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Research Article

Bacteriological profile and *in vitro* antibacterial activities of some liquid herbal preparations sold in Abia State, south-eastern Nigeria

Okechukwu G. Pipi [1], Emmanuel O. Nwankwo [b,*], Kelechi N. Onusiriuka [b]

[a] *Department of Zoology and Environmental Biology, Michael Okpara University of Agriculture, Abia State, Nigeria*
[b] *Department of Microbiology, Michael Okpara University of Agriculture, Abia State, Nigeria*

* **Corresponding author**: Department of Zoology and Environmental Biology, Michael Okpara University of Agriculture, P.M.B. 7267, Abia State, Nigeria; **Tel:** +234-802-3309146; **Email**: emmaonwubiko@yahoo.com

Background: The use of liquid herbal preparations in the treatment and management of human diseases has long been practiced before the advent of chemotherapy and is a fundamental component of the African traditional healthcare system.

Objectives: The objective of this study is to analyze the bacteriological profile and *in vitro* antibacterial activities of selected indigenous liquid herbal products sold in Abia State, Nigeria. METHODS: A total of 315 bacterial strains were isolated from 150 therapeutic liquid herbal preparations (LHPs) sold in different parts of Abia State. Pathogenic bacteria were isolated from these products; the isolates were evaluated for total aerobic plate count, Gram's reaction, biochemical reaction. Antibacterial activity was assessed using minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and inhibitory zone diameter (IZD).The MBC, MIC and invitro antibacterial activities of LHPs were carried out against 3 test clinical bacterial isolates; *Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa*, using agar well diffusion methods.

Results: The number of isolates obtained in this study were; 100 (31.4%), 84 (26.7%) 131 (41.6%) from Umuahia, Ohafia and Aba respectively. The genera isolated included *Salmonella, Bacillus, Escherichia, Klebsiella, Proteus, Staphylococcus, Streptococcus, Citrobacter* and *Pseudomonas*. Out of 150 LHPs processed, 20 showed no bacterial growth. Different concentrations of these LHPs were evaluated for their antibacterial activities. MIC for LHPs with antibacterial activities range from 6.25-100%. MBC ranged from 12.5-100%, while IZD had range of 6-20mm.

Conclusion: The study revealed that some tested liquid herbal preparations were grossly contaminated with bacteria while some had antibacterial activities.

Key words: Liquid herbal preparations, Antibacterial activity

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1. Introduction

Herbal medicines may be associated with a broad variety of microbial load and exert an important impact on overall quality of herbal products and preparations (Onyambu et al, 2013). Traditional herbalists in Nigeria use various herbal preparations to treat various types of ailments, including diarrhea, urinary tract infection, typhoid fever and skin diseases (Sofowora, 1993). Most of the herbal preparations are used in different forms and normally carry a large number of various kinds of microbial contaminants originating from soil and are normally adhered to leaves, stem, flowers, seeds and root of the herbs (Adeleye et al, 2005). Erich et al. (2001) studied 138 medicinal herbal drugs and reported association of several microbial pathogens

including enterohaemorrhagic *E. coli*, *Staphylococcus aureus*, *Candida albicans* and *Campylobacter*.

Higher humidity facilitates enhancement of microbial population during storage of herbal drugs and causes severe loss of medicinally important chemical components which are utilized as nutrient components by harbored heterogeneous microbial flora. Their count varies with storage period and climatic conditions even after best storing packages and conditions. Prasad et al. (2002) made a quantitative enumeration of microbial flora of certain herbal drugs and found that all the drug samples were highly contaminated. Herbal medicinal preparations, if unpreserved, readily become contaminated with adventitious microorganisms leading to spoilage (Tella, 1978). On one hand, such grossly contaminated herbal medicinal products may serve as potential sources of transmission of pathogenic organisms from products to consumers (Okunade A.O., 2001, Onyambu et al, 2013). In Abia State, however, even though, there is proliferation of herbal products in the market, not much has been done in this field. The use of contaminated herbal medicinal drugs could create problems for the patient who sometimes could develop acute diarrhea symptoms that could be life threatening.

