Microbial Contamination of Herbal Preparations on the Ghanaian Market, Accra

Samuel Darkwah1, Doris Agbettor2, Francis Codjoe2 and Eric S Donkor1

1Department of Medical Microbiology, University of Ghana Medical School, University of Ghana, Accra, Ghana. 2Department of Medical Laboratory Sciences, School of Biomedical and Allied Health Sciences, University of Ghana, Accra, Ghana.

ABSTRACT: In developing countries, an estimated 80% of the population use traditional herbal medicines as part of their primary health care. As the market for herbal medicine expands in many African countries, partly due to their use in the treatment of COVID-19, there is the need to address all the associated safety issues. The aim of the study was to evaluate the microbial contamination of locally prepared, as well as imported foreign herbal products sold in Accra. Standard microbiological methods were employed in the enumeration of coliforms and the identification of pathogenic microbes in 60 herbal preparations that were sampled. A larger proportion (76.7%) of local herbal preparations was contaminated with bacteria as compared with imported ones (63.3%). Bacillus species and Pseudomonas aeruginosa were the predominant bacteria obtained from foreign and locally manufactured herbal preparations, respectively. A proportion of 36.7% (11) of the local samples were positive for coliform and the coliform counts ranged from $3.0 \times 10^1\text{cfu/ml}$ to $2.0 \times 10^4\text{cfu/ml}$. Two foreign herbal samples (6.7%) were positive for coliforms; one had a count of $1.7 \times 10^5\text{cfu/g}$ while the other had $2 \times 10^4\text{cfu/g}$. Herbal preparations sold in markets of Accra harbour several microbial pathogens; the risk is relatively higher for locally produced herbal preparations compared to imported herbal preparations. As a result, it is recommended that quality assurance in the production of local herbal preparations should be thoroughly monitored from the beginning of production to the final selling of the preparations. There is also the need to strengthen microbiological safety monitoring of imported herbal preparations.

KEYWORDS: Herbal, contamination, safety, coliforms, Pseudomonas aeruginosa

Introduction

In developing countries, an estimated 80% of the population use traditional herbal medicines as part of their primary health care.1-4 Hygienic safety of herbal preparations is a major issue in the developing world and there are concerns that such preparations may be produced under poor hygienic conditions and pose risk to consumers. The major sources of microbial contamination of these herbal preparations are from raw materials used for the preparation, handling and processing, their storage and transportation.5-7 Some of the common microbial pathogens found in herbal preparations are Escherichia coli, Salmonella typhi, Pseudomonas aeruginosa, Staphylococcus aureus, Bacillus spp, Enterobacter spp, Klebsiella pneumoniae and Proteus mirabilis.8,9 As the market for herbal medicine expands in many African countries, partly due to their use in the treatment of COVID-19,10 there is the need to address all the associated safety issues. In Ghana, there is very limited information on the microbiological safety of herbal preparations available on the market.11-13 To help fill this research gap and also provide policy relevant information, this preliminary study was carried out. The aim of this study was to evaluate the microbial quality of both locally manufactured and imported herbal extracts sold in Accra (Ghana).

Methods

Collection and preparation of herbal extract samples

Thirty imported and 30 locally branded herbal preparations used for this study were purchased from 3 major markets across the capital city of Ghana (Accra) from herbal and pharmaceutical shops, drug stores and hawkers. All the purchased herbal preparations were transported into the laboratory and were kept in a clean, cool and dry cabinet away from direct sunlight, prior to testing. The type of herbal preparation, manufacturing and expiration dates were recorded. Samples obtained in suspensions were 10 times diluted with sterile phosphate buffered saline (PBS). Similarly, 1 g of powder, capsulated and tableted forms of herbal extracts were dissolved in 9 ml of sterile PBS. Ten-fold serial dilutions of the samples were prepared up to $10^{-4}$.

Coliform enumeration

Coliform contamination was assessed for all samples using the pour-plate bacteria culture method.14 Briefly, 1 ml of the diluted sample from $10^{-1}$ to $10^{-4}$ dilutions was aseptically added to 20 ml of molten MacConkey agar, previously cooled to 40°C and well mixed by gentle rocking. The mixture was allowed to set and incubated at 37°C for 24 hours. Pink colony-forming coliform bacteria growth were then enumerated manually.
Isolation and identification of bacterial contaminants

Dilution in fractions of $10^{-1}$ of the prepared samples were centrifuged at 5000 rpm for 5 minutes and the pellets were inoculated on culture plates of Blood agar, MacConkey agar, Deoxycholate Citrate agar and Eosin Methylene Blue agar. The plates were incubated at 35°C for 18 to 24 hours. Bacteria isolates were identified based on colonial morphology, gram staining and a battery of biochemical tests.\textsuperscript{15}

Results

Herbal remedies sold in Accra have diverse geographical origin

The 30 imported samples originated from 9 countries, representing 4 continents including Europe, Asia, North America and Africa (Table 1). All imported samples in the form of liquids, capsules, teabags, and tablets were branded (90%) however, 3 samples in powdered form were unbranded (10%). Locally manufactured herbal medicines obtained for this study also originated from various parts of the country. Of note, about 16.7\% of locally manufactured herbal preparations lacked appropriate registration details such as registration number, however, none of the preparations had expired.

