diseases [1]. Herbal medicine is becoming increasingly popular in both developing and developed countries [2]. A World Health Organization survey indicates that about 70–80% of the world population, particularly in developing nations; rely on non-conventional medicines mainly of herbal sources in their primary health care [3]. Medicinal plant materials normally carry a large number of microbes originating from the soil. Microorganisms of various kinds are normally adhered to leaves, stems, flowers, roots and seeds. Additional contaminants may also be introduced during harvesting, handling and production of various herbal remedies since no conscious efforts are made to decontaminate the herbs other than by washing them. [4]. Herbal medicines are therefore vulnerable to attack by microorganisms and as such are disposed to spoilage. Accordingly, gross microbial contamination of herbal medicinal products commonly consumed in Nigeria has been severally demonstrated [5, 6,7]. The presence of antibiotic resistant microbial isolates in the Herbal Medicinal Products (HMPs) could lead to transfer of antibiotic resistance traits to hitherto sensitive gut or oral micro flora of consumers [8].
The emergence of multiple drug resistant bacteria (MDR) has become a major cause of failure of the treatment of infectious disease [9]. As a result, society is facing one of the most serious public health dilemmas over the emergence of infectious bacteria displaying resistance to many and in some cases, effective antibiotic [10] much like the situation in human medicine. Bacteria and fungi resistance to antimicrobial drugs has continued to grow in the last decades [11]. The increased prevalence of their resistant is due to extensive use and misuse of antimicrobials. This has rendered the current available antimicrobial agents insufficient to control microbial infections and create major public health problem.
Resistant bacteria strains may develop almost anywhere particularly in a pressurized environment containing previously non-resistant bacteria strains as contaminants. One of such environments can be created by widespread use of HMP. HMPs have been previously implicated as a pool for such contaminations [12,13]. It is of utmost importance to both monitor and ascertain the microbial purity of HMPs given the huge medical and economic implications of any such microbial contamination especially with multiple drug resistant strains. Such surveillance will both help to identify microbial contamination of herbal products and slow down and prevent the emergence of drug-resistant strains. The present study evaluated the presence of contaminating organisms and the susceptibility-resistance pattern of the isolated organisms.

2. EXPERIMENTAL DETAILS / METHODOLOGY (ARIAL, BOLD, 11 FONT, LEFT ALIGNED, CAPS)

2.1 MATERIALS: HERBAL SAMPLES

A total of twenty herbal anti-infectives were purchased randomly from different shops and herbal outlets located within the five states that make up the south-east, Nigeria and were used in this study. The samples which were within their shelf lives and were kept at room temperature (as indicated by their manufacturers) were used within two weeks of collection.

2.2 METHODS

2.2.1 Isolation and Identification of Microbial Contaminants in the Herbal

The herbal anti-infectives were serially diluted and plated on nutrient agar and sabouroud dextrose agar plates in triplicate and incubated at 37^{o}C for 18-24 hours and 20^{o}C- 27 ^{o}C for 72-168hours for bacteria and fungi respectively. The resultant colonies were further purified, isolated and characterized using standard methods [14].

2.2.2 Characterization of Microorganisms Isolated From the Herbal Preparations

The bacteria isolates were characterized using the morphological appearance (macroscopy) of their colonies, their Gram stain reaction and confirmatory biochemical tests. The fungi isolates were also identified on the basis the morphological characteristics (macroscopy) of their colonies, microscopy, staining with ordinary stain and the appearance of their mycelia [15].

2.2.3 Antibiotics Susceptibility Testing

The susceptibility tests were performed following the method M2-A6 disc diffusion method recommended by the National Committee for Clinical Laboratory Standards [16] using Mueller Hinton and Sabouraud Dextrose Agar. The bacterial isolates from the samples were reactivated by sub-culturing from agar slant onto nutrient agar plate and was incubated for 18-24 hours. The inoculum was standardized by transferring three distinct and separate colonies of the pure culture of the test organism using sterile wire loop into 3mls of sterile nutrient broth. The suspension was incubated for 3 hours at 37°C to allow for the growth of test organism till the density was equivalent to the turbidity of 0.5 McFarland. The standardized inocula were swabbed onto Mueller-Hinton agar and Sabouraud Dextrose Agar plate and the discs were placed on the inoculated plates and pressed firmly onto the agar plate for complete contact. The bacterial strains were tested against the following discs:, ofloxacin (OFX, 5µg) ; ciprofloxacin (CIP,5µg) ; amoxicillin/clavulanic acid (AMC,20/10µg) ; gentamicin,(GN ,10µg) ; ceftazidime (CAZ,30µg) ; cefotaxime (CTX,30µg) ; trimethoprim-sulfamethoxazole (SXT,1.25/23.75µg); Ampicillin (AMP,10µg) ; tetracycline (TE, 30µg); ceftriaxone (CRO, 30µg).The fungal strains were tested against the following discs: nystatin (N,20µg) ; clotrimazole (C,20µg) ; griseofulvin (G,20µg) ; ketoconazole (K,20µg) and fluconazole (F,20µg). The Plates were inverted and left on the work table for 30 minutes to allow for pre-diffusion of antibiotics into the agar. The plates were incubated at 37°C for 18-24 hours and at 25^{o}C 24-48hours for bacteria and fungi respectively. The susceptibility of each isolate to each antibiotic was shown by a clear zone of growth inhibition and this was measured using a meter rule in millimeters and the diameter of the zones of inhibition was then interpreted using standard chart [17].

2.2.4 Determination of Multiple Antibiotics Resistance Index (MARI)

The Multiple Antibiotics Resistance Index (MARI) of ten antibiotics (ofloxacin, ciprofloxacin, gentamicin, amoxycillin-clavulanic acid, sulphamethoxazole-trimethoprim, ceftriazone, ceftazidime, cefotazime, tetracycline and ampicillin) were determined using the formula, MARI = a/b

Where

a = Represents the aggregate resistance of antibiotics to all isolates and

b = Represents the total number of antibiotics that was used

2.3 Statistical Analyses

The data were analyzed using students t-test with the aid of SPSS 10 software package and expressed as mean values ± [Standard error of Mean] of the three replicates of antibiotic susceptibility profile of the isolated organisms against panel of antibiotics.