The objective of this study is to evaluate the bacterial diversity of pathogens that could contaminate the liquid herbal medicinal preparations and also to ascertain if some of the herbs have in vitro antimicrobial activity against some clinical isolates.

2. Methods

2.1 Study Area and Sample Collection

A total of 150 samples were purchased from different places in Abia State (50 each from Umuahia, Ohafia and Aba). All the samples collected from the sites were analyzed in the Postgraduate Microbiology Laboratory, College of Natural Sciences, Michael Okpara University of Agriculture, Umudike, Abia State. These herbal preparations which had various therapeutic claims were purchased from different vendors to get as many different brands as possible. The samples were within their shelf lives, were kept at room temperature and were analyzed within two weeks of collection. The sealed bottles of herbal preparations were cleaned with 70% ethanol before opening to prevent contamination.

2.2 Materials

The herbal preparations used for this study were all in liquid formulations with different plant parts (i.e. bark, roots, wood etc) which are either active ingredients or preservatives. All herbal preparations were given codes - LHP and numbers for specificity.

2.3 Microbiological tests

2.3.1 Total aerobic plate count and identification of isolates

Surface viable count of some LHPs was carried out by the method of Miles and Misra (1938). A ten-fold serial dilution of the LHPs was made. A series of 9 McCartney bottles containing 9mL of sterile peptone water each were placed on a sterilized bench. With a sterile delivery pipette, 1mL of the collected sample was transferred into the first bottle of diluents (10^{-1}), with another fresh sterile 1mL pipette; 1mL from the first dilution was transferred into the second bottle of diluents (10^{-2}) dilution. This was done until 10^{-10} dilution was achieved. 0.02ml of each LHP was allowed to fall from a height of 2.5cm on to plate count agar and spread over an area of 1.5-2.0cm diameter. Counts were made in the drop areas showing the largest number of colonies without confluence (up to 20 or more). The plates were incubated for a maximum of 48hrs at 37^0C. Finally the counting of the bacterial colonies was done using a Digital colony counter (Harrigan et al, 1993). The Gram staining technique was used as the staining reaction to identify the different bacteria species in each liquid herbal preparation by their Gram reaction (Gram positive or Gram negative) and their morphology. All Gram stained smears of different colonies on different cultures were examined using oil immersion objectives (x100) of a compound microscope to check the staining reaction and morphology of the bacteria species and then with the oil immersion objective. A series of biochemical methods e.g., coagulase test, oxidase test, indole test, citrate test, sulphate reduction test, catalase test, methyl red test, triple sugar iron test, motility test, Voges-Proskauer test was used in identifying and classifying bacterial isolates (Baron et al, 1990, Bachoon et al,2008).

2.3.2 Tube dilution test for minimum inhibitory concentration (MIC)

Liquid herbal preparations with antibacterial activity were prepared in 1:2, 1:4, and 1:8 dilutions or by making serial twofold dilutions (i.e. 3.125 %, 6.25 %, 12.5 %, 25 %, 50 % and 100%) in sterile stoppered test tubes. To each series doubling dilutions one drop of clinical bacterial isolates (*Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa*) from an overnight nutrient broth culture was added aseptically to each tube including tubes of uninoculated LHPs to act as controls. These were incubated for 18-24h at 37^0C and examined for turbidity due to bacterial growth. The tube with the least dilution of LHP showing no visible turbidity was read as the one containing the minimum inhibitory concentration (MIC) of the LHP for the test bacterial isolate (CLSI, 2008).

