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MULTI-ANTIBIOTIC RESISTANCE PROFILE OF BACTERIA ISOLATED FROM STREET-VENDED HERBAL CONCOCTIONS

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Abstract
The multi-antibiotic resistance profile of bacteria isolated from street vended herbal concoctions in Mile 3 Area of Port Harcourt, Rivers State was investigated. Ten (10) different local herbal drugs were purchased from hawkers, examined for bacteriological quality and antibacterial activities. The samples were analysed using standard microbiological procedures as well as disc diffusion method for antibiotic sensitivity testing. The bacterial counts obtained from the street-vended herbal concoction ranged from 3 x 10^4 cfu/ml to 2.86 x10^5 cfu/ml while the coliform counts obtained from the street-vended herbal concoction ranged from no growth to 2.87 x10^5 cfu/ml. The bacteria isolated and identified are *Bacillus* sp., *Enterobacter* sp., *Staphylococcus aureus* and *Klebsiella* sp. The antibiotic profile of the test isolates reveals that augmentin, septrin, chloramphenicol and amoxicillin recorded the highest resistance; tarivid, perfloxacin, ciprofloxacin, gentamicin and sparfloxacin recorded the highest sensitivity and the multi-antibiotic resistance index (MARI) range between 0.1 and 1. Most of the bacterial counts exceed recommended limits for bacteria and coliform, posing a risk for consumers; the identified bacteria indicates prevalence of potential pathogens and most of the isolates were of high antibiotics polluted sources. Training of herbal drugs producers and prescribed antibiotic use advocacy is essential.

Keywords: Bacteria, Coliform, Herbal Concoction, Multi-Antibiotic Resistance Index, Street-Vended

Introduction

Herbal medicines also include industrially manufactured preparations in which the active ingredients are purely and naturally original plant substances, chemically unaltered and responsible for the overall therapeutic effect of the product (Alostad *et al*., 2018). They are not classified as drugs in some climes because the rules that apply to registering them are not as stringent as with conventional drugs (Thakkar *et al*., 2020).

There had been reported surge and rising interest across the globe in the use of herbal medicinal products in the treatment and prevention of diseases. This may be partly attributable to a widely held belief that herbal medicines are safe and devoid of adverse reactions, as natural products (Singh *et al*., 2021). It is estimated that approximately 80% of the population in developing countries uses traditional herbal medicines as part of their primary health care (Umir *et al*., 2017). However, deficient or nonexistent regulatory control of herbal medicines in most countries have raised concerns and awareness on the need to monitor safety and deepen understanding of possible harmful as well as potential benefits associated with the use of herbal medicines (Chikezie *et al*., 2015). Most conventional medicines contain known active ingredients whose identification are required as evidence of adherence to Good Manufacturing Practice, quality control and all such necessary regulatory protocols (Alostad *et al*., 2020). These are more clear-cut than for a single herbal remedy, which may contain hundreds of natural constituents, what more for polyherbal products containing mixtures of

various herbs containing several times more. Thus, producers of herbal products and regulatory agencies are confronted with challenges in terms of standardization, as well as technical, financial and ethical constraints (Alostad *et al*., 2020).

Studies have also revealed that not only are herbal medicines intrinsically not free from toxicities and adverse reactions but are also exposed to extraneous factors through contamination or adulteration which may render them unsafe for human consumption. These extraneous materials include microorganisms, metals, various toxins, pesticides, and agrochemicals organic and inorganic contaminants (Singh *et al*., 2020; De Sousa Lima *et al*., 2021); as well as adulterations with several approved and unapproved conventional drugs (Lin *et al*., 2018). A higher standard of hygienic practices during production is required to minimize contamination (Ya'aba *et al*., 2020). The majority of herbal producers in Nigeria lack the necessary skills to perform quality control and resolve safety concerns with the products they produce (Ya'aba *et al*., 2020). Some of pathogenic bacteria including coliforms reported in herbal medicine products in previous studies include *Escherichia coli*, *Staphylococcus aureus*, *Salmonella* spp., *Listeria monocytogenes*, *Bacillus* spp., *Mycobacterium* spp., *Campylobacter* spp., *Clostridium* spp., *Pseudomonas aeruginosa* and *Proteus mirabilis* (Meshack *et al*., 2013; Kira *et al*., 2021).