3. RESULTS AND DISCUSSION

3.1 RESULTS

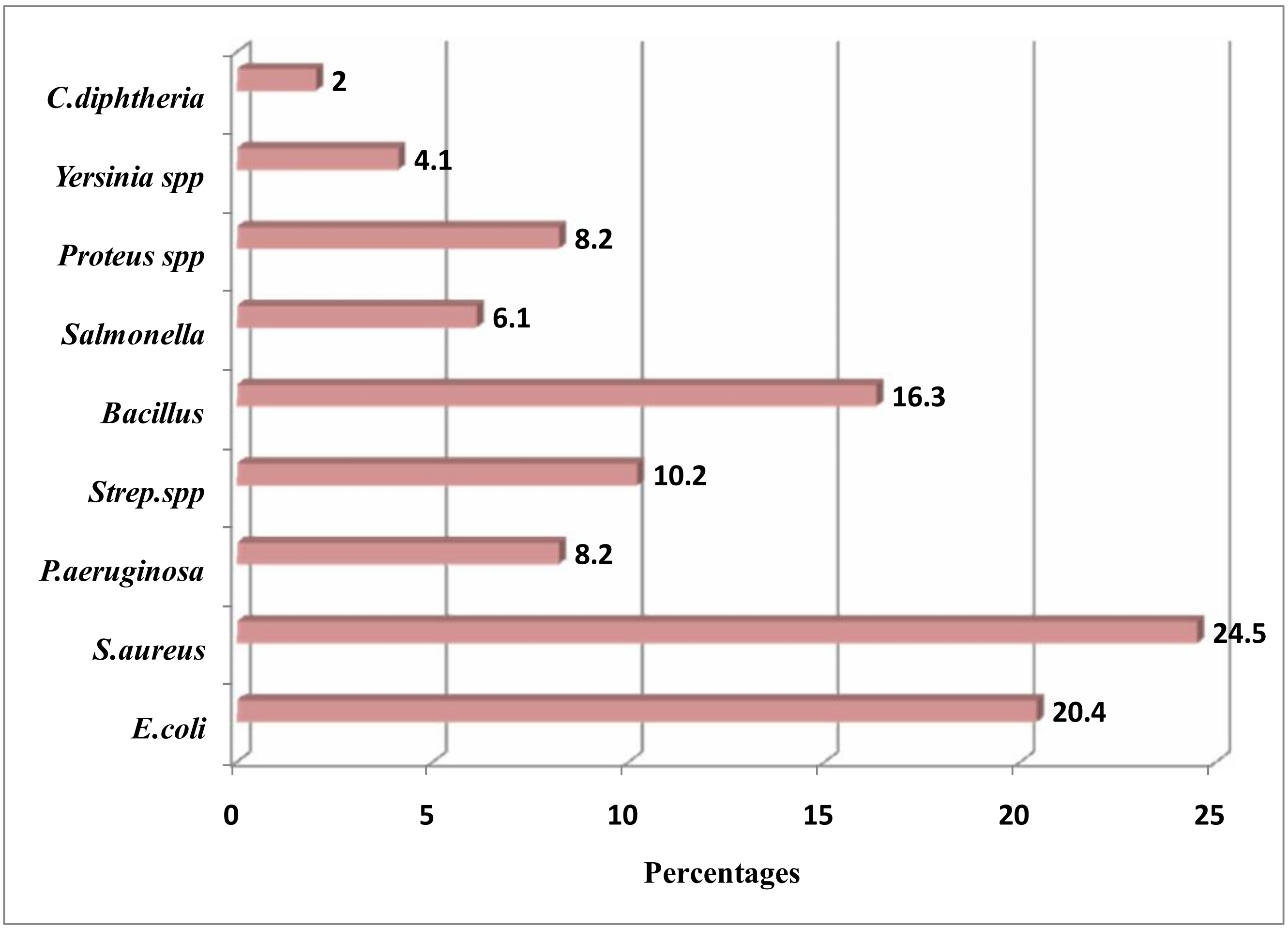

Figure 1: Percentage of Bacteria Species Isolated from the Herbal Anti-Infectives

