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Public Health Implications of Coliform Contaminants in Non-packaged, Commercially Hawked Herbal Remedies Sold in Port Harcourt

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The long history of use of coliforms as indicators of microbiological quality of water and food materials constitutes a veritable rationale for the ascertainment of the microbiological status of locally brewed, liquid herbal remedies, commercially hawked and widely consumed within Port Harcourt metropolis as envisaged in this study. Seventy two samples of twelve different non-packaged, oral herbal remedies commonly known as "Agbo" – the Yoruba word for medicine - were purchased from six different localities within Port Harcourt City and Obio/Akpor local governments of Rivers state. They were analyzed within two hours for total heterotrophic bacterial counts and total coliform counts. The bacterial isolates were characterized and identified by Gram staining and Biochemical tests using standard methods. The total coliform count is 4.53±1.11 Log_{10}cfu/ml ranging from 3.69±0.85 for Sexually Transmitted Diseases (STD) remedies to 5.46±1.15 Log_{10}cfu/ml for the cure-all (Gbogbonise) remedies. The mean total coliform counts (TCC) for the remedies are 4.53±1.00 Log_{10}CFU/ml. Gbogbonise had the highest TCC of 5.46±1.15 Log10 cfu/ml, while STD remedies had the least TCC of 3.69±0.85 Log_{10}cfu/ml. Out of a total of one hundred and fifty six isolates there were seventy three coliforms constituting 46.8% of all isolates. They include *Enterobacter aerogenes* (19; 12.2%), *Enterobacter cloacae* (18; 11.5%), *Klebsiella pneumoniae* (10; 6.4%) *Citrobacter rodentium* (7;4.5) *Enterobacter pyrinus:* (5;3.2%)

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Enterobacter hormaechei (3; 1.9%) *Klebsiella oxytoca* (3; 1.9%) *Serratia marcescens* (3;1.9%) *Serratia rubidaea* (3; 1.9%) *Hafnia alvei* (1; 0.6%) and *Pantoea dispersa* (1; 0.6%) The high prevalence of coliform contamination is an indication of poor microbial quality, implying that consumption of the products may pose potential health hazards. Education and enlightenment of handlers on the principles of basic hygiene and good manufacturing principles, is advised to ensure that products have tolerable levels of bacterial contaminants.

Keywords: Coliform contamination; Herbal remedies; microbial quality; Indicator bacteria.

1. INTRODUCTION

A World Health Organization definition presents herbal medicine as the use of plant-derived materials or preparations for the treatment, diagnosis or prevention of illnesses and to maintain well-being [1]. The use of herbal medicine is a common practice in different cultures of the world, particularly in developing countries, despite limited scientific evidence establishing the safety and efficacy of such products [2]. The World Health Organization estimates that 40% of all healthcare services in China comprise traditional medicines and up to 80% of Africans still use traditional medicine to meet their healthcare needs [1,2]. Herbal remedies as a major part of traditional or ethnic medicine imply an existence that dates back to antiquity. People worldwide have been using herbal medicine for treatment, control and management of variety of diseases since pre-historic times [3]. The variant of herbal remedies known as “Agbo” are locally brewed, non-packaged, non-labeled, liquid, oral herbal remedies commonly hawked on the streets of most cities and villages across Nigeria. “Agbo” – the Yoruba word for medicine - are decoctions made by boiling medicinal plant materials in water which serves as the medium of extraction for the active ingredients. The decoctions kept in plastic bottles that had been used for table waters, packed in basins or buckets of between eight to ten different remedies and dispensed to consumers in small plastic cups.

Coliform bacteria, a non-taxonomic grouping of aerobic or facultative anaerobic, Gram-negative, non-spore-forming rods capable of fermenting lactose with the production of acid and gas at 32–35°C within 48 hours, have been used as indicator organisms for over a hundred years. Indicator organisms are used as determinants for the general microbiological condition of water, food. [4]; the related term, index organism is a predictor for the possible presence of ecologically similar pathogens, implying a potential public health hazard [4,5]. The presence of coliforms in water, food or items meant for human consumption may therefore be a pointer to likely threat to public health.