Despite the growing number of herbal products in the market in Nigeria, there is still scarcity of information on microbiological quality of herbal drugs in some regions and the genetic basis of resistance in these bacteria (Ya'aba *et al*., 2020; Walusansa *et al*., 2022). On the other hand, presence of antibiotic-resistant microbial isolates in the herbal medicine products (HMPs) could lead to transfer of antibiotic resistance traits to hitherto sensitive gut or oral microflora of consumers (Esimone *et al*., 2007). Since the presence of microbes cannot be detected by organoleptic means and microbial contamination in herbal medicines practices is of major public health importance, there is need for product microbiological analysis. This study therefore investigated the multi-antibiotic resistance profile of bacteria isolated from street-vended herbal concoctions in Mile 3 Area of Port Harcourt, Rivers State.

Materials and Methods

The study was carried out in Mile 3 Diobu, Port Harcourt, Port Harcourt Local Government Area, Rivers State. Ten (10) herbal concoctions (drink) commonly sold in the morning hours within Mile 3 Diobu, Area of Port Harcourt were bought from a vendor (hawker). The samples were brought to the Biology/Microbiology Laboratory 1, Science Laboratory Technology Department, Captain Elechi Amadi Polytechnic, Rumuola within 2 hours of purchase for microbiological analysis. As identified by the street vendors, the herbal concoctions bought are: Ulcer, Typhoid, Malaria, Manpower, Purging, Infection, Washing and setting, Sugar and Real one for purging.

For the isolation of total heterotrophic bacteria, Nutrient Agar (TM Media, India) were prepared according to manufacturer's specification while MacConkey Agar were prepared total coliform. One millilitre (1 ml) of the each herbal concoction sample was measured and then transferred into a test tube containing 9ml of sterilised normal saline. This was serially diluted up to 10^{-2} using a sterile pipette in a ten-fold serial dilution technique; thereafter an aliquot, 0.1ml was inoculated on a freshly prepared Nutrient agar Plates and MacConkey agar plates employing a spread plate method with the aid of a sterile glass rod (spreader). The inoculated plates were then incubated at ambient temperature within the laboratory for 3-5 days. Discrete colonies that developed (30-300) were counted and recorded. The average colony forming unit per gramme was calculated using the formula:

$$\text{cfu/g} = \frac{\text{Average number of colonies x dilution factor}}{\text{Volume plated}}$$

Discrete colonies were picked and subcultured onto Nutrient Agar plates using streak plate method. Stock cultures were prepared on sterile Nutrient Agar in Bijou bottles, coded for ease of identification and stored in the refrigerator (4°C) until needed for further tests such as biochemical test. The isolates were identification on the basis of their cultural, morphological and physiological characteristics in accordance with schemes and methods described by Ogbulie *et al*. (2001) and Cheesborough (2006). Microscopic examination of isolates was carried out using oil immersion objectives (x100). The following tests were carried out: Gram staining and Biochemical tests which were catalase, citrate utilisation, indole, methyl red, Voges Proskauer and Hydrogen Sulphide.

The antibiotics status of bacteria isolated was determined using Kirby-Bauer disc diffusion method. Nutrient Agar (TM Media, India) was prepared according to manufacturer's specification. When the medium cooled to 40 – 50 °C, it was poured into plates and allowed to set. When the medium was set, the agar plates were dried for thirty (30) minutes at 35 °C by placing them upright in the incubator with the lid tilted. Inoculum of each isolate prepared from an 18 hour broth culture adjusted to obtained turbidity comparable to 0.5 Mcfarland Standard. The turbidity standard was made by adding 0.5 ml of 1.175 % (w/v) barium chloride dehydrate ($BaCl.2H_2O$) solution to 99.5 ml of 1 % tetraoxosulphate (VI) (H_2SO_4) acid, and used to standardise the bacteria inocula. Adjustment was made by diluting the 18 hour broth culture with sterile saline. Sterile swab stick was then dipped into standardized bacterial solution. The swab was then used to streak the entire dried surface of medium. The inoculated plates were incubated for five minutes to remove excess moisture to dry. Antibiotic disc (Maxicare Medical Laboratory) were then placed at equidistance from each other on the plate with the aid of a pair of sterile forceps. The discs were pressed firmly onto the agar with the sterile forcep to ensure complete contact with the agar. The plates were then incubated at 35 °C for 18-24 hours. The antibiotics discs that were used for this study include ciprofloxacin (30 µg), septrin (30 µg), gentamycin (30 µg), streptomycin (30 µg), perfloxacin (30 µg), Augmentin (10 µg), Chloramphenicol (30 µg), tarivid (10 µg), sparfloxacin (10 µg) and Amoxacillin (30 µg). After incubation, the diameters of the zone of inhibition were measured (using metre rule) to the nearest mm. These zones were interpreted using Clinical and Laboratory Standard Institute (CLSI) guidelines (CLSI, 2021). Using the chart, the organisms were classified as being resistant, intermediate or susceptible to the test antibiotic.

