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Full Length Research Paper

Susceptibility-resistance profile of micro-organisms isolated from herbal medicine products sold in Nigeria

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In order to evaluate the susceptibility and resistance pattern of bacteria and fungal isolates obtained from herbal medicine products (HMPs) marketed in Nigeria to conventional antibiotics, a total of seventy-five (75) bacteria and fifty-two (52) fungi isolated from the HMPs were screened for susceptibility to conventional antibiotics using the disc diffusion method. Most of the bacteria isolates were sensitive to the fluoroquinolones (ciprofloxacin, 85.3%, norfloxacin 93.3%) and the aminoglycosides (streptomycin 90%, gentamycin 89.3%). However, the isolates demonstrated significant resistance to common antibiotics like penicillins (augmentin [amoxycillin-cavulanic acid combination] 80%, cloxacillin 88.3%, ampicillin 56%), cephalosporins (rocephine [ceftriaxone] 65%, ceporex [cephalexin] 80%, cefuroxime 100%), chloramphenicol (66.7%), nitrofurantoin (100%) and cotrimoxazole (93.3%). Most of the fungal isolates were resistant to griseofulvin (67.3%) but susceptible to nystatin (73.1%), ketoconazole (98.1%), tioconazole (100%), clotrimazole (78.9%) and miconazole (88.5%). A significant proportion of bacteria and fungi isolated from these HMPs demonstrated resistance to conventional antibiotics. The present study therefore reveals that HMPs may represent novel routes of spread of antibiotic-resistant genes especially in developing countries. Efforts should therefore be geared at standardizing the quality of HMPs via strict adherence to Good Manufacturing Practice (GMP).

Key words: Susceptibility, antibiotic resistance, herbal medicine products.

INTRODUCTION

Herbal Medicine products (HMPs) are becoming increasingly popular (Fisher and Ward, 1994; Brevoort, 1998; Eisenberg et al., 1998). An estimated 80% of the world's population still depends on traditional herbal medicines for their health security (Carter, 2001). In most African countries including Nigeria, herbal medicine is recognized as an important component of health care system, especially among rural dwellers that constitute about 70% of the population (Esimone et al., 2002). Also, the ever increasing cost of orthodox health care services coupled with the side effects of certain synthetic drug therapies, has further caused a large proportion of patients in the developing countries to resort to alternative herbal health care which they feel is natural, safer, more accessible, more economical and takes into consideration the people's socio-cultural values (Nwaogu, 1997; Carter, 2001).

Although the World Health Organisation (WHO) has advocated for the integration of HMP into the primary health care system of developing countries (WHO, 1978, 1989), safety issues related to herbal drugs continue to be ignored by the herbalist whose methods of concocting herbal preparations for the public are usually unhygienic with the attendant microbiological hazards (Tella, 1977). Accordingly, gross microbial contamination of herbal medicinal products commonly consumed in Nigeria has been severally demonstrated (Onawunmi and Lamikanra, 1987; Lamikanra et al., 1992; Esimone et al., 2002; Esimone et al., 2003) On the one hand, such grossly

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contaminated HMPs may serve as potential sources of transmission of pathogenic spoilage organisms from product to consumers (Grigo, 1976; Mendie et al., 1993). On the other hand, presence of antibiotic resistant microbial isolates in the HMPs could lead to transfer of antibiotic resistance traits to hitherto sensitive gut or oral microflora of consumers.

The present study attempts to evaluate the potential health hazards associated with the consumption of herbal medicinal products vis-a-vis the susceptibility-resistance profile of microorganisms isolated from such products.

MATERIALS AND METHODS

Herbal samples

A total of twenty-six herbal samples were used in this study. Sixteen (16) of these samples were in solid dosage forms, while nine (9) were liquid and one (1) leaf herbal tea. They were purchased at random from different herbalists in Edo State, Nigeria.

Isolation and identification of microbial contaminants in the herbal preparations

A loopful of each of the liquid sample was streaked on nutrient agar, cetrimide agar, mannitol salt agar, MacConkey agar, Kligler iron agar and Sabouraud dextrose agar. Isolated organisms were identified by their morphological, physiological and biochemical characteristics (Cowan and Steel, 1974; Leanor and Carey, 1978). Solid samples were first agitated in sterile distilled water before streaking loopfuls into the culture media above.