The choice of coliforms is partly due to the dominant role of water in the preparation of herbal remedies being used in boiling the plant material as a medium of extraction as well as for soaking, washing and other activities. It is apparently, the source of many associated microbial contaminants particularly coliforms. The water quality is thus a problematic issue in herbal remedies, given the little or no regulatory control over the activities of herbal practitioners who are mostly not schooled in the principles good manufacturing practices and the absence of potable water in many localities. The greatest public health risk from microbes in water is associated with, contamination with human and animal excreta, although other sources and routes of exposure may also be significant [1].

The World Health Organization recommends *Escherichia coli* or thermotolerant coliforms indicators of food and water quality, and as index organisms for the potential presence of fecal contamination and waterborne pathogens; [6,7].While *Escherichia coli* is considered the most suitable indicator organism due to their specificity to fecal sources of contamination, thermotolerant coliforms are also recommended as an acceptable surrogate; despite the recognition that thermotolerant coliforms group includes coliform species of environmental origin, and is therefore not likely specific to fecal contamination of such products [6,7]. This study was conceived to ascertain the degree of coliform contamination, as a pointer to the microbiological status of locally brewed, liquid herbal remedies, commercially hawked and widely consumed within Port Harcourt metropolis.

2. MATERIALS AND METHODS

2.1 Collection and Preparation of Samples

Seventy two samples, consisting of six samples for each of twelve different home prepared herbal

remedies commonly hawked along the streets of Port Harcourt, mostly by young women were purchased from hawkers within various localities of Port Harcout and Obio/Akpo Local Governments of Rivers State. The "Agbo" remedies are identified by their indicated ailments. For the purposes of this study, the following remedies were used: Abdominal Pain, Aphrodisiac, Cough, Diabetes, Dysmenorrhea, Gbogbonise (Cure All), General Infection, Laxative, Malaria, Rheumatism, Sexually Transmitted Diseases (STD) and Typhoid. They were neither packaged nor labeled and have no regulatory numbers. The samples were examined and relevant particulars recorded. They analyzed within two hours of purchase at the Department of Microbiology Laboratory, Rivers State University, and Port Harcourt.

2.2 Enumeration and Isolation of Bacterial Contaminants

Each sample of the remedies was gently shaken by inverting a number of times, and 1ml of the sample was transferred into 9 ml of sterile distilled water and mixed to obtain a 1 in 10 dilution. Then 1.0 ml aliquot of which was serially diluted in ten folds serial dilution up to the 10^8 dilution. Inoculation of the plates were carried out by transferring 0.1ml of each dilution into duplicate plates of prepared Nutrient agar, Blood agar, MacConkey agar and Eosin methylene blue agar and incubated at 37^{o}c for eighteen hours. The colony counts were read from dilutions of each of the samples with suitable countable plates ranging from 25 and 250 colonies' and appropriately multiplied with the final dilution factors to obtain the bacterial counts for each sample, expressed in colony forming units per ml (cfu/ml) (Silvestri *et al.,* 2017). The colonial appearances of the isolates were examined carefully and recorded before being aseptically sub cultured in Nutrient agar plates. The cultures were purified by streaking of single colonies identified on the basis of colonial features.

2.3 Characterization of Bacterial Isolates

The total of one hundred and fifty six bacterial isolates recovered from the herbal remedies were characterized on the basis of morphological and biochemical scheme described by Benson's Microbiological Applications Laboratory Manual [8,9]. The isolates were Gram stained to establish the Gram reaction/ cellular features and subjected to biochemical analysis. The morphological, biochemical, and physiological parameters encompassed, colony size, colony elevation, colony texture, gram reaction, cellular morphology, cellular arrangement, haemolysis, spores formation, pigments production, motility, catalase, oxidase, ,indole, production, methyl red, Voges-prauskuer, citrate utilization, urease production, H_2S , nitrates reduction, gelatin hydrolysis, starch hydrolysis, glucose, lactose, maltose, manitol, succrose, gas and oxidation/ fermentation. The data were inputted into the ABIS online bacterial identification software and the best matching results were compared with those of standard organisms [10, 9].