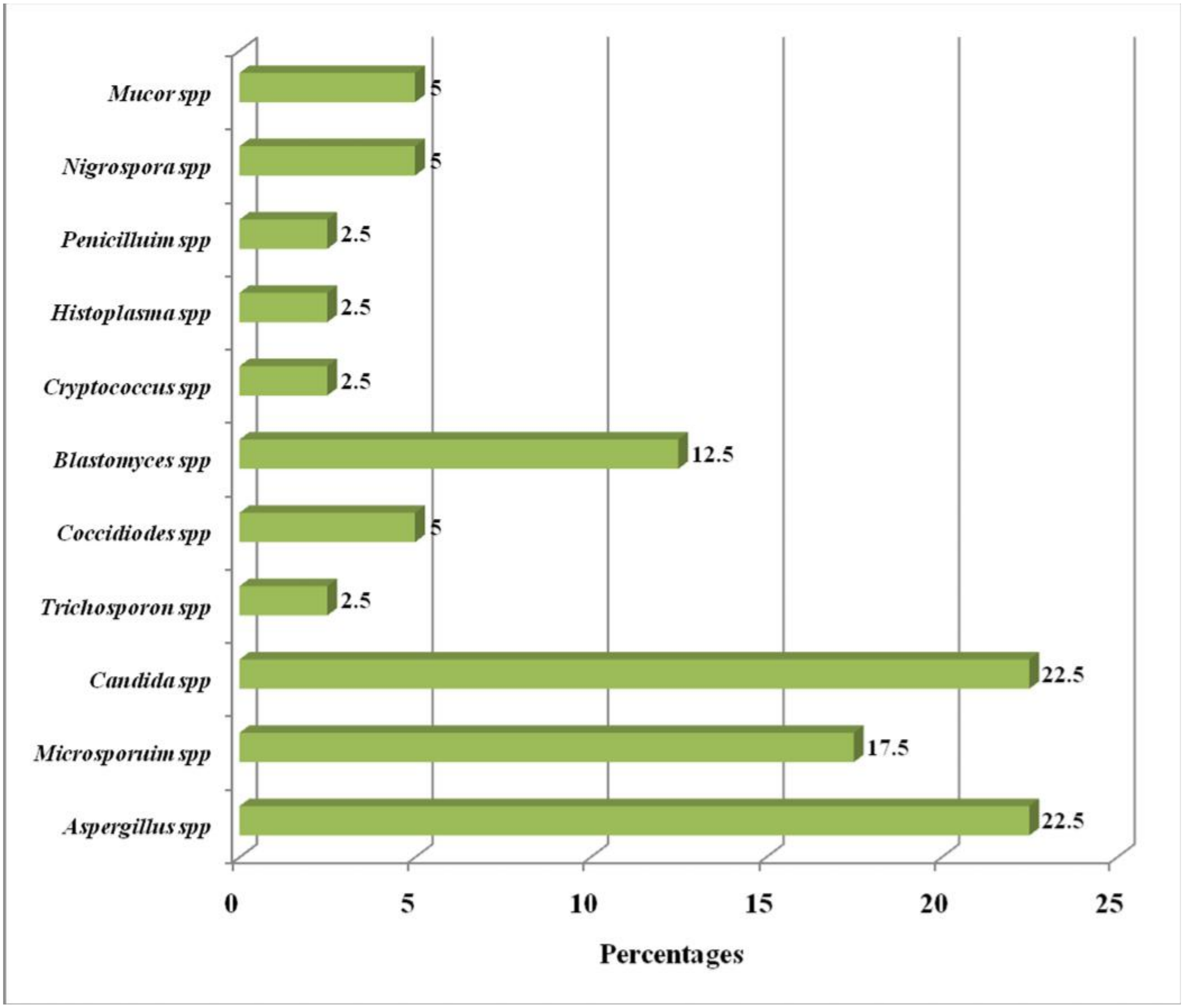

Figure 2: Percentage of Fungi Species Isolated from the Herbal Anti-Infectives

Table 1: Microorganisms Isolated From the Herbal Preparations.

Samples Code	Therapeutic Claims	Identity Of Bacteria Isolated	Identity Of Fungi Isolated
1	Antibacterial, Antimalarial, Ant rheumatic, infertility, Antiviral.	*a)Staphylococcus aureus* *b)Proteus spp*	*a) Microsporuim spp.* *b) Aspergillus spp* *c) Nigrospora spp*
2	Antibacterial, Antirheumatic, Antifungal and Antiviral.	*a)Escheria coli* *b)Staphylococcus aureus* *c)Staphylococcus epidermidis* *d)Pseudomonas aeruginosa* *e)Bacillus spp* *f)Proteus spp*	*a) Candida tropicalis* *b) Microsporum canis*
3	Antibacterial, Antirheumatic, Antifungal, Earlier Menopause, Painful and irregular menstruation.	*a)Staphylococcus aureus* *b)Bacillus subtilis* *c)Bacillus cereus* *d)2 Salmonella spps*	*a) Candida albicans* *b) Candida tropicalis* *c) Trichosporon spp*
4	Antibacterial, Antifungal.	*a)Escherichia coli* *b)Staphylococcus areus* *c)Streptococcus spp* *d)Bacillus spp*	*a) Coccidioides immitis* *b) Microsporuim audounii*

5	Antibacterials, Antimalarial, Antiparasitic, Internal heat, pile, and reduces sugar.	*a)Staphylococcus epidemidis* *b)Streptococcus spp* *c)Yersinia spp*	-
6	Antibacterial, Treatment of all form of eye infections.	*a)Escherichia.coli* *b)Bacillus spp* *c)Proteus spp*	*a)C.topicalis* *b) Microsporuim audounii* *c)Aspergillus niger*
7	Antibacterial, Antimalarial.	*a)Escherichia coli* *b)Staphyloccus spp* *c)Pseudomonas* *d)Bacillus spp*	*a) Blastomyces* *b) Microporuim canis*
8	Antibacterial.	*a)Staphylococcus spp* *b)Salmonella spp*	*a) Blastomyces*
9	Antibacterial Anti-malarial, HBP, Cough Antirheumatism, e.t.c.	*a)Staphylococcus areus*	*a) Blastomyces spp* *b) Cryptococcus neoformans* *c) Histoplasma*
10	Antibacterial, Hypertension, Antiviral, fibroid, stroke.	*a)Pseudomonas aeruginosa*	*a)Penicillum spp* *b)Aspergillus spp*
11	Antibacterial and Asthmatic cough.	*a)Escherichia coli* *b)Staphylococcus*	*a)Candida spp*
12	Anti-bacterial Antiviral, Diabetes, Reduces cholesterol.	*a)Staphylococcus areus* *b)Streptocoloccus spp*	*a) C.albicans* *b)Blastomyces*
13	Anti-bacterial, Anti-malaria, Antirheumatic. Antifungal	*a)Escherichia coli* *b)Corynebacteruim diphtheria*	*a)Aspergillus spp* *b) Nigrospora spp* *c) C.tropicalis*
14	Antibacterial, Antiviral, Antirheumatic, Antifungal, Antiparatic, internal heat, pile.	*a)Escherichia coli* *b)Streptococcus spps* *c)Yersinia spp*	*a)C.albicans* *b) Aspergillus spp*
15	Antibacterial, Antimalarial, Antirheumatic Antiviral.	*a)Escherichia coli*	*a)Microsporum spp* *b) Coccidioides spp* *c)Aspergillus spp.*
16	Antibacterial, Antirheumatic and Arthritis, Veneral diseases.	*a)Escherichia coli* *b)Pseudomonas aeruginosa*	*a)C.albicans* *b) Mucor spp*
17	Antibacterial, Treatment and prevention of toothache.	*a)Proteus spp*	*a) Aspergillus spp*
18	Antibacterial, Antirheumatism reduces sugar and cholesterol.	*a)Escherichia coli*	*a)Mucor spp*
19	Antibacterial, Antiviral, Purifies blood, Detoxifies toxins, Builds immune system, Stops dizziness, weakness.	*a) Bacillus spps*	*a) Yeast/Blastomyces* *b)Aspergillus spp* *c) Microsporum spp*
20	Antibacterial, Antiparasitic, ulcer, constipation, fibroid, internal heat heart burn and diabetes	*a) Staphylococcus epidermidis*	*a)Aspergillus spp*