Antibiotic susceptibility testing

Susceptibility tests were performed following the M2-A6 disc diffusion method recommended by the National Committee for Clinical Laboratory Standards (NCCLS, 1997) using nutrient agar and Sabouraud dextrose agar. The bacteria strains were tested against the following discs: nitrofurantoin (N), 100 µg; cefuroxime (CF), 20 µg; norbactin [norfloxacin] (NB), 10 µg; cotrimoxazole (CO), 50 µg; gentamycine (GN), 10 µg; tetracycline (TE), 50 µg; ciprofloxacin (CIP), 5 µg; nalidixic acid (NA), 30 µg; chloramphenicol (C), 10 µg; and ampiclin (AM), 25 µg (polytes® Nigeria); augmentin (AG), 30 µg; cloxacillin (CXL), 5 µg; septrin [a brand of cotrimoxazole by Welcome, Nigeria] (SXT), 25 µg; rocephine [ceftriaxone] (ROC), 30 µg; erythromycin (E), 5 µg; ampicillin (PN), 10 µg; streptomycin (S), 10 µg; gentamycin (CN), 10 µg; claforan (CTX), 30 µg; and ciproxin [a brand of ciprofloxacin by Bayer, Nigeria] (CPX), 10 µg (Jireh(R), Nigeria); tarivid [ofloxacin] (OFX), 10 µg; peflacine [pefloxacin] (PEF), 10 µg; ciprofloxacin (CPX), 10 µg; augmentin (AU), 30 µg; gentamycin (GN), 10 µg; streptomycin (S), 30 µg; ceporex (CEP), 10 µg; nalidixic acid (NA), 30 µg; septrin (SXT), 30 µg and ampicillin (PN), 30 µg (Optun,® Nigeria). The fungal strains were tested against the following discs: nystatin (N), 20 µg; clotrimazole (C), 20 µg; griseofulvin (G), 20 µg; ketoconazole (K), 20 µg; tioconazole (T), 20 µg; and miconazole (M), 20 µg. The plates were incubated inverted at 37°C for 24 h (antibacterial evaluation) and 28°C for 2 - 5 days (antifungal evaluation) and the corresponding inhibition zone Diameters (IZD) that developed were measured and recorded.

RESULTS

A total of 127 strains (75 bacterial and 52 fungal strains) were isolated from the herbal preparations (Table 1). Eleven (11) samples (43.3%) contained pathogenic micro-organisms or faecal indicators such as Escherichia coli, Staphylococcus aureus and Pseudomonas aeruginosa. The organism most commonly isolated from the herbal medicines was Bacillus (28.4%), Torulopsis (9.5%), Staphylococcus (8.7%), Aspergillus (7.1%) and Penicillium (7.9%).

Tables 2 and 3 show the antibiogram summary of all the strains isolated. The bacterial isolates were very sensitive to the fluoroquinolones (ciprofloxacin, norfloxacin) and aminoglycosides (streptomycin, gentamycin). Also susceptibility was recorded against tetracycline, septrin and erythromycin.

It is observed that Bacillus strains had 86.1% resistance to claforan, 80.6% resistance to tarivid (a brand of ofloxacin) and 8.3% resistance to gentamycin. Bacillus subtilis presented the highest number of strains resistant to all penicillins, cephalosporins and septrin (a brand of cotrimoxazole). Staphylococcus spp. demonstrated a high percentage of resistance to ampicillin (54.6%), augmentin® (81.8%), cloxacillin (90.9%), ceporex (90.9%) and erythromycin (72.7%). All the gram-negative rod strains were resistant to β–lactam antibiotics and nitrofurantoin. All Pseudomonas aeruginosa strains were resistant to chloramphenicol. Escherichia coli strains were resistant to cotrimoxazole, nalidixic acid, nitrofurantoin and ampicillin.

The fungi isolates were very sensitive to tioconazole (100%) and ketoconazole (98.1%), while the least susceptibility was shown towards griseofulvin (32.7%). Susceptibility was also recorded against miconazole (88.5%), clotrimazole (78.9%) and nystatin (73.1%). The results of the inhibition zone diameters (IZD) of the antibiotics against 50, 90 and 95% range of the organisms (IZD_{50}, IZD_{90} and IZD_{95} respectively) is presented in Table 4.