2.3.3 Tube dilution test for minimum bactericidal concentration (MBC)

The tube dilution test was used to measure the minimum bactericidal concentration (MBC) of potent LHPs. Before incubating each series of inoculated tubes from the tube dilution test for minimum inhibitory concentration, 0.02ml, from the tube without any dilution of LHPs was spread uniformly over a plate of nutrient agar. After incubation of the tubes, a loopful from each tube not showing growth was spread over nutrient agar plates. The plate cultures were incubated for 24hours. The tube culture containing the least dilution that yields no growth in the plate subculture was read as the MBC of the LHP for each test bacteria isolate. The plate sub-cultures yielding growth was compared with that cultured from the control tube cultures before incubation to determine whether there

was some bactericidal action on only bacteriostasis (CLSI, 2008).

2.3.4 Inhibition zone diameter measurement (Agar well diffusion assay)

Three clinical bacterial isolates (*Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa*) were obtained from the Microbiology Laboratory, Federal Medical Centre, Umuahia, Abia State, Nigeria; for the antimicrobial screening of the liquid herbal products. Four morphologically similar colonies of these clinical bacterial isolates was collected with a sterile loop and transferred for growth in peptone broth and incubated with shaking at 35-37°C until the visible turbidity is equal to or greater than the 0.5 McFarland standard.

Adequate light to visually compare the inoculum tube and the 0.5 McFarland standard against a card with a white background and contrasting black line (Hugo and Russel, 2004, CLSI, 2012). Intermediate dilutions of LHPs were prepared by making successive 1:2, 1:4, and 1:8 dilutions or by making serial twofold dilutions. One part of these dilutions was added to nine parts of molten Mueller–Hinton agar (MHA) (Oxoid, UK) and allowed to equilibrate in a water bath to 45 to 50°C. the molten Mueller–Hinton agar and dilutions of LHPs was mixed thoroughly and poured aseptically into Petri dishes on a level surface to result in an agar depth of 3 to 4 mm. Mueller–Hinton agar plates without LHPs was used as controls. Six wells (6mm) were made in the agar with aid of cork borer No. 4. The wells were sealed at the bottom with molten sterilized agar, Aliquot of each well-mixed adjusted and diluted bacterial suspensions (10^7 CFU/mL) was placed into the corresponding wells with standardized pipettes. A growth-control plate (no LHPs) was inoculated and a second growth control plate to ensure there was no contamination or significant antimicrobial carryover during the inoculation. Antibiotic disc (Ciprofloxacin- 5µg) used as control was placed on the agar aseptically.

The plates were then incubated at 37°C for 24 hours. The zone diameters of inhibition produced by each dilutions of the liquid herbal product and that of the antibiotic disc was measured and recorded in millimetres (CLSI, 2013).

2.3.5 Antibiotics susceptibility test

Antimicrobial susceptibility testing of the isolated organisms was performed by the disk diffusion technique following the method recommended by the Clinical Laboratory Standards Institute (CLSI, 2013). All the microbial strains were sub-cultured on a freshly prepared nutrient agar plate 24hour prior to antimicrobial test. Inocula were prepared by transferring several single colonies of bacteria to a sterile broth. A sterile, non-toxic cotton swab was dipped into the standardized Inocula and used to spread the entire surface of Mueller Hinton agar plates (Bauer et al, 1996, CLSI, 2013). Antibiotics discs were placed aseptically on the surface of the agar plates using sterilized forceps and thereafter incubated at 37^0C for 24hours.

Antibiotic discs used to determine the antibacterial susceptibility include; Amoxicillin/Clavulanate (AMC) 30μg, Ofloxacin (OFL) 5µg,Gentamicin (GEN) 10µg, Peflacin (PEF) 10 μg, Ciproflox (CIP) 30µg, Streptomycin (S) 30 μg, Ceftriaxone (CRO) 30μg, Amoxil (AML) 20μg, Cefuroxime (CXM) 30 μg and Cefotaxime (CTX) 30 μg. Erythromycin (ERY) 5μg, Cloxacillin (CXC) 5µg and Chloramphenicol (CH) 30μg. All disks were obtained from Oxoid Ltd. (Basingstoke, UK)

The diameters of the zones of inhibition were read after 24hours of incubation of 37^0C. Data for resistant were reported.