Table 1 shows the products/samples code, therapeutic claims and the organisms isolated from the various herbal products. Figures 1 and 2 show the percentage of the microbial species isolated. A total of 89 microbial strains (49 bacterial and 40 fungal strains) were isolated from the herbal preparations. The identified microbial isolates consists of nine (9) bacterial genera and eleven (11) fungal genera which include *Staphylococcus, E. coli, Bacillus, Streptococcus, Pseudomonas*, *Proteus, Salmonella, Yersinia*, *Corynebacteruim diphtheria* and *Aspergillus, Candida, Microsporuim, Trichosporon* , *Coccidiodes, Blastomyces*, *Cryptococcus, Histoplasma, Penicilluim, Nigrospora*, *Mucor* respectively. The most frequently isolated bacteria and fungi specie were *Staphylococcus spp* (24.5%) and *Aspergillus spp/Candida spp*

(22.5%) respectively. The least frequently isolated bacteria specie was *Corynebacteruim diphtheria* (2.0%) and that of fungi were *Trichosporon spp, Cryptococcus spp, Histoplasma* and *Penicilluim spp* (2.5%).

Table 2: The Antibiotic Susceptibility Profile of the isolated bacteria

Isolates and Samples Code	Drugs' Mean Inhibition Zone Diameters (IZD in mm) ± Standard Error in the Mean (SEM)									
	OFX Ẋ ±SEM	CIP Ẋ ±SEM	SXT Ẋ±SEM	AMC Ẋ± SEM	GN Ẋ ±SEM	CTX Ẋ ±SEM	CAZ Ẋ± SEM	TE Ẋ ±SEM	AMP Ẋ± SEM	CRO Ẋ±SEM
E15	19±0.58	24±0.00	0±0.00	0±0.00	10±0.33	11±0.00	0±0.00	23±0.00	0±0.00	34±0.00
E14	20±0.00	27±1.15	0±0.00	10±0.00	22±0.00	0±0.00	0±0.00	0±0.00	0±0.00	14±0.00
E4	34±1.15	34±0.58	0±0.00	10±0.00	25±0.58	20±0.58	0±0.00	0±0.00	0±0.00	17±1.15
E7	19±0.00	22±1.15	0±0.00	6±1.00	21±0.88	12±1.15	0±0.00	8±0.00	0±0.00	23±0.00
E11	10±1.15	23±1.15	0±0.00	0±0.00	21±0.00	20±0.58	0±0.00	8±0.00	10±0.00	36±0.33
E16	19±0.58	26±0.00	0±0.00	7±0.00	20±0.58	15±0.00	24±0.00	0±0.00	0±0.00	12±0.00
E2	21±0.00	29±0.00	0±0.00	0±0.00	20±0.67	16±1.00	22±3.61	8±0.00	8±0.58	22±0.58
E6	14±1.53	23±1.73	0±0.00	0±0.00	20±0.00	12±0.00	0±0.00	9±1.15	10±0.58	23±0.58
E13	20±0.00	23±0.00	0±0.00	6±0.33	21±0.00	20±0.00	0±0.00	0±0.00	0±0.00	20±0.00
E18	20±0.58	25±0.00	0±0.00	0±0.00	21±0.58	21±0.00	0±0.00	6±0.00	10±0.00	30±0.58
Sa2	24±0.00	27±0.33	0±0.00	0±0.00	8±0.00	20±0.00	17±0.88	10±1.00	0±0.00	21±0.00
Sa3	21±0.00	23±0.33	0±0.00	7±0.00	20±0.00	0±0.00	20±0.00	0±0.00	0±0.00	15±0.58
Sa4	14±0.00	22±0.00	0±0.00	6±0.00	18±0.58	11±0.58	0±0.00	19±0.58	0±0.00	16±1.00
Sa8	23±1.75	24±0.00	0±0.00	18±0.00	21±0.00	7±0.58	23±0.58	15±0.00	0±0.00	27±0.00
Sa5	14±0.58	22±0.00	0±0.00	16±0.58	21±0.00	12±0.00	21±6.33	15±0.00	0±0.00	29±0.58
Sa1	13±1.15	17±0.00	0±0.00	10±0.00	16±0.00	13±0.88	20±0.67	0±0.00	0±0.00	16±0.00
Sa12	21±0.00	29±0.58	0±0.00	7±1.53	20±0.00	21±0.58	17±0.00	11±0.58	0±0.00	28±0.58
Sa11	20±0.00	23±0.00	0±0.00	12±0.00	21±0.58	15±0.00	18±0.58	11±0.00	0±0.00	32±1.00
Sa7	17±0.58	24±0.00	0±0.00	0±0.00	19±0.00	17±0.58	0±0.00	0±0.00	0±0.00	34±0.58
Sa9	18±0.58	21±0.58	0±0.00	0±0.00	20±1.00	21±0.00	0±0.00	15±0.58	9±0.58	26±0.00