3. RESULTS

The results for the enumeration of the bacterial population are shown on Figs. 1 & 2. The mean total heterotrophic bacterial count (THBC) for the remedies is 5.00±1.04 Log_{10}cfu/ml. The most contaminated remedy is Gbogbonise remedy with a THBC of 5.99±0.88 Log_{10}cfu/ml, while the least contaminated is Typhoid fever remedy with a THBC of 3.93±0.58 Log_{10}cfu/ml. (Fig. 1). The mean total coliform counts (TCC) for the remedies are 4.53±1.00 Log_{10}cfu/ml. Gbogbonise remedies had the highest TCC of 5.46±1.15 Log_{10}cfu/ml, while STD remedies had the least TCC of 3.69±0.85 Log_{10}cfu/ml. (Fig. 2)

The results of the frequency and occurrence of the isolates are shown on Table 1**.** A total of one hundred and fifty six bacterial contaminants were recovered from all the herbal remedies comprising of seventy three (46.8%) coliform bacterial isolates. The coliform also constitutes a majority of the nineteen bacterial species with a dominant 11 (57.9%) species, while the other bacteria recorded 8 (42.1%) of the species. The most dominant of the coliform isolates was *Enterobacter aerogenes* (19; 12.2%) found in (7; 58.3%) of the remedies, followed by *Enterobacter cloacae* (18;11.5%), the most widespread coliform occurring in 9 (75%) remedies. The others include: *Klebsiella pneumoniae* (10 ;6.4%) with occurrence of (5; 41.7%), *Citrobacter rodentium* (7;4.5) occurrence: (2;16.7%), *Enterobacter pyrinus:* (5;3.2%) occurrence: (4; 33.3%), *Enterobacter hormaechei* (3; 1.9%) occurrence; (2;16.7%) *Klebsiella oxytoca* (3; 1.9%) occurrence: (1;8.3%) *Serratia marcescens* (3;1.9%) occurrence; (2; 16.7%), *Serratia rubidaea* (3; 1.9$) occurrence; (2; 16.7%) *Hafnia alvei* (1; 0.6%) occurrence; (1; 8.3%) and *Pantoea*

dispersa (1; 0.6%) occurrence: (1; 8.3%). Other bacteria isolated from the herbal remedies amounts to a frequency of 83 (53.2%) and 8 (42.1%) of the species.

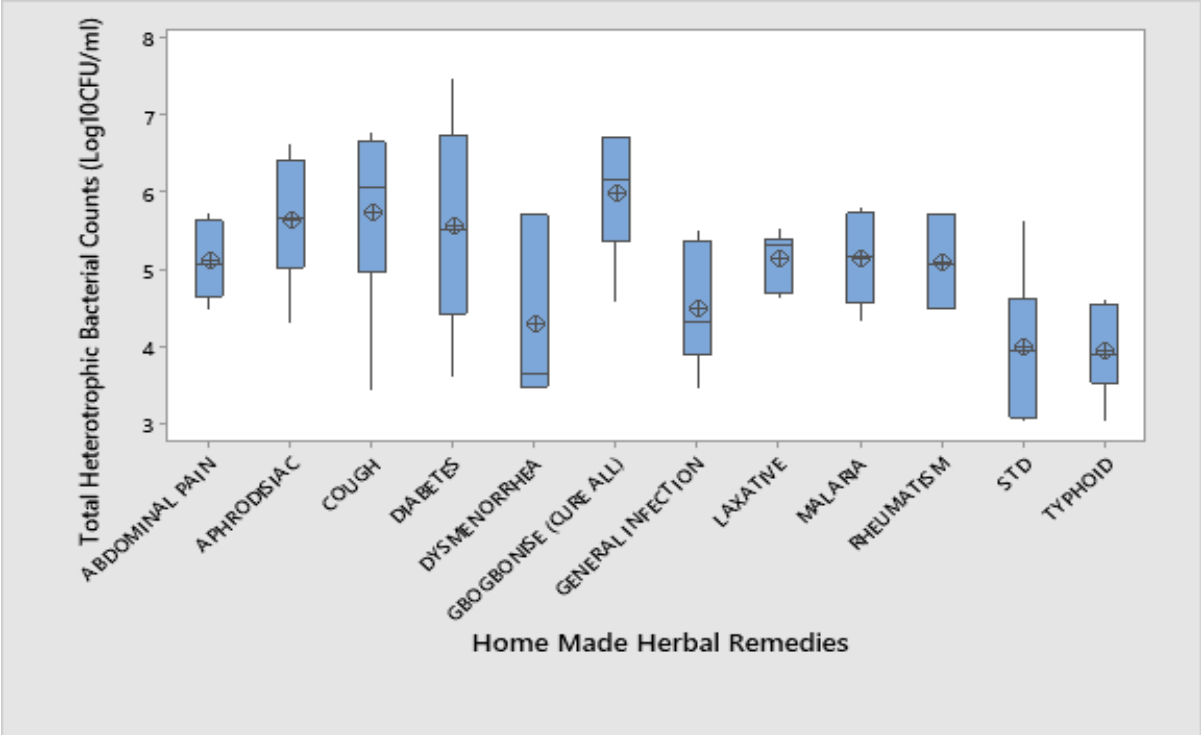

Fig. 1. Variations in mean Total Heterotrophic Bacteria Counts (THBC) of the studied Non-packaged Herbal Remedy Samples