2.4 Multiple antibiotic resistances calculation

Calculation of Multiple Antibiotic Resistance (MAR) index was done by the method of Riaz et al, 2011, using the formula:

$$MAR = a/(b.c)$$

Where:

a = the aggregate resistance of antibiotics to all isolates,

b = the total number of antibiotics

c = the number of isolates from the specimen site.

3. Results

Table 1 shows the incidence and distribution of bacterial contaminants isolated from all sampled liquid herbal preparations (LHPs). A total of 315 bacterial strains were isolated, with 100 strains isolated from 50 LHPs in Umuahia, 84 strains isolated from 50 LHPs in Ohafia and 131 strains isolated from 50 LHPs collected in Aba, all in Abia State. Bacteria genera isolated cultured and identified include *Salmonella, Bacillus, Escherichia, Klebsiella, Proteus, Staphylococcus*, *Streptococcus*, *Citrobacter* and *Pseudomonas* all occurring in varying viable counts per milliliter. The incidence of species of Enterobacteriaceae was significant in most LHPs collected. Also significant is the high occurrence of spore forming *Bacillus* spp., in 49 (16%) liquid herbal preparations, which indicates the unhygienic preparation and handling of these products.

Table 2 presents the minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and antibacterial activities measured in inhibitory zone diameter (in millimeters) of some liquid herbal preparations (LHPs) against 3 test clinical bacteria isolates, namely *Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa*.

MIC for LHPs with antibacterial activities range from 6.25-50%, for *S. aureus*, 6.25-100%, for *E. coli* and 6.25-100% for *P. aeruginosa*. MBC of LHPs against test isolates range from 12.5-100% for *S. aureus, E. coli, P. aeruginosa.* Inhibitory zone diameters (IZD) of test isolates against LHPs range from 6-19mm for *S. aureus,* 6-20mm for *E. coli* and *P. aeruginosa.*

Table 1: Bacteria Contaminants of Some Liquid Herbal Preparations in Abia State

Bacterial Isolates	Number (%) of strains isolated			TOTAL (%) (n=315)
	Umuahia (n=100)	Ohafia (n=84)	Aba (n=131)	
Salmonella spp	5(5)	5(6)	6(5)	16(5)
Bacillus spp.	13(13)	15(18)	21(16)	49(16)
Escherichia coli	13(13)	11(13)	23(18)	47(15)
Klebsiella pneumoniae	0(0)	19(23)	10(8)	29(9)
Proteus vulgaris	11(11)	0(0)	0(0)	11(4)
Staphylococcus aureus	14(14)	11(13)	16(12)	41(13)
Streptococcus spp	0(0)	12(14)	13(10)	25(8)
Citrobacter freundii	9(9)	0(0)	10(8)	19(6)
Pseudomonas aeruginosa	22(22)	11(13)	21(16)	54(17)
Coagulase Negative Staphylococci	13(13)	0(0)	11(8)	24(8)

Table 2: Minimum Inhibitory Concentration (MIC), Minimum Bactericidal Concentration (MBC) and Antibacterial Activities (Inhibitory Zone Diameter) of Some Liquid Herbal Preparations on Test Bacteria Isolates

SAMPLE CODE (LHP)	*Staphylococcus aureus*			*Escherichia coli*			*Pseudomonas aeruginosa*		
	MIC (%)	MBC (%)	IZD (mm)	MIC (%)	MBC (%)	IZD (mm)	MIC (%)	MBC (%)	IZD (mm)
11	12.5	25	13	50	100	6	100	100	6
18	12.5	25	15	50	100	10	100	100	8
20	6.25	12.5	17	100	100	7	12.5	25	16
24	12.5	25	11	6.25	12.5	20	12.5	25	14
25	25	50	9	12.5	25	10	25	50	15
27	6.25	12.5	19	100	100	6	25	50	12
30	6.25	12.5	14	25	50	15	25	50	13
42	25	50	9	25	50	14	12.5	25	20
58	12.5	25	16	12.5	25	20	25	50	11
59	25	50	13	50	100	16	50	100	8
62	25	50	16	100	100	7	50	100	7
73	25	50	10	50	100	8	50	100	10
81	25	50	14	25	50	10	25	50	9
86	50	100	6	12.5	25	14	25	50	14
91	50	100	9	25	50	13	12.5	25	15
103	25	50	11	50	100	9	12.5	25	15
112	25	50	13	100	100	9	12.5	25	11
125	25	50	10	50	100	8	6.25	12.5	16
136	12.5	25	18	25	50	15	6.25	12.5	18
146	6.25	12.5	19	12.5	25	13	6.25	12.5	14