DISCUSSION

The 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. Similar resistance to cephalosporins especially cefuroxamine and rocephine® (ceftriaxone) was also observed. More worrisome is the resistance to trimethoprim–sulphamethoxazole (cotrimoxazole) observed especially against the gram negative isolates. Bacillus spp. were the most frequently found in these medicaments because they are widely distributed in the soil, dust and air because they are resistant to

Table 1. Microbial contaminants of the herbal preparations.

Sample code	Nature of sample /therapeutic claims (herbal usage)	Identity of bacteria isolated	Identity of fungi isolated
1	Liquid: Antityphoid fever	a) Corynebacterium xerosis b) Bacillus subtilis c) Micrococcus luetus	a) Hansenula anomala b) Torulopsis glabrata
2.	Liquid: Effective against arthritis,rheumatism, gout, muscular pains and anaemia.	a) Corynebacterium pseudodiphtheriticum	a) Trichosporon cutaneum b) Torulopsis glabrata
3.	Liquid: Anti malaria	a) Streptococcus faecalis b) Bacillus pumilus c) Micrococcus luteus d) Bacillus subtilis	a) Candida albicans b) Penicillium spp.
4.	Liquid: Anti malaria fever	a) Klebsiella pneumoniae b) Staphylococcus saprophyticus c) Bacillus polymyxa d) Bacillus megaterium e) Bacillus subtilis	a) Candida albicans b) Penicillium spp
5.	Liquid: Anti asthma	a) Serratia marcescens	a) Mucor spp
6.	Liquid: Effective against general fever	a) Staphylococcus saprophyticus b) Lactobacillus casei	a) Hansenula anomala
7.	Liquid: Effective against difficulty in urinating and stooling	a) Bacillus subtilis b) Bacillus cereus c) Citrobacter intermidius d) Listeria murrayi e) Listeria grayi f) Klebsiella pneumoniae	a) Aspergillus niger b) Mucor spp c) Torulopsis glabrata d) Actinomadura madurae
8.	Liquid: Effective against all forms of illnesses	a) Bacillus subtilis	a) Madurella mycetomatis b)Penicillium spp c)Aspergillus fumigatus
9.	Liquid: Effective against lack of sleep (Insomnia)	a)Proteus vulgaris b)Klebsiella pneumoniae c)Bacillus subtilis d)Bacillus megaterium	a)Aspergillus flavus b)Aspergillus oryzae c)Penicillium spp
10.	Dry leaves (herbal tea) Effective against hypertension, insomnia, rheumatism, malaria and general fever.	a)Bacillus subtilis b)Klebsiella pneumoniae	a)Aspergillus niger b)Penicillium spp
11.	Solid: Anti malaria	a)Bacillus pumilus b)Listeria murrayi c)Escherichia coli	a)Torulopsis candida b)Hansenula anomala c)Penicillium spp
12	Solid: Effective against food poisoning, constipation, intestinal disorder and general health.	a)Micrococcus luteus b)Bacillus polymyxa	a)Rhodotorula glutinis b)Aspergillus fumigatus

Table 1. Contd.

