**Short Communication**

**Comparative distribution of bacterial contaminants of packaged and unpackaged polyherbal products sold in Nnewi, Nigeria**

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**Abstract:**

**Background:** The use of herbal medicine continues to remain popular despite advances in orthodox medicine largely as a result of affordability and availability. However, contaminated and potentially toxic polyherbal preparations remain a public health challenge despite regulations instituted by concerned agencies in Nigeria. The objective of this study was to determine and compare the bacterial contaminants of different polyherbal products sold in Nnewi, Nigeria.

**Methodology:** This study evaluated the bacteriological profile of 22 packaged and 22 unpackaged polyherbal preparations sold in Nnewi, Nigeria. The samples were collected from different herbal medicine shops in Nnewi by simple random sampling and were assayed for comparative bacterial loads with chromogenic media and their total viable counts evaluated following standard method for microbial load analysis.

**Results:** Bacterial contaminants were isolated from 9 of 22 (40.9%) packaged polyherbal samples while 13 of 22 (59.1%) samples were bacteriologically sterile. For the unpackaged polyherbal, bacterial contaminants were isolated from 18 of 22 (81.8%) samples while 4 of 22 (18.2%) were bacteriologically sterile (OR 0.1538, \( p=0.0122 \)). The most frequently isolated bacterial contaminant in the packaged polyherbal samples was *Enterococcus faecalis* with 33.3% (6/18) while *Salmonella* sp was the least frequently isolated with 5.6% (1/18). For the unpackaged polyherbals, the most frequently isolated bacterial contaminant was *Staphylococcus aureus* with 25% (7/28) while *Salmonella* sp and *E. faecalis* were the least frequently isolated with 10.7% (3/28) each. The median total viable count of the packaged group of the polyherbal products was 1.48x10^6 CFU/ml, while the median total viable count for unpackaged group of polyherbals was 1.95x10^6 CFU/ml.

**Conclusion:** This study shows that many polyherbal products sold in Nnewi are potentially contaminated with bacterial agents. It is therefore imperative that herbal medicine practitioners be enlightened on hygienic ways of preventing microbial contamination during polyherbal production.

**Keywords:** Bacterial contaminants, herbal products, Nnewi, Nigeria

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**Contexte:** L’utilisation de la phytothérapie continue de rester populaire malgré les progrès de la médecine orthodoxe en grande partie en raison de l’abordabilité et de la disponibilité. Cependant, les préparations à base de plantes contaminées et potentiellement toxiques restent un problème de santé publique malgré les réglementations mises en place par les agences concernées au Nigeria. L’objectif de cette étude était de déterminer et de comparer les...
Bacterial contaminations of polyherbal products

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Mots-clés: Contaminants bactériens, produits à base de plantes, Nnewi, Nigéria

Introduction:

Herbal medicine is a medication made from herbs and has long been used as a source of alternative medicines in developed, developing and underdeveloped countries. Throughout the ages, humans have turned to herbal medicine for healing. All societies have folk medicine traditions that include the use of plants and plant products. Many licensed drugs used today in conventional medicine originated from herbal products.

The World Health Organization (WHO) estimates that about 4 billion or at least 80% of the world’s population use herbal preparations for some aspects of primary health care (10). In Nigeria, herbal therapy remains a popular alternative in many traditional communities where orthodox medicine is not affordable (2). Herbal medicine practitioners in Nigeria use various herbal preparations to treat various types of ailments including diarrhoea, urinary tract infections, typhoid fever and skin diseases (8). Unfortunately, many of these herbal medicine practitioners do not follow hygienic procedures in preventing microbial contaminants during production of their polyherbal products. The study is designed to comparatively evaluate bacteriological contaminants of packaged and unpackaged polyherbals sold in Nnewi, Nigeria.

Materials and method:

Collection of polyherbal samples

A total of 44 samples of liquid formulations of the polyherbals produced in Nigeria were purchased from 16 different herbal shops and trade-medical hawkers, who were selected by simple random sampling within Nnewi town. The samples of the packaged polyherbal preparations (n=22) were purchased while samples of the unpackaged extemporaneous polyherbal preparations (n=22) were collected in polythene bags that are used to dispense products to customers by the herbal medicine practitioners. All samples were labeled and immediately transported to the Faculty of Health Science Laboratory of the Nnamdi Azikiwe University, Okofia, Nnewi.

Estimation of total viable count of bacteria

A tenfold dilution of each sample of polyherbal was achieved by adding 1ml of each sample to 9ml of sterile normal saline in the first test tube of a row of 10 sterile tubes. One (1) ml from the first tubes on each row was then transferred to the 2nd test tube after proper mixing continuing up to the 10th tube where one (1) ml of the mixture was discarded to achieve a 1/101 to 1/1010 dilutions. One (1) ml of the dilution from each test tube was then transferred into a sterile Petri dish and molten nutrient agar was added, the constituents were well mixed and incubated aerobically at 37°C for 24 hours. The number of colonies on each plate was counted and the mean for each sample was established and recorded as the mean colony forming units (CFU) per ml.