Local brands of herbal remedies are likely to be contaminated with coliforms

Twenty-eight (93.3\%) out of 30 foreign herbal samples were negative for coliform; 2 coliform positive samples were from China ($1.7 \times 10^3 \text{ cfu/g}$) and Thailand ($2 \times 10^4 \text{ cfu/g}$). Eleven (36.7\%) of the 30 local samples were positive for coliform and the coliform counts ranged from $3.0 \times 10^1$ to $2.0 \times 10^4 \text{ cfu/ml}$, with an average of $3.3 \times 10^3 \text{ cfu/ml}$.

Herbal preparations sold in Accra contain bacterial and fungal pathogens

Overall, 12 different microbial pathogens were isolated from the herbal preparations (Table 2): both foreign and local herbal preparations yielded 10 organisms each (Table 3). A larger proportion (76.7\%) of local herbal preparations were contaminated with bacteria as compared with imported ones (63.3\%). \textit{Bacillus spp} and \textit{Pseudomonas aeruginosa} were the predominant bacteria obtained from foreign and locally manufactured herbal preparations, respectively. \textit{Candida species} was the only fungus identified in the study, obtained from 3.3\% and 10\% of local and foreign herbal preparations, respectively (Table 2).

Discussion

In this study, we observed that herbal preparations sold on the Ghanaian market, specifically in Accra, were diverse in origin.
and presented in many forms. Locally manufactured herbal preparations were sold mainly as liquids whereas imported ones had a variety of forms ranging from liquid suspension to capsules and hard tablets. We identified potential contamination of both local and imported herbal preparations with local brands implicated in a relatively higher potential. The general absence of coliform in the foreign herbal samples may be due to the continual monitoring of the microbiological safety of imported commodities in Ghana by the Food and Drugs Authority (FDA). The relatively high contamination of locally produced herbal preparation in this study is similar to that reported in several other developing countries such as Iran, Togo and Nigeria. In Nigeria, Olonitola et al reported that about 47% of herbal remedies sampled from the Kaduna metropolis, Nigeria were contaminated with Salmonella typhi, Shigella, Staphylococcus aureus and Escherichia coli.

Contaminants of herbal preparations may be introduced in various ways; through processing, handling and storage of herbal preparations. Plants, including herbs and their exudates, have high levels of different types of carbohydrates, minerals and vitamins; key factors that influence the growth of microbes. Coliform enumeration of herbal preparations is an indicator of their hygienic quality and safety. Pseudomonas aeruginosa, the most predominant bacteria isolated from contaminated local herbal samples in the study is a soil derived bacterium implicated in urinary tract and respiratory infections. Its presence suggests improper washing and handling of herbs. Intestinal bacteria such as E. coli, Enterobacter and Citrobacter species isolated indicate contamination by faces along the line of production and is a sign of poor hygienic practices and inadequate handling of the herbal preparations. Bacteria such as E. coli and Salmonella species should never be present in preparations that will be ingested, according to European pharmacopoeia, 2011. The presence of Bacillus, the second dominant bacterium, may be due to inadequate cleaning and handling processes in their preparations, to eliminate sporoc-containing soil that contaminate the raw materials. Staphylococcus aureus is a normal flora of the nasal passage and skin, however, their presence in herbal preparations to be ingested orally may result in food poisoning and gastro-intestinal related infections. Worthy of mention is the bacterium Shigella sonnei isolated from one of the locally branded samples, that causes shigellosis. Individuals infected with this bacterium present bloody diarrhoea, fever and stomach cramps which can be fatal, especially in children and patients with health co-morbidities. Shigella sonnei contamination is alarming because the bacterium is known to have high infectivity, thus, even small amount of the bacteria (as low as 10-200 bacteria) can be enough to warrant infection in humans. Yeast and moulds such as Candida potentiate the quick spoilage of the herbal medicine and introduction of toxins by fungal activity (mycotoxigenic) into packaged products. Fungi secrete enzymes like proteases, lipases and amylases that are used in the degradation of proteins, lipids and starch present in the preparations.

In conclusion, herbal preparations sold in markets of Accra harbour several bacteria, including microbial pathogens of medical and public health importance such as Shigella spp, Escherichia coli and Enterobacter spp. Such findings precipitate the potential risk of severe microbial infections gastroenteritis, to consumers of contaminated herbal preparations. The potential risk, from our results is relatively higher for locally produced herbal preparations compared to imported herbal preparations sold in Accra. As a result, it is recommended that quality assurance in the production of local herbal preparations should be thoroughly monitored from the beginning of production to the final selling of the preparations. Additionally, further studies targeted at investigating the risk to consumers using appropriate risk assessment models is imperative to better understand the public health impact of microbial contamination of herbal preparations. There is also the need to strengthen microbiological safety monitoring of imported herbal preparations.