		c)Bacillus megaterium d)Bacillus pumilus e)Proteus vulgaris	c)Penicillium spp
13.	Solid: Effective against all kinds of intestinal disorders	a)Bacillus subtilis b)Bacillus megaterium c)Micrococcus luteus d)Acinetobacter calcoaceticus	a)Saccharomyces cerevisiae
14.	Solid: Effective against hypertension	a)Bacillus megaterium b)Staphylococcus aureus c)Listeria grayi	a)Candida tropicalis
15.	Solid: Effective against sexually transmitted diseases (STD)	a)Bacillus polymyxa b)Staphylococcus aureus	a)Torulopsis candida
16.	Solid: Anti diabetes	a)Bacillus pumilus b)Bacillus polymyxa	a)Candida albicans b)Hansenula anomala
17.	Solid: Anti typhoid fever	a)Bacillus polymyxa b)Bacillus subtilis	a)Candida albicans b)Candida pseudotropicalis
18.	Solid: Purgative	a)Staphylococcus aureus	a)Penicillium spp
19.	Solid: Anti malaria	a)Bacillus polymyxa b)Escherichia coli	a)Torulopsis candida b)Trichosporon cutaneum
20.	Solid: Effective against stomach problems	a)Staphylococcus aureus b)Staphylococcus epidermidis c)Bacillus subtilis d)Bacillus polymyxa	a)Aspergillus flavus b)Penicillium spp c)Aspergillus niger
21.	Solid: Effective against enlarged spleen	a)Klebsiella pneumoniae b)Bacillus pumilus c)Bacillus megaterium	a)Saccharomyces cerevisiae b)Torulopsis glabrata c)Torulopsis candida
22.	Solid: Anti rheumatism	a)Staphylococcus aureus b)Pseudomonas aeruginosa	a)Rhodotorula glutinis b)Aspergillus niger
23.	Solid: Effective against appendicitis	a)Pseudomonas aeruginosa b)Bacillus subtilis c)Listeria murrayi	a)Torulopsis candida b)Toruplosis glabrata
24.	Solid: Effective against diaphragm problems	a)Staphylococcus aureus b)Bacillus subtilis	a)Candida albicans b)Torulopsis glabrata
25.	Solid: Effective against alcoholism	a)Bacillus subtilis b)Bacillus polymyxa c)Staphylococcus aureus	a)Torulopsis candida
26.	Solid: Anti dysentery and diarrhoea	a)Staphylococcus aureus b)Bacillus pumilus c)Klebsiella pneumoniae d)Bacillus subtilis e)Bacillus cereus	a)Penicillium spp

Table 2a. Antibiogram summary for bacteria isolated from HMPs. Inhibition zone diameter (izd) values in millimeter (mm).

Isolate No	AG	CXL	SXT	CRO	PN	S	CN	CTX	E	CPX
1a	0	0	30	0	18	14	22	0	22	23
1b	0	0	0	0	0	22	19	0	20	23
1c	16	0	0	0	12	0	0	0	0	0
3a	0	0	0	0	0	20	18	0	0	16
3b	0	0	0	0	30	13	22	0	21	25
3d	0	0	27	0	23	26	26	0	22	28
4b	0	0	0	0	16	24	24	18	20	22
4e	0	0	0	0	0	20	19	0	18	30
6a	0	0	0	0	0	0	17	0	19	0
6b	0	0	0	0	12	15	23	21	22	28
7b	0	0	30	20	19	14	21	0	23	21
7d	0	0	0	0	0	19	22	0	20	23
7e	0	0	23	16	27	14	18	0	18	32
9d	13	0	20	24	0	18	14	25	18	22
11a	19	0	24	21	0	18	22	0	21	25
11b	0	0	0	0	0	13	16	0	18	28
12a	0	14	17	13	16	0	14	15	14	14
12d	0	0	0	0	0	18	22	0	19	0
13b	0	17	22	20	22	20	25	14	17	14
13c	0	0	31	0	15	0	23	0	29	24
14a	0	0	0	18	22	21	18	0	15	28
14b	0	0	20	21	0	16	0	0	0	16
14c	0	19	12	23	18	17	0	0	0	20
15a	0	0	20	22	20	20	21	0	0	25
16a	0	0	0	0	0	20	15	0	0	0
16b	0	0	22	21	18	17	14	18	0	26
17a	0	0	21	18	12	20	20	15	0	16
17b	0	0	27	0	23	26	26	0	22	24
18	0	0	21	16	0	19	18	17	0	20
19a	0	0	0	21	0	16	22	0	16	18
20a	0	0	0	0	0	16	23	0	0	0
20c	0	0	0	0	0	14	19	0	28	23
20d	0	0	0	0	0	23	18	0	25	23
21b	0	14	28	16	15	23	21	0	20	20
21c	0	0	0	0	13	19	18	0	0	23
22a	0	0	0	16	0	17	12	0	0	16
23b	0	0	0	0	0	16	31	0	20	33
23c	0	0	0	0	0	16	31	0	20	33
24b	0	0	0	0	0	18	19	0	0	18
25a	0	0	0	0	0	16	13	0	0	0
25b	0	0	0	0	0	0	20	0	18	15
26a	0	18	22	12	12	14	22	12	20	18
26d	0	15	27	15	20	23	25	0	0	21
26e	0	15	30	20	19	14	20	0	17	21

Gentamycin (GN), augmentin (AG), cloxacillin (CXL), septrin (SXT), chloramphenicol (CRO), erythromycin (E), penicillin G (PN), streptomycin (S), claforan (CTX), and ciproxin (CPX).
Source of disk: Jireh® Laboratories (Nig.) lot no. 9903.