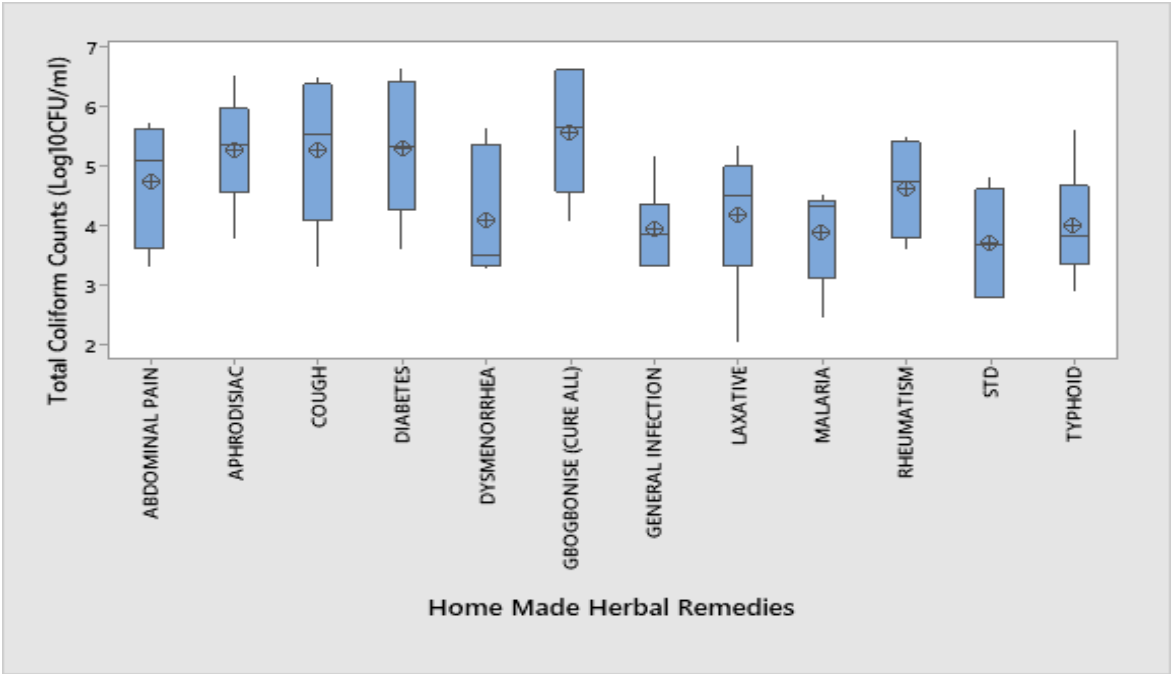

Fig. 2. Variations in mean Total Coliform Counts (TCC) of the studied Non-packaged Herbal Remedy Samples

Table 1. Frequencies and Occurrence of Bacterial Contaminants Isolated from Non-packaged Herbal Remedy Samples

Species of Contaminants	Frequency (%)	Occurrence in Herbal Remedies (%)
Staphylococcus aureus	47 (30.1)	11 (91.7)
Enterobacter aerogenes	19 (12.2)	7 (58.3)
Enterobacter cloacae	18 (11.5)	9 (75)
Klebsiella pneumoniae	10 (6.4)	5 (41.7)
Staphylococcus epidermidis	9 (5.8)	3 (25)
Bacillus subtilis	8 (5.1)	4 (33.3)
Citrobacter rodentium	7 (4.5)	2 (16.7)
Bacillus cereus	6 (3.9)	2 (16.7)
Pseudomonas aeruginosa	5 (3.2)	3 (25)
Enterobacter pyrinus	5 (3.2)	4 (33.3)
Enterobacter hormaechei	3 (1.9)	2 (16.7)
Klebsiella oxytoca	3 (1.9)	1 (8.3)
Proteus mirabilis	3 (1.9)	2 (16.7)
Serratia marcescens	3 (1.9)	2 (16.7)
Serratia rubidaea	3 (1.9)	2 (16.7)
Streptococcus pyogenes	3 (1.9)	1 (8.3)
Salmonella pullorum	2 (1.3)	1 (8.3)
Hafnia alvei	1(0.6)	1 (8.3)
Pantoea dispersa	1(0.6)	1 (8.3)
Total	**156** (**100**)	