This was carried out as described by Matyar *et al*. (2007) with slight modification. MARI = Resistant Antibiotics ÷Total Antibiotics. MARI value > 0.2 indicate existence of isolate(s) from high-risk contaminated source with frequency use of antibiotic(s) while values ≤ 0.2 show bacteria from source with less antibiotics usage (Krumperman, 1985).

All the data obtained during the study period was analyse using mean and presented in tables.

Results

The results of bacterial and coliform counts obtained from street-vended herbal concoction are presented in Table 1. The bacterial counts are in the following order: 3×10^4 cfu/ml(ulcer) < 3.14×10^4 cfu/ml (typhoid) < 6×10^4 cfu/ml (pile and manpower) < 1.07×10^5 cfu/ml (malaria) < 1.40×10^5 cfu/ml (real one for purging) < 1.96×10^5 cfu/ml (infection) < 2.29×10^5 cfu/ml (purging) < 2.43×10^5 cfu/ml (Washing and Setting) < 2.86×10^5 cfu/ml (purging). The coliform counts are on the following

order: no growth (ulcer, typhoid, malaria) < 3.15 x 10^4 cfu/ml (pile) < 8.85 x10^4 cfu/ml (manpower) < 1.98 x10^5 cfu/ml (purging) < 1.99 x10^5 cfu/ml (infection) < 2.23 x10^5 cfu/ml (washing and setting) < 2.68 x10^5 (sugar) < 2.87 x10^5 (real one for purging).

Table 1 Bacterial Counts obtained from Street-Vended Herbal Concoctions

Herbal Sample	THB Counts (cfu/ml)	Coliform Counts (cfu/ml)
Ulcer	3 x 10^4	-
Typhoid	3.4 x 10^4	-
Pile	6 x 10^4	3.15 x 10^4
Washing and Setting	2.43 x10^5	2.23 x10^5
Manpower	6 x10^4	8.85 x10^4
Malaria	1.07 x 10^5	-
Sugar	2.86 x10^5	2.68 x10^5
Infection	1.96 x10^5	1.99 x10^5
Real one for Purging	1.40 x10^5	2.87 x10^5
Purging	2.29 x10^5	1.98 x10^5

Key cfu/g Colony forming unit per gramme

The characterisation and identification of bacteria isolated from street-vended herbal concoctions shows that the bacteria isolated and identified are *Bacillus* sp., *Enterobacter* sp., *Staphylococcus aureus* and *Klebsiella* sp.

The antibiotics profiles and multi-antibiotic resistance index (MARI) of bacteria isolated from street-vended herbal concoctions are presented in Table 2. *Bacillus* sp. 1 was resistant to all the tested antibiotics (100 %). *Bacillus* sp. 2 was resistant to septrin, streptomycin, amoxacillin and chloramphenicol (40 %); intermediate reaction to ciprofloxacin (10 %) while being sensitive to augmentin, gentamicin, perfloxacin, tarivid and sparfloaxcin (50 %). *Bacillus* sp. 3 was resistant to augmentin and amoxacillin (20 %) while being sensitive to gentamicin, tarivid, sparfloaxcin, perfloxacin, septrin, sparfloaxcin, streptomycin and ciprofloxacin (80 %). *Bacillus* sp. 4 was resistant to chloramphenicol (10 %); intermediate reaction to septrin (10 %) while being sensitive to augmentin, amoxacillin gentamicin, tarivid, sparfloaxcin, perfloxacin, streptomycin and ciprofloxacin (80 %).

Enterobacter sp. was resistant to augmentin (10 %); intermediate reaction to gentamicin (10 %) while being sensitive to perfloxacin, tarivid, septrin, streptomycin, sparfloxacin, ciprofloxacin, chloramphenicol and amoxacillin (80 %). *Staphylococcus aureus* 1 was resistant to augmentin, tarivid, septrin, streptomycin, ciprofloxacin, chloramphenicol and amoxacillin (70 %); intermediate reaction to gentamicin (20 %) and perfloxacin while being

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Table 3 Antibiotics Profiles and Multi-Antibiotic Resistance Index (MARI) of Bacteria from Street-Vended Herbal Concoctions