Table 2b. Antibiogram summary for bacteria isolated from HMPs. Inhibition zone diameter (izd) values in millimeter (mm).

Isolate No.	OFX	PEF	CPX	AU	CN	S	CEP	NA	SXT	PN
2a	30	34	20	14	19	20	25	16	20	19
3c	0	14	10	16	0	0	14	0	14	14
4c	29	22	15	17	15	19	18	11	17	15
7a	0	26	0	0	0	14	22	0	21	0
8	20	18	20	15	0	21	18	18	17	18
9c	0	27	0	0	25	16	26	0	26	0
10a	24	20	24	12	0	28	26	26	24	14
12b	24	21	25	13	19	24	23	19	11	16
12c	33	32	18	12	18	13	17	10	10	10
13a	14	17	19	0	17	14	16	15	17	0
15b	20	26	23	0	24	19	0	15	22	16
20b	30	29	18	12	22	14	26	18	24	15
24a	18	20	30	12	17	18	0	12	14	13
25c	18	18	20	0	14	0	0	0	0	0
26b	19	22	16	6	12	20	19	16	10	0

Gentamycin (GN), nalidixic acid (NA), augmentin (AG), septrin (SXT), penicillin G (PN), streptomycin (S), ciproxin (CPX), tarivid [ofloxacin] (OFX) and peflacine [pefloxacin] (PEF).
Source of disk: Optun® Laboratories Nigeria Limited.

Table 2c. Antibiogram summary for bacteria isolated from HMPs. Inhibition zone diameter (izd) values in millimeter (mm).

Isolate No.	N	CF	NB	CO	GN	TE	CIP	NA	C	AM
4a	0	0	26	0	0	37	0	0	26	0
5	0	0	18	0	22	19	27	0	0	0
7c	0	0	30	0	25	20	30	0	0	0
7f	0	0	20	0	0	17	28	0	13	0
9a	0	0	0	0	23	20	19	23	0	0
9b	0	0	22	40	12	19	24	0	12	0
10b	0	0	26	0	28	20	23	0	0	0
11c	0	0	18	0	18	20	0	0	12	0
12e	0	0	11	0	18	23	26	0	0	0
13d	0	0	19	0	21	0	0	7	18	0
19b	0	0	32	0	26	20	28	0	0	0
21a	0	0	14	0	17	14	28	0	0	0
22b	0	0	24	0	18	9	0	0	0	0
23a	0	0	24	0	11	10	30	0	0	0
26c	0	0	17	0	24	20	25	0	0	0

Nitrofurantoin (N), cefuroxime (CF), norbactin [norfloxacin] (NB), cotrimoxazole (CO), gentamycin (GN), tetracycline (TE), ciprofloxacin (CIP), nalidixic acid (NA); chloramphenicol (C) and ampicllin (AM).
Source of disk: Poly-Tes® Multo-Disks (Nig.) lot no. PS003.

environmental destructive factors (Devleeschouwer and Dony, 1979; Garcia-Arribas et al., 1986). 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 (Cotton et al., 1987; Sliman et al., 1987; Kramer and Gilbert, 1989).

The Staphylococcus strains 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 fre-

Table 3. Antibiogram summary for fungi isolated from HMPs. Inhibition zone diameter (izd) values in millimeter (mm).