Isolation and identification of bacterial contaminants in the polyherbal preparations.

The isolation and identification of the bacterial agents was done by culture on two commercial chromogenic media; CHROMagar™ Orientation and HARDYCHROM™ SS NPRO agar, which have been validated to have positive and negative predictive values of 99.3% and 100% respectively for the isolation and identification of the bacterial organisms such as Klebsiella pneumoniae, Enterococcus faecalis, Staphylococcus aureus, Staphylococcus epidermidis, Escherichia coli, Proteus sp, Salmonella sp, Citrobacter sp, Serratia marcescens, Providencia sp, Acinetobacter sp, and...
Pseudomonas aeruginosa which are common contaminants in polyherbal preparations (1,5). The media were prepared according to the manufacturer’s instructions.

Briefly, labeled individual samples of both the packaged and unpackaged herbals were well mixed to ensure complete homogenization before culture. A loopful of each of the samples was streaked on the prepared agar plates using sterile wire loop. Incubation was done aerobically at 37ºC for 24 hours. A sterile non-inoculated plate was also placed in the incubator for quality control during incubation (3). Plates were read after 24 hours and bacteria were identified by their peculiar and different colony colours and chromogenic attributes using the colour charts as a guide (7), in determining the bacterial isolates (1). The identification parameters of bacterial colonies on the media based on colour are; Klebsiella sp colonies appeared metallic blue, Enterococcus faecalis colonies as red, Proteus mirabilis colonies as clear and brown halo, and Salmonella spp as pink.

Analysis of data

The data were presented in frequency tables and statistical analysis performed with IBM SPSS 20.0 version. Chi square test was used to measure association of bacterial contamination with packed and unpackaged polyherbals, and p value less than 0.05 was considered to be statistically significant.

Results:

The frequency of distribution of bacterial contaminants in the packaged and unpackaged polyherbal samples is shown in Table 1.

Data obtained from this study showed that bacterial agents such as K. pneumoniae, E. faecalis, S. aureus, E. coli, P. mirabilis and Salmonella sp were isolated at varying frequencies in both groups of polyherbals. These

Table 1: Frequency distribution of bacterial isolates in packaged and unpackaged polyherbal samples

| No of polyherbals/bacterial isolates | Packaged (%) (n=22) | Unpackaged (%) (n=22) | X² | p |
|--------------------------------------|---------------------|----------------------|----|---|
| No bacteria isolate                  | 13 (59.1)           | 4 (18.8)             | 7.8| 0.01* |
| No positive for bacterial isolate    | 9 (40.9)            | 18 (81.2)            |
| Klebsiella pneumonia                 | 2 (11.1)            | 5 (17.9)             |
| Enterococcus faecalis                | 6 (33.3)            | 3 (10.7)             |
| Staphylococcus aureus                | 3 (16.7)            | 7 (25)               |
| Escherichia coli                     | 3 (16.7)            | 5 (17.9)             |
| Proteus sp                           | 3 (16.7)            | 5 (17.9)             |
| Salmonella sp                        | 1 (5.6)             | 3 (10.7)             |
| **Total isolates**                   | **18 (100)**        | **28 (100)**         |

X²= Chi square; *statistically significant

Bacterial contaminants were isolated from 9 of 22 (40.9%) packaged polyherbal samples while 13 of 22 (59.1%) samples were bacteriologically sterile. For the unpackaged polyherbals, bacterial contaminants were isolated in 18/22 (81.8%) samples while 4/22 (18.2%) were bacteriologically sterile (OR 0.1538, p =0.0122). Of the 9 positive samples in the packaged polyherbals, a total of 18 bacterial isolates were recovered, E. faecalis was the most frequent with 33.3% (6/18), followed by S. aureus 16.7%, E. coli 16.7%, Proteus sp 16.7%, K. pneumoniae 11.1% and Salmonella sp 5.6%. Of the 18 positive samples in the unpackaged polyherbals, a total of 28 bacterial isolates were recovered, S. aureus was the most frequent with 25% (7/28), followed by K. pneumonia 17.9%, E. coli 17.9%, Proteus sp 17.9%, E. faecalis 10.7% and Salmonella sp 10.7% (Table 1).

Table 2 shows the distribution of the polyherbal products from the 16 herbal shops and trado-medical hawkers, and the bacteria isolates recovered from those positive. Table 3 shows the total viability count (TVC) for each of the packaged herbal product that cultured positive for bacteria with a mean TVC of 1.48 x 10⁶ CFU/ml, while Table 4 shows the TVC for each of the unpackaged herbal product that cultured positive for bacteria, with a mean TVC of 1.85 x 10⁶ CFU/ml.