Table 2. Distribution of Bacterial Isolates among the respective Herbal Remedies

Remedies	Isolates
Abdominal pains	*Enterobacter cloacae* (2) *Pantoea dispersa* (1), *Klebsiella pneumonia*e (3)
Aphrodisiac	*Citrobacter rodentium* (2), *Enterobacter cloacae* (2), *Klebsiella pneumonia*e (1)
Cough	*Hafnia alvei* (1), *Klebsiella oxytoca* (3), *Enterobacter hormaechei* (1)
Diabetes	*Enterobacter aerogenes* (2),*Enterobacter pyrinus* (1), *Serratia rubidaeae* (2)
Dysmenorrhea	*Enterobacter cloacae* (1), *Klebsiella pneumonia*e (2), *Serratia marcescens* ()
Gbogbonise (Cure all)	*Enterobacter aerogenes* (3),*Enterobacter cloacae* (3)
General infection	*Enterobacter aerogenes* (5), *Enterobacter cloacae* (3), *Serratia marcescens* ()
Laxative	*Enterobacter aerogenes* (3), *Enterobacter cloacae* (1)
Malaria	*Citrobacter rodentium* (5), *Enterobacter aerogenes* (3), *Enterobacter cloacae* (1), *Enterobacter pyrinus* (2)
Rheumatism	*Enterobacter aerogenes* (1), *Enterobacter pyrinus* (1), *Klebsiella pneumonizae* (2)
STD	*Enterobacter cloacae* (4), *Enterobacter pyrinus* (1), *Klebsiella pneumonia*e (2)
Typhoid	*Enterobacter aerogenes* (2), *Enterobacter cloacae* (1), *Enterobacter hormaechei* (2), *Serratia rubidaea* (1)

4. DISCUSSION

A total of seventy four isolates, constituting 46.8% of the one hundred and fifty six bacterial contaminants found in the non-packaged herbal remedies were coliforms. They were spread among all (100%) of the herbal remedies. This compares substantially to some previous studies on herbal remedies where total coliforms were

recovered from 77.8% of the herbal medicines samples [11]; also coliforms were recovered from 81.7% of traditional liquid herbal medicinal products in Kenya [12]. In four (33.3%) of the herbal remedies, the total coliform counts exceeded the WHO maximum limit of 10^3/ml of herbal remedies for enterobacteriae [13]. This closely aligns with studies where 46.1% of remedies had coliform levels above acceptable limits [14]. In all these results are pointers to the high prevalence of coliform contaminants in herbal remedies.

The coliform isolates were distributed among six genera and eleven species. The population was predominated by the four species of the *Enterobacter* namely *E. aerogenes* (12.2%)*, E. cloacae,* (11.5%), E. *pyrinus* (3.2%). *a*nd *E. hormaechei* (1.9%). The presence of this genus aligns relatively with previous studies where *Enterobacter* species had been isolated from herbal remedy samples. *Enterobacter cloacae* (12%) and *Enterobacter aerogenes* (2.7%), had been reported in herbal remedies [15]; *while* Walther et al. [12] isolated *Enterobacter aerogenes* (29.2%) and *Enterobacter cloacae* (19.1%) from various herbal products. Many studies have reported different members of the genus particularly *E. cloacae* and *E. aerogenes,* in herbal products [16,12], *Enterobacter* in various terrestrial and aquatic environments such as water, sewage, and soil, as well as the intestinal tracts of endotherms are well-known nosocomial pathogens. They have been linked to bacteremia, endocarditis, septic arthritis, osteomyelitis, and skin/soft tissue infections, and lower respiratory tract- urinary tract and intra-abdominal infections and a contaminant of various medical, intravenous, and other hospital devices [17]. *E. aerogenes* is reported to be an important human opportunistic pathogen found in the gastrointestinal tract and environmentally; and associated with hospital-acquired infections such as pneumonia, bacteremia, urinary tract infection, surgical site infection, and meningitis, exhibiting resistance to multiple common antibiotics [18].