Isolate	C	K	N	T	M	G
1a	27	28	30	28	28	-
1b	13	13	-	14	-	-
2a	-	27	-	15	20	-
2b	14	13	-	11	-	-
3a	8	21	14	25	21	-
3b	10	16	12	24	16	15
4a	12	23	12	19	22	-
4b	16	20	18	19	19	21
5	14	15	15	11	-	18
6a	11	21	18	29	14	-
7a	10	10	-	15	12	-
7b	-	18	20	24	22	-
7c	14	12	-	13	-	-
7d	-	20	18	16	14	20
8a	20	22	-	20	-	12
8b	-	15	11	22	25	23
8c	18	20	20	24	26	22
9a	19	21	11	12	15	12
9b	8	11	16	17	12	-
9c	-	18	19	23	21	17
10a	8	10	-	10	10	-
10b	19	-	14	17	13	11
11a	14	30	19	31	22	-
11b	23	28	28	23	22	-
11c	-	22	24	22	10	14
12a	-	22	19	22	11	-
12b	17	15	20	22	24	19
12c	14	15	-	21	22	9
13a	20	18	28	34	25	-
14a	18	20	14	25	21	-
15a	20	25	15	24	18	-
16a	15	20	13	21	23	-
16b	23	25	31	26	24	-
17a	15	30	19	28	20	-
17b	12	18	-	14	19	-
18a	24	17	18	20	17	12
19a	17	23	16	22	16	-
19b	-	20	21	20	16	-
20a	21	20	11	13	15	10
20b	17	18	-	21	27	18
20c	16	13	-	13	10	-
21a	18	18	26	30	27	-
21b	-	16	10	11	17	-
21c	14	16	20	21	16	-
22a	-	19	18	19	13	-
22b	12	10	-	19	15	
23a	15	20	18	17	12	-
23b	15	13	-	19	-	-
24a	16	20	15	17	20	-
24b	-	18	8	10	15	-

Table 3. Contd.

25a	18	19	21	19	15	-
26a	29	25	12	22	21	22

Nystatin (N), clotrimazole (C), griseofulvin (G), ketoconazole (K), tioconazole (T, and miconazole (M).

Table 4. Percentiles for sensitivity test results for the isolated microorganisms.

Antibiotic	N		Percentiles		
	Valid	Missing	50	90	95
Gram positive bacteria					
AG	63	0	0	12.60	15.80
CEP	63	0	0	18.60	25.80
CN	63	0	19.00	25.00	25.80
CPX_J	63	0	20.00	28.00	31.60
CPX_O	63	0	0	20.00	24.80
CRO	63	0	0	21.00	22.00
CTX	63	0	0	18.00	18.80
CXL	63	0	0	14.00	16.60
OFX	63	0	0	22.60	25.00
E	63	0	14.00	22.60	25.00
NA	62	1	0	16.00	18.85
PEF	63	0	0	22.00	28.60
PN_{10}	63	0	0	22.00	23.80
PN_{30}	63	0	0	15.00	17.60
S_{10}	63	0	16.00	23.00	24.00
S_{30}	63	0	0	19.00	20.80
SXT_{30}	62	1	0	17.00	23.70
SXT_{25}	63	0	0	27.00	30.00
Gram negative bacteria					
AM	15	0	0	0	0
C	15	0	0	21.20	0
CO	15	0	0	16.00	0
CF	15	0	0	0	0
CIP	15	0	25.00	30.00	0
GN	15	0	18.00	26.80	0
N	15	0	0	0	0
NA	15	0	0	13.40	0
NB	15	0	20.00	30.80	0
TE	15	0	20.00	28.60	0
Fungi					
Clotrimazole	52	11	14.00	22.40	25.05
Griseofulvin	52	11	0	19.70	22.00
Ketoconazole	52	11	19.00	26.40	28.70
Miconazole	52	11	16.50	25.00	27.00
Nystatin	52	11	15.00	23.10	28.70
Tioconazole	52	11	20.00	28.00	30.35

N = Nitrofurantoin, CEP = ceporex, CF = cefuroxime, NB = norbactin [norfloxacin], CO = cotrimoxazole, GN = gentamycin, TE = tetracycline, CIP = ciprofloxacin, NA = nalidixic acid, C = chloramphenicol, AM = ampicllin, AG = augmentin, CXL = cloxacillin, SXT30, 25 = septrin (30 µg or 25 µg), ROC = rocephine [ceftriaxone], E = erythromycin, PN30,10 = penicillin G (30 or 10 µg), S30,10 = streptomycin (30 µg or 10 µg), CTZ = claforan, CPXj or CPXo = ciproxin (Jireh or Optune disks), OFX = tarivid [ofloxacin] and PEF = peflacine [pefloxacin].