Sa2	15±0.00	19±0.58	0±0.00	15±0.88	10±0.00	0±0.00	24±1.00	0±0.00	14±0.00	0±0.00
Sa20	25±1.15	27±0.00	0±0.00	14±0.00	23±0.58	27±0.58	0±0.00	22±0.58	0±0.00	0±0.00
Pa2	24±0.00	32±0.00	0±0.00	0±0.00	20±0.00	20±0.00	24±0.00	12±0.00	0±0.00	20±0.00
Pa16	0±0.00	0±0.00	0±0.00	0±0.00	0±0.00	0±0.00	12±0.58	0±0.00	0±0.00	0±0.00
Pa7	20±0.00	29±0.00	0±0.00	0±0.00	23±0.58	16±0.00	24±1.00	14±1.00	0±0.00	25±0.00
Pa10	21±1.45	29±0.33	0±0.00	0±0.00	18±0.00	17±0.00	0±0.00	13±0.00	0±0.00	23±1.73
St5	28±0.00	28±0.58	0±0.00	0±0.00	21±0.00	9±0.58	0±0.00	0±0.00	0±0.00	26±0.00
St14	23±1.00	27±0.00	0±0.00	0±0.00	24±1.15	28±0.00	23±0.00	7±0.00	0±0.00	30±0.58
St12	21±0.00	24±0.88	0±0.00	17±1.15	24±0.00	0±0.00	0±0.00	11±0.58	0±0.00	24±0.00
St4	17±0.00	24±0.00	0±0.00	10±0.00	15±1.15	18±0.58	17±0.88	0±0.00	0±0.00	12±1.00
St14	13±1.15	18±0.88	0±0.00	7±0.33	21±0.00	26±0.00	23±0.00	24±0.58	14±0.00	13±0.00
Ba19a	23±0.00	26±0.00	0±0.00	11±0.58	21±1.00	0±0.00	0±0.00	24±0.00	13±1.15	15±0.58
Ba7	16±0.00	26±0.58	0±0.00	6±0.00	21±1.15	15±0.00	25±0.58	0±0.00	0±0.00	23±0.00
Ba19b	14±0.58	28±0.33	0±0.00	0±0.00	22±0.58	11±0.58	0±0.00	0±0.00	0±0.00	15±0.58
Ba3a	17±0.00	24±0.00	0±0.00	16±1.15	12±0.00	20±0.58	0±0.00	24±0.58	12±0.00	13±0.00
Ba4	14±0.00	17±0.00	0±0.00	15±0.00	20±1.15	12±0.00	18±0.00	17±0.00	16±1.00	25±0.58
Ba6	14±1.53	18±1.00	0±0.00	0±0.00	17±0.00	17±0.33	0±0.00	0±0.00	0±0.00	30±0.00
Ba2	13±1.15	18±0.58	0±0.00	0±0.00	11±1.15	20±0.00	17±0.00	13±0.58	0±0.00	15±1.15
Ba3b	18±0.00	22±0.00	0±0.00	0±0.00	22±0.00	0±0.00	0±0.00	0±0.00	0±0.00	25±0.00
S3a	17±1.15	21±0.88	0±0.00	14±0.00	22±0.58	27±0.58	22±0.00	23±0.00	0±0.00	26±1.15
S3b	0±0.00	19±0.00	0±0.00	10±0.58	21±0.00	17±0.00	0±0.00	0±0.00	0±0.00	18±0.00
S8	16±0.00	18±0.58	0±0.00	0±0.00	20±0.00	28±0.58	18±0.58	27±3.33	12±0.00	32±0.00
P1	20±1.00	16±0.00	0±0.00	10±0.00	19±1.15	25±0.00	20±0.00	24±1.15	10±0.00	13±0.00
P17	14±0.00	20±0.00	0±0.00	15±1.15	22±0.58	0±0.00	0±0.00	0±0.00	0±0.00	16±1.15
P2	22±1.15	25±0.58	0±0.00	13±0.00	24±0.58	6±0.00	22±0.58	0±0.00	0±0.00	13±0.00
P6	20±0.00	24±0.58	0±0.00	0±0.00	8±0.00	11±1.15	16±0.33	24±0.00	0±0.00	18±0.58
Y5	15±0.00	24±0.00	0±0.00	0±0.00	19±0.00	16±1.00	19±0.00	0±0.00	0±0.00	10±0.00
Y14	14±0.58	20±0.58	0±0.00	0±0.00	16±0.00	12±0.00	0±0.00	14±0.58	21±0.58	0±0.00
C13	15±1.15	22±0.00	0±0.00	0±0.00	16±1.00	0±0.00	20±0.58	0±0.00	26±0.00	0±0.00

Notes for Table 2: E = *E.coli*, Sa = *Staphylococcus spp*, Pa = *Pseudomonas aeruginosa*, St = *Streptococcus spp*, Ba = *Bacillus spp*, S = *Salmonella*, P = *Proteus spp*, Y = *Yesinia spp*, C = *Corynebacteruim spp*. OFX = Ofloxacin, CIP - =

Ciprofloxacin, SXT = Sulphamethoxazole, AMC = Amoxycillin-Clavulanic Acid, GN = Gentamicin, CTX = Ceftazidime, CAZ = Cefotaxime, TE = Tetracycline, AMP = Ampicillin, CRO = Ceftriaxone. Table 2 above shows the antibiotic susceptibility profile of the bacterial isolates from the herbal samples against ten conventional antibiotics.

Table 3: Antibiogram of Bacterial Isolates from the Herbal Anti-infectives.