Organism	Antibiotics (mm)													MARI
	AU	CN	PEF	OFX	S	SXT	CH	SP	CPX	AM	R	I	S	
Bacillus 1	0R	12R	14R	0R	14R	0R	14R	14R	0R	0R	10	0	0	1
Enterobacter sp.	14R	16I	22S	30S	22S	24S	24S	28S	28S	26S	1	1	8	0.1
Staphylococcus aureus 1	0R	16I	16I	12R	14R	10R	0R	22S	14R	14R	7	2	1	0.7
Bacillus 2	20S	22S	24S	30S	12R	14R	0R	22S	18I	12R	4	1	5	0.4
Klebsiella sp. 1	0R	10R	14R	20S	18I	0R	0R	16I	18I	14R	6	3	1	0.6
Klebsiella sp. 2	0R	8R	22S	18I	14R	0R	0R	18I	22S	14R	6	2	2	0.6
Bacillus 3	12R	22S	24S	30S	28S	24S	30S	28S	24S	12R	2	0	8	0.2
Staphylococcus aureus 2	18I	20S	24S	22S	22S	10R	18I	18I	14R	20S	2	3	5	0.2
Klebsiella sp. 3	12R	12R	18I	20S	14R	12R	20S	18I	20S	16I	4	3	3	0.4
Bacillus 4	22S	24S	22S	30S	28S	16I	0R	22S	22S	26S	1	1	8	0.1

Key: mm Millimetre
CPX ciprofloxacin **SXT** septrin **CN** gentamycin **S** streptomycin **PEF** perfloxacin **AU** Augmentin **CH** Chloramphenicol **OF**X Tarivid
SP Sparfloxacin **AM** **Amoxacillin**
Zone of inhibition Resistant = 0-14mm Intermediate = 15-19 mm Sensitivity = ≥20 mm

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sensitive to sparfloaxcin. *Klebsiella* sp. 1 was resistant to augmentin, gentamicin, perfloxacin, septrin, chloramphenicol and amoxacillin (60 %); intermediate reaction to septrin and sparfloaxcin (20 %) while being sensitive to tarivid (10 %). *Klebsiella* sp. 2 was resistant to augmentin, gentamicin, streptomycin, septrin, chloramphenicol and amoxacillin (60 %); intermediate reaction to tarivid and sparfloaxcin (20 %) while being sensitive to perfloxacin and ciprofloxacin (60 %). *Staphylococccus aureus* 2 was resistant to septrin and ciprofloxacin (20 %); intermediate reaction to augmentin, chloramphenicol and sparfloaxcin (30 %) while being sensitive to gentamicin, perfloxacin, ofloxacin, streptomycin and amoxacillin (50 %). *Klebsiella* sp. 3 was resistant to augmentin, gentamicin, streptomycin and septrin (40 %); intermediate reaction to perfloxacin, sparfloaxcin and amoxacillin (30 %) while being sensitive to tarivid, chloramphenicol and ciprofloxacin (30 %).

The results also shows that the test bacteria recorded a multi-antibiotic resistance index (MARI) were in the following order: The results also shows that the test bacteria recorded a multi-antibiotic resistance index (MARI) were in the following order: *Bacillus* 1 recorded 0; *Bacillus* 2 recorded 0.4; *Bacillus* sp. 3 recorded 0.2 while *Bacillus* sp. 4 recorded 0.1. *Enterobacter* sp. recorded MARI of 0.1; *Staphylococcus aureus* 2 recorded 0.2; *Klebsiella* sp. 3 recorded a MARI of 0.4 and *Klebsiella* sp. 2 and *Klebsiella* sp. 3 recorded a MARI of 0.6 while *Staphylococcus aureus* 1 recorded MARI of 0.7.

Discussion

The present study investigated the multi-antibiotic resistance profile of coliforms isolated from street vended herbal concoctions in Mile 3 Area of Port Harcourt, Rivers State.

The bacterial counts obtained from street-vended herbal concoction ranged from 3 x 10^4 cfu/ml (ulcer) to 2.86 x10^5 cfu/ml (purging). Olaniran *et al*. (2022) reported that the least total mean bacterial load was 1.0x10^4 cfu/ml while the highest total mean bacterial load was 2.6x10^7 cfu/ml which is higher than those recorded in this study; however, the bacterial count in this study exceeds the recommended World Health Organisation (2017) standard of ≤10^5 cfu/ml, demonstrating increased risks in the intake of these products.