The antibiotic resistance profiles of bacteria isolates from liquid herbal preparations (LHPs) are given in **Table 3**. The resistance pattern of LHP isolates varied among antibiotics used and shows 47 (17.9%) isolates were resistant to Amoxycillin-Clavulanate, while 5 (1.9%) isolates were resistant to Ciprofloxacin. **Table 4** shows the multiple antibiotic resistance (MAR) index of the isolates. The MAR ranged from 0.24 – 0.34.

Table 3: Antibiotic Resistance Profile Bacteria Isolated From Some Liquid Herbal Preparations

	Number and (%) Resistant												
Bacterial Isolates	**AMC**	**OFL**	**GEN**	**PEF**	**CIP**	**S**	**CTX**	**CXM**	**CRO**	**ERY**	**CXC**	**AML**	**CH**
Salmonella spp. (n=16)	2(12.5)	2(12.5)	2(12.5)	1(6.3)	4(25)	2(12.5)	1(6.3)	11(68.8)	5(31.3)	NT	NT	8(50)	NT
Escherichia coli (n=47)	25(52.5)	5(10.5)	10(20.1)	3(6.3)	10(20.1)	15(31.5)	13(27.3)	23(48.3	4(8,4)	NT	NT	32(67.2)	NT
Proteus vulgaris (n=11)	4(36.4)	2(18.2)	1(9.1)	4(36.4)	4(36.4)	2(18.2)	6(54.6)	6(54.6)	1(9.1)	NT	NT	7(63.7)	NT
Citrobacter freundii (n=19)	6(31,8)	4(21.2)	7(37.1)	7(37.1)	5(26.5)	5(26.5)	5(26.5)	8(42.4)	2(10.6)	NT	NT	8(42.4)	NT
P. aeruginosa (n=54)	54(100)	6(11.4)	7(13.3)	3(5.7)	1(1.9)	4(7.6)	54(100)	54(100)	3(5.7)	NT	NT	54(100)	NT
Klebsiella pneumonia (n=29)	14(49)	5(17.5)	4(14.5)	6(21)	3(10.5)	4(14.5)	4(14.5)	15(52.5)	1(3.5)	NT	NT	16(56)	NT
Staph. aureus (n=49)	27(54)	12(24)	5(10)	5(10)	7(14)	10(20)	20(40)	29(58)	20(40)	4(8)	2(4)	40(80)	32(64)
Strep. spp. (n=25)	10(40)	12(48)	10(40)	5(20)	5(20)	8(32)	10(40)	5(20)	11(44)	4(16)	4(16)	10(40)	15(60)
Coagulase Negative Staphylococci (n=24)	12(50.4)	15(63)	5(21)	6(25.2)	7(29.4)	10(42)	10(42)	1(4.2)	10(42)	2(16.8)	2(16.8)	20(84)	15(63)

AMC= Amoxycillin-Clavulanate 30μg, OFL= Ofloxacin 5μg, GEN= Gentamicin 10μg, PEF=Peflacin 10 μg, CIP= Ciproflox 10μg, S=Streptomycin 30 μg, CTX=Cefotaxime 30 μg, CXM =Cefuroxime 30μg, CRO= Ceftriaxone 30μg, ERY= Erythromycin 5μg, CXC= Cloxacillin 5µg, AML= Amoxil 20μg, CH= Chloramphenicol 30μg, NT = NOT TESTED