Isolates and Samples Code	Antibiotics Tested									
	OFX	CIP	SXT	AMC	CN	CTX	CAZ	TE	AMP	CRO
E15	S	S	R	R	R	R	R	S	R	S
E14	S	S	R	R	S	R	R	R	R	R
E4	S	S	R	R	S	R	R	R	R	R
E7	S	S	R	R	S	R	R	R	R	S
E11	R	S	R	R	S	R	R	R	R	S
E16	S	S	R	R	S	R	S	R	R	R
E2	S	S	R	R	S	R	S	R	R	I
E6	I	S	R	R	S	R	S	R	R	S
E13	S	S	R	R	S	R	R	R	R	I
E18	S	S	R	R	S	R	R	R	R	S
Sa2	S	S	R	R	R	I	R	R	R	S
Sa3	S	S	R	R	S	R	I	R	R	I
Sa4	I	S	R	R	S	R	R	S	R	I
Sa8	S	S	R	R	S	R	R	I	R	S
Sa5	I	S	R	R	S	R	R	R	R	S
Sa1	R	I	R	R	S	R	R	R	R	I
Sa12	S	S	R	R	S	I	S	R	R	S
Sa11	S	S	R	R	S	R	I	R	R	S
Sa7	I	S	R	R	S	R	R	R	R	S
Sa9	S	S	R	R	S	R	R	I	R	I
Sa2	I	I	R	R	S	R	S	R	R	S
Sa20	S	S	R	R	S	R	R	S	R	R
Pa2	S	S	R	R	S	I	S	R	R	I
Pa16	R	R	R	R	R	R	R	R	R	R
Pa7	S	S	R	R	S	I	S	R	R	S
Pa10	S	S	R	R	S	R	R	I	R	S
St5	I	S	R	R	I	R	R	I	R	S
St14	S	S	R	R	S	S	S	R	R	R
St12	S	S	R	I	S	R	R	R	R	R
St4	S	S	R	R	S	R	I	R	R	R
St14	I	I	R	R	S	S	S	S	R	R
Ba19a	S	S	R	R	S	R	R	S	R	I
Ba7	S	S	R	R	S	R	R	R	R	S
Ba19b	I	S	R	R	S	R	R	R	R	S
Ba3a	I	S	R	I	R	R	R	S	R	R
Ba4	I	I	R	R	S	R	S	S	I	S
Ba6	I	I	R	R	S	R	R	S	R	R
Ba2	I	I	R	R	R	I	I	I	R	S
Ba3b	S	S	R	R	S	R	R	R	R	S
S3a	I	S	R	R	S	S	S	S	R	S
S3b	R	I	R	R	S	R	R	R	R	R
S8	S	I	R	R	S	S	S	R	R	S
P1	I	I	R	R	S	S	S	S	R	R
P17	S	I	R	R	S	R	R	R	R	I
P2	S	S	R	R	S	R	R	R	R	R
P6	S	S	R	R	R	R	I	S	R	I
Y5	I	S	R	R	S	R	S	R	R	R
Y14	I	I	R	R	S	R	R	R	R	I
C13	I	S	R	R	S	R	S	R	R	I

Key: S = Susceptible, **I** = Intermediate, **R** = Resistance. Table 3 above shows the antibiogram, of all the bacterial strains isolated from the Herbal products - a representation of the bacteria that are Susceptible, Intermediate or Resistant to the different antibiotics using the NCCLS breakpoints[17].

Table 4: Antibiotic Susceptibility Profile of the Organisms Isolated from the Herbal Anti- infectives

Drugs and Strength (µg)			OFX-5	CIP-5	SXT-1.25/ 23.75	AMC-20/10	GN-10	CTX-30	CAZ-30	TE-30	AMP-10	CRO-30
			N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)
BACTERIA ISOLATES	*E. coli*	**S**	8 (80)	10 (100)	0 (0)	0 (0)	9 (90)	0 (0)	3 (30)	1 (10)	0 (0)	5 (50)
		I	1 (10)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2 (20)
		R	1 (10)	0 (0)	10 (100)	10 (100)	1 (10)	10 (100)	7 (70)	9 (90)	10 (100)	3(30)
	P. aeruginosa	**S**	3 (75)	3 (75)	0 (0)	0 (0)	3 (75)	0 (0)	2 (50)	0 (0)	0 (0)	2(50)
		I	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	3 (75)	0 (0)	0 (0)	0 (0)	1 (25)
		R	1 (25)	1 (25)	4 (100)	4 (100)	1 (25)	1 (25)	2 (50)	4 (100)	4 (100)	1(25)
	Staphylococcus spp	**S**	8 (67)	10 (83)	0 (0)	0 (0)	10 (83)	0(0)	2 (17)	2 (17)	0 (0)	7(58)
		I	4 (33)	2 (17)	0 (0)	0 (0)	0 (0)	2 (17)	1 (8)	2 (17)	0 (0)	4 (33)
		R	0 (0)	0 (0)	12 (100)	12 (100)	2 (17)	10 (83)	9 (75)	8 (67)	12 (100)	1(8)
	Salmonella spp	**S**	1 (33)	1 (33)	0 (0)	0 (0)	3 (100)	2(67)	2 (67)	1 (33)	0 (0)	1(33)
		I	1 (33)	2 (67)	0 (0)	0 (0)	0 (0)	0(0)	0 (0)	0 (0)	0 (0)	1(33)
		R	1 (33)	0 (0)	3 (100)	3 (100)	0 (0)	1 (33)	1 (33)	2 (67)	3 (100)	1(33)
	Streptococcus spp	**S**	3 (60)	4 (80)	0 (0)	0 (0)	4 (80)	2 (40)	2 (40)	1 (20)	0 (0)	1(20)
		I	1 (20)	1 (20)	0 (0)	1 (20)	1 (20)	0 (0)	1 (20)	1(20)	0 (0)	0(0)
		R	1 (20)	0 (0)	5 (100)	4 (80)	0 (0)	3 (60)	2 (40)	3 (60)	5 (100)	4 (80)
	Bacillus spp.	**S**	3 (38)	5 (63)	0 (0)	0(0)	6 (75)	0 (0)	1 (13)	4 (50)	0 (0)	5(63)
		I	5 (63)	3 (38)	0 (0)	1(13)	0 (0)	1 (13)	1 (13)	1 (13)	1 (13)	1(13)
		R	0 (0)	0 (0)	8 (100)	7(88)	2 (25)	7 (88)	6 (75)	3 (38)	7 (88)	2(25)
	Proteus spp	**S**	2 (50)	1 (25)	0 (0)	0 (0)	2 (50)	1 (25)	2 (50)	2 (50)	0 (0)	1 (25)
		I	2 (50)	3 (75)	0 (0)	0 (0)	0 (0)	0 (0)	1 (25)	0 (0)	0 (0)	2 (50)
		R	0 (0)	0 (0)	4 (100)	4 (100)	2 (50)	3 (75)	1 (25)	2 (50)	4 (100)	1(25)
	Yersinia spp	**S**	0 (0)	1 (50)	0 (0)	0 (0)	2 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
		I	2 (100)	1 (50)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (50)
		R	0 (0)	0 (0)	2 (100)	1 (100)	0 (0)	2 (100)	1 (50)	2 (100)	2 (100)	1 (50)
	C. diphtheria	**S**	0 (0)	1 (100)	0 (0)	0 (0)	1 (100)	0 (0)	1 (100)	0 (0)	0 (0)	0 (0)
		I	1 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1(100)