The coliform counts obtained from street-vended herbal concoction ranged from no growth to 2.87 x10^5 cfu/ml. Olaniran *et al*. (2022) reported counts which ranged from 1.3 x 10^4 cfu/ml to 9.0 x 10^6 cfu/ml which are slightly above those recorded in this study. The coliform counts exceeded the World Health Organisation (2017) standard of 0 cfu/ml, indicating that consuming these products poses a risk. Generally, herbal medicines in liquid pharmaceutical form for oral use has been known to have the highest microbial contamination and were the most commonly consumed products among the elderly (de Sousa Lima *et al*., 2020). The contaminations were attributed to low education, lack of formal training in the process and poor hygienic level in processing and packaging (Walter *et al*., 2016). Presumably, the proliferation of microorganisms may result from the failure to control moisture levels of herbal medicines during transportation and storage, as well as from the failure to control the temperatures of liquid forms and finished herbal products (WHO, 2007).

The bacteria isolated and identified are *Bacillus* spp., *Enterobacter* spp., *Staphylococcus aureus* and *Klebsiella* spp. These organisms identified in this study are in conformity with

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Ndukwu *et al*. (2021) while Olaniran *et al*. (2022) reported *Staphylococcus aureus* and *Klebsiella* sp. Also, Enoch and Diane (2019) that in another study reported *Bacillus, Enterobacter* sp., *Staphylococcus aureus* and *Klebsiella* sp. amongst other bacteria identified as contaminants of herbal drugs. The contaminations were attributed to low education, lack of formal training in the process and poor hygienic level in processing and packaging (Walter *et al*., 2016). Moreover, most medicinal plants are prepared in an open environment in nonhygienic conditions that gradually lead to contamination with enteric pathogens with public health importance (Zank and Hanazaki, 2017). The microbial contamination of herbal drugs might cause biodeterioration and reduction and the efficacy of the herbal drugs. Bacteria might also produce toxin that may render herbal drugs unsafe for human consumption (Noor *et al*., 2013).

Coliforms are bacteria of major public health concern and one of the largest groups of bacteria, associated with faecal pollution (Mishra *et al*., 2018), thus their presence in the remedies may be attributable to contaminated water used in washing and boiling, although three herbal concoction recorded no growth/contamination. Coliform bacteria are known to flourish in the gut of endothermic animals, and indefinitely discharged into the environment through the faeces. Their presence is thus amply suited as circumstantially indicative of the likely prevalence of potential pathogens, a factor that has sustained their choice for more than a century as indicators of fecal pollution (Mishra *et al*., 2018). Also, Onyemelukwe *et al*. (2019) and De Sousa Lima *et al.* (2020) stated that the presence of coliforms in the herbal remedies could thus been seen as indicative of poor microbial quality.

The results show that tarivid, perfloxacin, sparfloxacin, ciprofloxacin and gentamicin were the best antibiotics in this study. The *Bacillus* sp. 1 isolated from the examined local herbal drugs was resistant to all the conventional antibiotics tested against it; this is in line with the report of Enoch and Diane (2019). The efficacy of ciprofloxacin is in line with the findings of Manhondo *et al*. (2014). *Enterobacter* sp. showed the highest sensitivity while *Staphylococcus aureus* 1 and *Klebsiella* sp. 1 showed the least sensitivity. Generally, the resistance to the commercial antibiotics was high which is in agreement with Olaniran *et al*. (2022) as augmentin and septrin showed highest resistant value of 83.33 %.

The multi-antibiotic resistance index (MARI) in this study is between 0.1 and 1 with *Bacillus* sp. 1. having the highest of MARI of 1. Multi-antibiotic resistance indexing (MARI) has been an effective and efficient tool in tracing bacteria exposure (Osundiya *et al*., 2013). When the use of antibiotics is rare or under dosed for animal treatment, the MARI value is ≤ 0.2; Subashkumar *et al*. (2006) stated that isolates that exhibit multi antibiotic resistance index of (MARI) value greater than 0.2 depicts high level of antibiotic resistance due to either indiscriminate use of antibiotic or horizontal gene transfer, particularly those that are non-biodegradable (Al-abedi *et al*., 2024). Multidrug resistant bacteria are responsible for serious infections in humans and the rate is on increase globally (Enoch and Diane, 2019).

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Conclusion

The bacterial and coliform counts obtained from most of the street-vended herbal concoction exceeded the World Health Organisation standard, indicating that consuming these products poses a risk. The presence of coliform and other bacteria sufficiently in most samples indicates the likely prevalence of other potential pathogens and confirms poor microbial quality for human consumption. Gentamicin, perfloxacin, tarivid and sparfloxacin were the best commericial antibiotics in this study while most of the isolates were of high antibiotics polluted sources.

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