quency (Archer et al., 1986; Davies and Stone, 1986). It seems probable that S. epidermidis serves as a reservoir for resistance, which can be transferred to S. aureus. Also, intergeneric transfer of resistance among different genera of gram-positive cocci and between Bacillus species and Staphylococci and Streptococci has been reported on several occasions (Schaberg and Zerros, 1986). Studies of clinical strains of S. aureus have often reported multi- resistance. In this study, however, medicament strains were susceptible to some antimicrobial agents. Similar results have also been reported (Devleeschouwer and Dony, 1979). S. aureus can originate from handlers, as its habitat is human skin. Micrococcus roseus and M. luteus have also been found in liquid and solid drugs (Willense-Collinet et al., 1981; Garcia-Arribas et al., 1983). The number of strains of Streptococci found was very few. These results are in agreement with other authors that found a similar number of these bacteria in different Pharmaceuticals (Devleeschouwer and Dony, 1979; Garcia-Arribas et al., 1983). These gram-positive cocci are found mainly in raw materials, air and water and on human beings and like Bacillus spp., they can survive in the environment and thus contaminate the medicaments.

The high level of resistance to many antimicrobial agents shown by gram negative rods is well known. Serratia and Proteus spp. are of increasing importance, as resistance to newer β-lactams may be acquired by mutation in addition to plasmids (O'Brien and Acar, 1987). Acinetobacter spp. are also known to be very resistant to aminoglycosides (Devleeschouwer et al., 1980). This is most often due to the presence of plasmid-mediated modifying enzymes. All the strains of Pseudomonas isolated were resistant to β-lactam antibiotics. Inducible β-lactam activity is a general property of Pseudomonas cepacia (Prince et al., 1988). Resistance to chloramphenicol was similar to those of clinical strains in the study of Burns et al. (1989).

The number of bacterial and fungal strains capable of causing infections is increasing, and many of them are resistant to one or more of the antimicrobial agents used in therapy. This problem has snowballed to a serious public health concern with economic, social and political implications that are global in scope, and across all environmental and ethnic boundaries. The acquisition of resistance may be due to chromosomal mutations or plasmids that are often capable of transfer from one strain of organism to another, even across the species barrier. Furthermore, the resistance genes are found on mobilizable genetic elements called transposons (O'Brien and Acar, 1987). The ability of transposons to integrate into either conjugative plasmids or the organisms' chromosome enhances the transferability of a given resistant determinant. This process is a natural, unstoppable phenomenon exacerbated by the abuse, overuse and misuse of antimicrobials in the treatment of human illness and in animal husbandry, aquaculture and agriculture (O'Brien and Acar, 1987; Lexchin, 2000; Stohr, 2000). The importance of surveying resistant environmental strains is that under favourable situations, they may transfer their resistance plasmids to pathogens. The problem is especially serious in hospitals where the environment can be a factor in the selection of multiresistant strains (O'Brien and Acar, 1987, Prince et al., 1988; Bryan, 1989; Burns et al., 1989). If such organisms are present in medicaments, they could behave as opportunist pathogens and initiate an infection, particularly in immuno-compromised patients. Our challenge is to slow the rate at which resistance develops and spreads.

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.

REFERENCES

Archer GL, Laughter JP, Johnston JL (1986). Plasmid encoded Trimethoprim resistance in Staphylococci. Antimicrob. Agents Chemother. 29: 733-740.

Brevoort P (1998).The booming US Botanical Market. A new overview. Herbalgram 44: 33-36.

Bryan LE (1989). Microbes causing problems of antimicrobial resistance. In: Bryan LE, ed. Microbial Resistance to drugs, Berlin Springenverlag, pp. 421-440.

Burns JL, Hedin LA, Lieu DM (1989). Chloramphenicol resistance in Pseudomonas cepacia because of decreased permeability. Antimicrob. Agents Chemother. 33: 136-141.

Carter I (2001). Footsteps: A quarterly newsletter linking development workers around the world. Tearfund, England, No. 48, September 3.

Cotton DJ, Marshall DJ, Gress J, Thaler M, Pizzo PA (1987). Clinical features and therapeutic intervention in 17 cases of Bacillus bacteremia in an immuno-suppressed Patient Population. J. Clin. Microbiol. 25: 672-674.

Cowan ST, Steel KJ (1974). Manual for identification of medical bacteria: Vol. 2, 2nd Edn. London: Cambridge University press.

Davies AJ, Stone JW (1986). Current problems of Chemotherapy of infections with coagulase – negative Staphylococci. Eur. J. Clin. Microbiol. 5:277-281.

Devleeschouwer MJ, Dony J (1979). Microbial contamination of drugs. Ecology and antibiotic resistance. J. Pharm. Belg. 34: 189-203.