Table 4: Multiple antibiotic resistances (MAR) of isolates

Isolate	**MAR**
Salmonella spp.	0.24
Escherichia coli	0.30
Proteus vulgaris	0.34
Citrobacter freundii	0.30
Pseudomonas aeruginosa	0.44
Klebsiella pneumonia	0.25
Staphylococcus aureus	0.33
Streptococcus spp.	0.34
Coagulase Negative Staphylococci	0.34

4.0 Discussion

In developing nations, low income individuals use herbal remedies for the treatment of common infections (Rojas et al, 2006). Most of these herbal preparations are widely distributed, hawked by vendors and consumed orally in most localities in Nigeria. In this study 150 liquid herbal preparations (LHPs) were collected from different representative towns in Abia State, Nigeria namely Umuahia (Abia Central), Ohafia (Abia North) and Aba (Abia South). These LHPs were hawked in transparent plastic containers by mostly female vendors on daily basis for medicinal purposes and from rate of sales; these herbal products have wide acceptance and demand. The level of bacterial contaminants of LHPs is quite evident in this study as 130 (86.7%) LHPs were found to harbor different species of bacteria. This agrees with the studies by Ujam, et al, 2013, Esimone et al, 2007 and Ampofo 2012. Agbo et al, 2012 identified different bacteria species in LHP consumed in Nigeria *i.e. Bacillus spp* (23.81%), *Proteus vulgaris* (14.280%), *Klebsiella spp*. (9.52%), *staphylococcus aureus* (9.52%), *Escherichia coli* (4.76%) and *Pseudomonas, aeruginosa* (4.76%). The proportion of *Klebsiella* spp from this Agbo et al, 2012 agrees with ours, while isolates were disproportionate..

The frequency of bacterial species agrees with results of previous studies on microbial contamination of traditional herbal medicinal products (Nakajima et al, 2005, Baba-Moussa et al, 2013). The high rates of bacterial contamination which have been reported severally (Khanyile et al, 2009, Kulkarni et al, 1999), maybe due to lack of stringent regulations of LHPs by regulatory agencies. Ampofo et al, 2012 in a similar study of microbial quality of 31 herbal preparations indicates 39% contamination with diverse heterotrophic bacteria, only 19 herbal samples in the study by Ampofo et al, 2012 were found to be microbiologically safe to be consumed.

Salmonella spp. also occurred in 5% of all assessed LHPs from Aba and is of health concern having been reported to be mostly associated with juvenile gastroenteritis. This is less when compared to findings of Ampofu et al, 2012, in which *Salmonella* spp., occurred in 9.7% of herbal medical products assayed in Ghana.

The high incidence of *Bacillus* spp., in 16% of LHPs suggest contamination from soil, air and dust where it's highly distributed. Similar studies by Oleghe et al, 2011 reported 39% *Bacillus* spp., occurrence in herbal preparation sold in Nigeria.

E. coli was isolated in 15% of LHPs, which is less when compared to 20% occurrence reported by Ujam et al, 2013 of *E. coli* isolated from herbal medicinal products in south east Nigeria. This also constitutes health hazard as reports have shown that *E. coli* is a major diarrhogenic pathogen (Gregory, 2005, Forest, 2004). Other Gram negative bacteria isolated in different counts from LHPs include *K. pneumonia*, *P. vulgaris*, *Citrobacter freundii* and *Pseudomonas aeruginosa* have also been reported (Abba et al, 2009, Kolajo, 2000).

Klebsiella pneumoniae occurred in 9% of LHPs and this agree with the report of Adenike et al; 2007 and has been implicated in bronchitis (Nordmann et al, 2009). *Proteus vulgaris* was isolated from 4% of LHPs which is less than 8% reported by Ujam et al, 2013. *Proteus* serovars have been reported as well to be associated with diarrhea diseases (Jiva et al, 1988).