Discussion:

Data obtained from this study showed that bacterial agents such as K. pneumoniae, E. faecalis, S. aureus, E. coli, P. mirabilis and Salmonella sp were isolated at varying frequencies in both groups of polyherbals. These
Table 2: Distribution of bacterial contaminants in polyherbals purchased at the herbal shops

| Herbal shops       | Polyherbals                                      | Bacterial isolates                                      |
|--------------------|--------------------------------------------------|---------------------------------------------------------|
| Dan-Ilyke          | Katoka, Ruzu bitters, Yoyo bitters                | No growth                                               |
| Blessed Mother     | Blood purifier, Super bitters                     | No growth                                               |
| Dr Chiagozie       | Nando mixture, Super bitters, Mako cleanser,      | Enterococcus faecalis                                   |
|                    | Super 7, Ruzu bitters                            |                                                         |
| Panx               | Goko cleanser, dukun care, Dr Igah cleanser       | E. coli, Klebsiella sp, E. faecalis, S. aureus          |
| Fesco              | Deep root, Bitterkinga                           | No growth                                               |
| Dr Agnes           | J.M.I herbal, Museya, Jalin herbal               | E. faecalis, Klebsiella sp, E. coli, Proteus sp         |
| Eze                | Eze herbal mixture, new beta cleanser            | E. coli, Proteus sp                                     |
| Dr Benbella        | Weifa body defense                                | No growth                                               |
| Baba Oyo           | Olori herbal mixture                              | E. faecalis, S. aureus, Proteus sp                      |
| Yemi               | Anti-pile, Anti-diabetic, fibroid                 | E. coli, Klebsiella sp, E. faecalis, Salmonella sp      |
| Dan Obitube        | Convulsion formula, general well-being, blood     | E. coli, E. faecalis                                    |
|                    | booster, energy booster                           |                                                         |
| Titi               | Fertility preparation, Laxative preparation       | S. aureus, Proteus sp                                   |
| Barakat            | Anti-ulcer, STI preparation, Abdominal preparation| S. aureus, Proteus sp                                   |
| Laide              | Skin infection, menstruation prep                 | Proteus sp                                              |
| Baba Osun          | Male fertility, anti-pile, back pain              | E. coli, S. aureus, Klebsiella sp, Salmonella sp        |
| Sunny              | Anti-gonorrhoea, STD preparation, anti-malaria    | E. coli, Klebsiella sp                                  |

Table 3: Total Viable Counts in packaged polyherbal samples and their bacteriological safety

| Packaged polyherbals | Total Viable Count (CFU/ml) | Bacteriological Safety Level (10^6) |
|----------------------|----------------------------|------------------------------------|
| Deep Root            | No Isolate                 | Safe                               |
| Blood purifier       | No Isolate                 | Safe                               |
| Jalin herbal mixture | 1.5x10^6                   | Unsafe                             |
| J.M.I herbal mixture | 3.6x10^6                   | Unsafe                             |
| Mako Cleanser        | No Isolate                 | Safe                               |
| Super 7              | 1.4x10^6                   | Unsafe                             |
| Museya bitters       | No Isolate                 | Safe                               |
| Dr Igah Bitter cleaner | 0.7x10^6                 | Unsafe                             |
| Goko Cleanser        | No Isolate                 | Safe                               |
| New Beta cleanser    | No Isolate                 | Safe                               |
| Infection destroyor  | 1.3x10^6                   | Unsafe                             |
| Dr sunny Gonorhea herbal | 1.8x10^6              | Unsafe                             |
| Dukun Care           | 1.7x10^6                   | Unsafe                             |
| Katoka Mixture       | No Isolate                 | Safe                               |
| Eze herbal           | 3.2x10^6                   | Unsafe                             |
| Dr Nando             | No Isolate                 | Safe                               |
| Super bitters        | No Isolate                 | Safe                               |
| Ruzu bitters         | No Isolate                 | Safe                               |
| Weifa body defense   | No Isolate                 | Safe                               |
| Bitterkinga          | No Isolate                 | Safe                               |
| Yoyo bitters         | No Isolate                 | Safe                               |

Mean Total Viable Count (TVC) = 1.4845x10^6
results are similar with the findings of Esimone et al., (4) and Tatfeng et al., (9), both of whom detected bacterial agents at varying frequencies in herbals and polyherbals samples in Nigeria. The predominance of *E. faecalis* and *S. aureus* in the packaged and unpackaged herbals respectively is in consonance with the study by Esimone et al., (4) who investigated the microbiological quality of liquid herbal preparations in south-eastern Nigeria and isolated arrays of microbial contaminants including *S. aureus* and *E. faecalis* as the most predominant bacterial contaminants of herbal medicines.

The higher frequency of contamination observed in the unpackaged group of polyherbals (81.8%) and higher median total viable count (TVC) compared to those of the packaged group may be attributable to contamination due to lack of standardization and quality control, poor personnel hygiene and handling, and use of contaminated water and raw materials (4). These findings could also be due to the fact that the packaged polyherbal products are comparatively better regulated by government agencies such as the National Agency for Food and Drug Administration and Control (NAFDAC) and the State Ministries of Health who usually mandate the manufacturers of packaged polyherbals to adopt some level of good manufacturing procedure, safe handling measures during production and quality control (6).

This study shows that many polyherbal products sold in Nnewi are potentially contaminated by bacterial agents, some of which are potential pathogens of man. It is imperative that herbal medicine practitioners be enlightened on hygienic ways of preventing microbial contamination during polyherbal production.

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