R	0 (0)	0 (0)	1 (100)	1 (100)	0 (0)	1 (100)	0 (0)	1 (100)	1 (100)	0 (0)

Key: S = Sensitive, **I** = intermediate, **R** - Resistance, **N** = number of organisms, OFX= Ofloxacin, CIP = Ciprofloxacin, CAZ = Ceftazidime, TE = Tetracycline, AMP = Ampicillin, SXT = Trimethoprim-Sulfamethoxazole, GN = Gentamicin, CTX = Cefotaxime, CRO = Ceftriaxone, AMC = Amoxycillin-Clavulanic Acid.

Table 6: Antibiogram of Fungi Isolated From The Herbal Anti-Infectives,.

Samples Code	**Isolates**	**Inhibition Zone Diameter (IZD) In Millimeter (mm)**				
		Griseofulvin	Nystatin	Ketoconazole	Clotrimazole	Fluconazole
1	*Microsporuim spp.*	0	25	0	0	0
	Aspergillus spp	0	32	10	12	0
	Nigrospora spp	0	30	20	14	12
2	*Candida tropicalis*	0	26	23	7	15
	Microsporum canis	0	25	0	0	0
3	*Candida albicans*	0	22	12	10	7
	Candida tropicalis	0	23	11	10	8
	Trichosporon spp	7	30	32	18	8
4	*Coccidioides immitis*	0	30	0	0	0
	Microsporuim audounii	0	29	0	0	0
6	*C.topicalis*	0	27	12	11	8
	Microsporuim audounii	0	8	0	0	0
	Aspergillus niger	0	29	0	7	0
7	*Blastomyces*	0	32	17	18	0
	Microporuim canis	0	30	0	0	0
8	*Blastomyces*	0	31	18	18	0
9	*Blastomyces spp*	0	29	16	19	0
	Cryptococcus neoformans	0	0	0	0	0
	Histoplasma	0	30	29	12	15
10	*Penicillum spp*	0	31	15	7	0
	Aspergillus spp	0	23	0	12	0
11	*Candida spp*	0	22	12	10	7
12	*Candida albicans*	0	20	14	12	9
	Blastomyces	0	30	15	19	0
13	*Aspergillus spp*	0	32	17	8	0
	Nigrospora spp	0	28	14	15	0
	Candida tropicalis	0	35	25	20	0
14	*Candida albicans*	0	22	13	10	10
	Aspergillus spp	0	26	0	7	0
15	*Microsporum spp*	0	25	0	0	0
	Coccidioides spp	0	30	0	0	0
	Aspergillus spp.	0	31	10	0	0
16	*C.albicans*	0	24	23	8	0
	Mucor spp	0	26	0	0	0
17	*Aspergillus spp*	0	31	10	0	0
18	*Mucor spp*	0	25	0	0	0
19	*Yeast/Blastomyces*	0	28	18	16	0
	Aspergillus spp	0	29	0	9	0
	Microsporum spp	0	0	0	0	0
20	*Aspergillus spp*	0	36	0	0	0

Table 7: Multiple Antibiotics Resistance Index (MARI) of the Isolated Bacteria.

Grouping	**Isolates and Samples Code**	**Multiple Antibiotics Resistance Index (MARI)**
Group A	Pa16	1
Group B	S3b	0.8
Group C	E4,11,14,15,Sa1,P2	0.7
Group D	E7,15,16,13,18,Sa2,Sa5,Sa7,Sa20,St12,St4,Ba7,Ba19b,Ba3a,Ba6,Ba3b,P17,Y5	0.6

Group E	E2,6,Sa3,Sa4,Sa8,Sa11,Sa9,Sa2,Pa10,St5,St14,Ba9a,P6,Y14,C13	0.5
Group F	Sa12,Pa2,Pa7,St14,Ba2,S8,P1	0.4