Staphylococcus aureus was isolated from 13% of LHPs which is more than the 9% occurrence reported by Esimone et al, 2007.Contamination with *Staphylococcus aureus* is indicative of improper handling during processing, distribution or storage of LHPs Abba et al, 2009). The occurrence of *Streptococcus* spp in 8% of LHPs corresponds slightly with the findings of Ujam et al, 2013 and has health implications. The presence of bacterial contaminants in non-sterile medicinal preparations can reduce and even inactivate the therapeutic activity of the products and has the potential to adversely affect individuals taking it (Nakajima et al, 2005, Okunlola et al, 2007).

This study shows that 20(13%) LHPs were found to exhibit in-vitro antibacterial activity. Studies by Esimone et al, 2007 and Ujam et al, 2013, reports 58% and 60% antibacterial potency of herbal products respectively.

Analysis of the multiple antibiotic resistance index of the isolates showed that seven (7) out of the ten (10) genera of bacteria had MAR values in the risk zone. This agrees with the findings of Riaz et al, 2011 who reported that *E. coli* and *Klebsiella* isolates obtained from their study equally showed MAR index in the risk zone and they were resistance to some antibiotics of cephalosporin group.

In evaluating the MIC and MBC potent LHPs were tested against 3 clinical isolates; *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*. MIC for LHPs with antibacterial activities range from 6.25-50%, for *S. aureus*, 6.25-100%, for *E. coli* and 6.25-100% for *P. aeruginosa*. This corresponds with the findings of Shermin et al, 2014 and Abba et al, 2009. Mean MBC of LHPs against test isolates range from 12.5-100% for *S. aureus, E. coli, P. aeruginosa.* Mean inhibitory zone diameters (IZD) of test isolates against LHPs range from 6-19mm for *S. aureus,* 6-20mm for *E. coli* and P. *aeruginosa.* This agrees favorably with the findings of Sharmin et al, 2014 and Ujam et al, 2013.The proportion of antibiotic resistant bacterial isolates from LHPs differ, with findings of Esimone et al, 2007 as it indicated high values for augmentin (amoxicillin-clavuanate-80%), cloxacillin (88.3%), chloramphenicol (66%), erythromycin (34.1%), cefotaxime (79.5%) cefuroxime (100%) and ciprofloxacin (147%). Also the proportion of resistance isolated in our studies *i.e* streptomycin (21.9%), gentamycin (15.3%), ofloxacin (23%) and peflacin (14.6%) were higher and differ from the findings of Esimone et al, 2007.

Consequently the observed inhibitory effects of the LHPs in the present study on the tested bacterial isolates are a justification for the need to explore the various traditional modes of diseases treatments in order to determine their various antimicrobial efficacies.

There is high level of bacterial contamination in liquid herbal preparations in Abia State. The contaminations of liquid herbal preparations were attributable to both individuals and system-centered risk factors. This

confirms observations of earlier studies on the use and consumption of herbal remedies in the treatment of diseases (Abba et al*,* 2009, Adeleye et al*,* 2005). Despite the increasing use of herbal preparations in most countries, there still remain numerous issues about the microbial quality and safety of these phytotherapeutics. The presence of diverse range of bacteria strains in LHPs poses serious public health implication as it has been known to be implicated in serious infection induced disease which could assume high level of morbidity and mortality (Hugo and Russell, 2004).Thus, the urgent need for the appropriate regulation and standardization of herbal preparations. Therefore, there is an urgent need to have specific programs, policies and regulations addressing herbal medicine safety which are specifically focused on prevention of microbial contamination, so as to prevent the possibility of these pathogens to be involved in deadly invasive infections.

5.0 Conclusion

The liquid herbals drugs were collected from 3 major locations in the State namely, Umuahia, Ohafia and Aba and showed the most frequently isolated bacteria as *Pseudomonas aeruginosa* 22%, *Klebsiella pneumonia* 23%, *and E. coli* 18% respectively. The MIC and MBC of some selected herbal medicinal preparations against *Pseudomonas aeruginosa*, *E. coli* and *S. aureus* showed very encouraging results.

Conflict of Interest declaration

The authors declare no conflict of interest.

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