Two species of the genus *Klebsiella namely K oxyteca* (1.9%) and *K pneumoniae* (6.4%) were isolated from the remedies in the present study. A number of previous studies have also reported the presence of this genus in herbal remedies. Walther *et al.* [12] reported that 34.8% of contaminants found in traditional liquid herbal medicinal products were *Klebsiella pneumoniae*, while Yusuf *et al.,* [15] identified *Klebsiella pneumoniae* as amounting to 7.3% of contaminants. This genus is known to reside in the gastrointestinal tracts from where they spread and cause infections [19]. *Klebsiella pneumoniae* is an opportunistic bacteria linked to urinary tract infections, bacteriamia, pneumonia, lung disorders, nosocomial infections [19]. *Klebsiella* spp. has been reported as a contaminant in herbal remedies in several studies [16,12].

The 4.5% of *Citrobacter rodentium* found in this study follows previous reports of *Citrobacter* species as contaminants in herbal remedies, especially the reported 8.0% of contaminants [15]. Some other studies have also repoted *Citrobacter* spp in herbal medicines [20]. *Citrobacter rodentium* is an extracellular enteric mucosal murine pathogen, found shares several pathogenic mechanisms with enteropathogenic *Escherichia coli* and enterohaemorrhagic *E. coli*, which are clinically important human gastrointestinal pathogens. It is associated with a self-limiting infection, which may resolve without antimicrobial therapy, and elicit forceful colitis, or other colonic conditions [21,22].

While the two species of *Serratia* namely *S. marcescens* and *S rubidaea* equitably contributed 3.8 % to the prevalence of the contaminants, a close prevalence of 3.3% had been reported in studies of herbal remedies [15]. *Serratia* are widely distributed in the environment, being found in the soil, food and water among others. Previously considered non-pathogenic, *Serratia marcescens* was reported to have assumed prominence as opportunistic pathogens associated with nosocomial outbreaks in neonatal intensive care Units, and linked with cases of bacteremia, urinary tract infections, meningitis, pneumonia, and surgical site infections [23,24]. *Serratia rubidaea* is usually nonpathogenic as been associated with invasive infections, such as pulmonary infection and traumatic infection immune-compromised persons [25].

Pantoea dispersa is a rare pathogen in clinical settings, reported to cause respiratory infections, neonatal sepsis, and bloodstream infections [26]. They have been reported in herbal remedies [16].

Hafnia alvei is a rare pathogen that has been implicated in both nosocomial and community-acquired infection; predominantly isolated from

the respiratory and gastrointestinal tract, with a few cases involving the blood, urinary tract, central venous catheters, and skin [27]. Its isolation may not out of place given its ecological antecedents, though it was not found to have been reported as a common contaminant in herbal remedies.

The absence of the archetypal faecal coliforn *Escherichia coli* among the isolates may appear to be a pointer that the samples are free from faecal contamination; however the relatively high levels of the thermotolerant species of *Klebsiella, Enterobacter* and *Citrobacter* as well as the presence of *Salmonella* in the remedies leads to considerations of the ability of *Escherichia coli* to survive for long outside the body of endotherms. The persistence of *Escherichia coli* and other faecal indicator bacteria particularly coliforms in the environment has been attributed to be dependent on a number of factors including the physicochemical status of the environment such as changes in temperature, pH, humidity, salinity the presence of other bacteria, viruses and predators and the metabolic capacities of the bacteria [28]. *Escherichia coli* is a commonly reported contaminant in herbal products in varying prevalences such as 4.5% [12] 6.7% [15] and 25.8% [11] while in this study like in a number of others *E coli* were not recovered from herbal remedies [29, 16].

5. CONCLUSION

The isolation of eleven species of coliform bacteria representing 46.8% of the total bacterial contaminants in the non-packaged herbal remedies is certainly a cause for concern. The degree of coliform contamination was high and every tested remedy yielded some strains of coliforms. This has obvious public health implications as indicator and index organisms. Their prevalence in the remedies is a pointer to a poor bacteriological quality of the products and a high likelihood of being contaminated with pathogenic bacteria. It is thus suggested that producers of the remedies need to be educated and enlightened on the principles of basic hygiene and good manufacturing principles to ensure the production of products with tolerable bacterial contaminants.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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