3.2 DISCUSSION

Antimicrobial Susceptibility testing of the isolated microorganisms was carried out to evaluate the activity of conventional antibiotics against the isolated bacteria and fungi strains. The bacteria contaminants isolated from these herbal preparations showed wide resistance to penicillins, especially ampicillin, augmentin (amoxycillin-clavulanic acid combination) and cloxacillin, suggesting that they could be producers of penicillinases. The resistance to trimethoprim-sulphamethoxazole (Co-trimoxazole) by all the isolates especially the Gram-negative isolates calls for attention. The findings of this study agree with an earlier work [12]. Staphylococci strains were the most frequently isolated bacteria species and it probably originated from handlers, as its habitat is human skin. *Staphylocococcus* showed wide resistance to penicillins suggesting possibly that they are producers of penicillinases. Resistance to trimethoprim by *S. aureus* and *S. epidermidis* has been reported with increasing frequency [18,19, 20]. It seems probable that *S. epidermidis* serves as a reservoir for resistance, which can be transferred to *S. aureus*. Also, inter-generic transfer of resistance among different genera of Gram-positive cocci and between Bacillus species and Staphylococci and Streptococci has been reported [20, 21]. *Escherichia coli* were the second most frequently isolated species in these medications which is an intestinal bacterium and an indicator of faecal contaminant. Presence of *Escherichia coli* in the sample indicates poor hygiene practices and lack of adequate handling of the products. According to the European pharmacopoeia 2007 [22], no *Salmonella spp or Escherichia coli* strain should be present in oral medicines. The presence of *E. coli* in herbal drugs had been reported by another researcher [23]. The *Escherichia coli* isolates showed a wide resistance to ampicillin, ceftazidime, sulphamethoxazole-trimethoprim, amoxycillin-clavulanic acid, and tetracycline. *Bacillus spp*. were the third most frequently found in these herbal medicaments because they are widely distributed in the soil, dust, air and because they are resistant to environmental destructive factors [20, 24]. A number of reports have described serious human infections caused by members of the genus Bacillus even though they have been regarded as non-pathogenic [25,26,27]. All the strains of *Pseudomonas* isolated were resistant to β-lactam antibiotics; Inducible β-lactamase activity is a general property of *Pseudomonas cepacia* [28]. Gram negative rods usually have wide resistance against antimicrobial agents [20] (Esimone *et al*., 2007a). *Streptococcus spp* showed high resistance to sulphamethoxazole-trimethoprim and ampicillin. *Salmonella spp*, were resistant to sulphamethoxazole-trimethoprim, amoxycillin clavulanic acid, and ampicillin. *Proteus spp*, *Yersinia spp* and *Corynebacteruim diphtheria* showed wide resistance to sulphamethoxazole-trimethoprim, amoxycillin clavulanic acid, ceftazidime and ampicillin (Table 3). On the other hand, the bacterial isolates were susceptible to some groups of the antibiotics (ofloxacin, ciprofloxacin, gentamicin and ceftriaxone).

Multiple Antibiotic Resistance Index (MARI) evaluation (Table 7) revealed that species of *Escherichia coli* showed high level of multiple antibiotic resistances to the panel of antibiotics used in this study. The MARI value ranged from 0-5 -0.7, with three (30%) resistant to seven antibiotic out of the ten used, six (60%) resistant to six of the antibiotics used and two (20%) resistant to five. *Staphylococcus spp* have MARI values ranging from 0.4-0.7, with one (8.3%) resistance to seven antibiotic, four (33.3%) resistant to six antibiotics, six (50%) resistant to five antibiotics and one (8.3%) resistance to four antibiotics. The MARI result of *Bacillus spp* ranged from 0.4 – 0.6, with five (62.5%) resistant to six antibiotics, One (12.5%) being resistance to five, four and three antibiotics each. *Proteus spp* MARI value is from 0.4 - 0.7, with one (25%) each of the four isolates resistant to seven, six, five and four respectively. *Pseudomonas spp* had MARI value ranging from 0.4 - 1.0, one (25%) showed high resistance index, being resistance to ten of the antibiotics used in this study, one (25%) resistant to five antibiotics and two (50%) resistance to four antibiotics. The three species of *Streptococcus* isolated showed MARI values from 0.3-0.8, that is, one (33.3%) resistant to eight antibiotics, one (33.3%) to four antibiotics and one (33.3%) to three out of the ten antibiotics. We had two isolates of *Yersinia spp* and the MARI values are 0.5 and 0.6, which is one (50%) resistance to five and one (50%) resistance six antibiotics. Lastly, *Corynebacteruin diphtheria* isolate is resistance to five antibiotics out of the ten antibiotics used in this study. This MAR index also recommended that all isolates, somehow, originated from the environment where antibiotics were over used [29].

Fungal infections are becoming an increasing cause of morbidity and mortality especially among immunocompromised patients. With the increased incidence of systemic fungal infections and the growing number of antifungal agents, laboratory methods to guide and select antifungal therapy have gained greater attention. However, determining antifungal susceptibilities of filamentous fungi is fraught with difficulties associated with slow growth of filamentous forms and subjectivity of interpreting visual endpoints [30]. In the present study, antifungal susceptibility testing of 40 fungi isolates was observed against five common antifungal agents (Griseofulvin, Nystatin, Ketoconazole, Clotrimazole, and Fluconazole) using disc diffusion method, presence of inhibition zone was considered as sensitive while absence of inhibition zone was recorded as resistance. The fungi isolates were very sensitive to Nystatin, Ketoconazole and Clotrimazole. Resistance to Fluconazole was observed among the isolates and high resistance recorded against Griseofulvin (Table 6).

The importance of surveying resistant environmental strains is that under favourable situations, they may transfer their resistance plasmids to pathogens [31,32]. If such organisms are present in medicaments, such as herbal medicinal products they could behave as opportunist pathogens and initiate an infection, particularly in immuno-compromised patients as well as lead to transfer of antibiotic resistance traits to hitherto sensitive microorganisms co-habiting within the consumers of those products. Given the increasing rate of development of resistant bacteria strains, our challenge is to slow the rate at which resistance develops and spreads. In order to decrease the spread of resistance among antibiotics, physicians, pharmacists, researchers and consumers alike need to be more aware of the selective pressures driving these bacteria to decrease their susceptibility [33]. These selective pressures include the abuse, overuse and misuse of antimicrobials in therapy, improperly manufactured and mishandled HMPs [13, 34] as well as other numerous socioeconomic factors that govern the development of multi-drug resistant bacteria strains [35].

4. CONCLUSION:

The high rate of resistance to antimicrobial agents of strains isolated from these herbal preparations may indicate a widespread antibiotic resistance among microorganisms from different sources. It is therefore mandatory that herbal medicines should not be taken indiscriminately and that current good manufacturing practices (cGMPs) must be observed by these herbal practitioners in the production of the medicines.

Authors' Contributions:

Oli Angus N - drafting of the manuscript/Corresponding author, Esimone Charles O – conceptualized and designed the work as well as revising the manuscript critically for important intellectual content, Ujam Nonye T – carried out the experiments and did analysis and interpretation of data, Adikwu Michael Umale - revised the manuscript critically for important intellectual content, Ikegbunam MN- carried out some part of the experimental work

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