Devleeschouwer MJ, Akin A, Dony J (1980). Microbial contamination of drugs. 2. Ecology and antibiotic resistance. J. Pharm. Belg. 35: 266-272.

Eisenberg D, David RB, Ettner SL, Appel S, Van Rompay M, Kessler RC (1998). Trends in alternative medicine use in the United States, 1990 – 1997. JAMA 280: 1569-1575.

Esimone CO, Chah KF, Ikejide SC (2002). Microbiological quality of herbal preparations marketed in Southeast Nigeria. J. Nat. Remedies 2:42-48

Esimone CO, Oleghe PO, Ibezim EC (2003). Effect of preservative agents on the microbial stability of some indigenous herbal preparations. Niger. J. Pharm. 34: 37-42.

Fisher P, Ward A (1994). Complementary Medicine in Europe. Br. Med. J. 309: 107-111.

Garcia-Arribas ML, de la Rosa MC, Mosso MA (1983). Characterization and antibacterial sensitivity of strains of Gram –positive cocci isolated from orally administered solid drugs. J. Pharm. Belg. 38: 140-146.

Garcia- Arribas ML, de la Rosa MC, Mosso MA (1986). Characterization

of the strains of Bacillus isolated from orally administered solid drugs. Pharm. Acta Helv 61: 303-307.
Grigo J (1976). Microorganisms in drugs and cosmetics: Occurrence, harms and consequences in hygienic manufacturing. Zentbl. Bakt. Hyg. Abt.1 Orig. B. 162: 233-287.
Kramer JM, Gilbert RJ (1989) Bacillus cereus and other Bacillus species. In: Doyle MP, ed. Foodborne Bacterial pathogens. New York: Marcel Dekker, pp. 21-70.
Lamikanra A, Orafidiyi L, Adediji JA (1992). A study of the microbiological quality of 'Ogun efu'. Afr. J. Pharm. Pharm. Sci. 22: 222-228.
Leanor DH, Carey SC (1978). Laboratory methods in medical mycology. US Department of Health Education and Welfare, Public Health Service Center for Disease Control.
Lexchin J (2000). Promoting resistance? World Health Organization Essential Drug Monitor, Geneva.; Nos. 28 and 29, 11.
Mendie UE, Ifudu ND, Brown SA (1993). How safe are non-sterile liquid preparations? J. W. Afr. Pharm. 7: 8-11
NCCLS (1997). Performance Standards for Antimicrobial Disk Susceptibility Tests, 6th Edn, Approved Standards. Document M2-A6. Wayne, PA: NCCLS.
Nwaogu MA (1997). Insight into herbal medicine: An approach to a healthier life. Aba: Pan Aluminium Co. Ltd, pp. 1-85.
O'Brien TF, Acar JF (1987). Antibiotic resistance worldwide. In; Peterson PK, Verhoef J eds. Antimicrobial Agents Annual 2nd ed. Amsterdam Elsevier, pp. 457-470.
Onawunmi GO, Lamikanra A (1987). Microbial quality of locally produced herbal preparations. Niger. J. Pharm. Sci. 3: 56-63.
Prince A, Wood MS, Sooug G, Xingchin N (1988). Isolation and characterization of a penicillinase from Pseudomonas cepacia. Antimicrob. Agents Chemother. 32: 838-843.
Schaberg BR, Zerros MJ (1986). Intergeneric and interspecies gene exchange in Gram positive Cocci. Antimicrob. Agents Chemother. 30: 817-822.
Sliman R, Rehn S, Shlaces DM (1987). Serious infections caused by Bacillus species. Medicine 66: 218-223.
Stohr K. (2000). Problems from antimicrobial use in farming. World Health Organization: Essential Drugs Monitor, Geneva. Nos. 28 and 29: 10-11.
Tella A (1977) Traditional medicine: Safety and Efficacy. Niger. J. Pharm. 8: 89-90.
WHO (1978) WHO Technical Report Series No 622, WHO, Geneva.
WHO (1989) Document No. WHO/TRM 19.1, Geneva, pp. 7-9.
Willense-Collinet MF, Turnbull PCB, Hosperg GT, Van Oppenraay AB (1981). Computer assisted method for identification of Bacillus species isolated from liquid antacids. Appl. Environ. Microbiol. 41: 169-172.