Author Contributions
Conceptualization: ESD. Methodology: ESD, DA and FC. Validation: ESD, DA and FC. Data curation: SD and DA. Data analysis: SD, DA and ESD. Investigation/Laboratory work: SD, DA. Resources: ESD, FC, SD and DA. Supervision: ESD and FC. Writing—original draft preparation: SD and DA. Writing—review and editing: SD, ESD, FC and DA. All authors have read and approved the final version of the manuscript.

REFERENCES
1. Umair M, Altaf M, Abbasi AM. An ethnobotanical survey of indigenous medicinal plants in Hafizabad district, Punjab-Pakistan. PLoS One. 2017;12: e0177912.
2. WHO. Traditional Medicine and Modern Health Care: Progress Report by the Director General. World Health Organization; 1991. Document No. A 44/10. March 22, 1991.
3. WHO. Traditional Medicine Strategy 2002–2005. World Health Organization; 2002.
4. Kretchy IA, Koudah A, Oponi KFM, et al. Prevalence, patterns and beliefs about the use of herbal medicinal products in Ghana: a multi-centre community-based cross-sectional study. Trop Med Int Health. 2021;26:410-420.
5. Rajkumar Dattatraya B, Sriram P. Evaluation of microbial load of herbal raw materials: a necessary quality control measure to ensure safety of finished herbal preparations. Adv Biotech & Micro. 2016;16:55934.
6. Abtahi F, Nourani SL. The most important fungal diseases associated with some useful medicinal plants. In: Ghorbanpour M, Varma A, eds. Medicinal Plants and Environmental Challenges. Springer International Publishing AG; 2017:279-293.
7. Khattak F. Microbiological quality assessment of commercially available medicinals plants in Peshawar city, Pakistan. Pak J Bot. 2012;44:1203-1208.
8. de Sousa Lima CM, Fujishima MAT, de Paula Lima B, Mastroianni PC, de Sousa FFO, da Silva JO. Microbial contamination in herbal medicines: a serious health hazard to elderly consumers. BMC Complement Med Ther. 2020; 20:17.
9. Yesuf A, Woodmeneny E, Gebrechorkos T, Moges F. Occurrence of potential bacterial pathogens and their antimicrobial susceptibility patterns isolated from herbal medicinal products sold in different markets of Gondar town, Northwest Ethiopia. Int J Bacteriol. 2016;2016;1959418.
10. Denke CA, Woldeyohannis AE, Kifle ZD. Herbal medicine use for the management of COVID-19: a review article. Metafor Open. 2021;12:100141.
11. Dei-Tutus A, Amuna P, Rahman MA. Rapid detection of microbial contamination in Ghanaian herbal medicines by PCR analysis. Ghana Med J. 2014;48:106-111.
12. Turkson BK, Mensah MLK, Sam GH, et al. Evaluation of the microbial load and heavy metal content of two polyherbal antimalarial products on the Ghanaian market. Evid Based Complement Alternat Med. 2020;2020:1-5.
13. Ampofo JA, Andoh A, Tetteh W, Bello M. Microbiological profile of some Ghanaian herbal preparations – safety issues and implications for the health professions. Open J Med Microbiol. 2012;02:121-130.
14. Sanders ER. Aseptic laboratory techniques: plating methods. J Vis Exp. Published online May 11, 2012. doi:10.3791/3064
15. Cheesbrough M. District Laboratory Practice in Tropical Countries, Part 2. Second Edition. Cambridge University Press; 2006.
16. Easvatierd R, Asgarirad H, Kazemi-Sani B. Microbial quality of some herbal solid dosage forms. Afr J Biotechnol. 2010;9:1701-1705.
17. de Souza C, Ameyapoh Y, Karou SD, Anani KT, Kpodar ML, Gbeassor M. Assessing market-sold remedies in lomé (togo) for hygienic quality. Biotechnol Res Int. 2011;2011:572521.
18. Abba D, Inabo HI, Yakubu SE, Olonitola OS. Contamination of herbal medicinal products marketed in Kaduna Metropolis with selected pathogenic bacteria. Afr J Trad CAM. 2010;6:70-77.
19. Brimecombe MJ, De Leij FA, Lynch JM. The effect of root exudates on rhizosphere microbial communities. In: Pinton R, Varanini Z, Nannipieri P, eds. The Rhizosphere: Biochemistry and Organic Substances at the Soil and Plant Interface. Marcel Dekker; 2001;95-140.
20. Ratajczak M, Kubicka MM, Kamińska D, Sawicka P, Długaszewska J. Microbiological quality of non-sterile pharmaceutical products. Saudi Pharm J. 2015;23:303-307.
21. Famewo EB, Clarke AM, Afolayan AJ. Identification of bacterial contaminants in polyherbal medicines used for the treatment of tuberculosis in Amotile district of the Eastern Cape province, South Africa, using rapid 16S rRNA technique. J Health Popul Nutr. 2016;35:27.
22. De Freitas Araujo MG, Bauab TM. Microbial quality of medicinal plant materials. In: Akyar I, ed. Latest Research into Quality Control. IntechOpen; 2012;67-81.
23. Guidi F, Duranti A, Gallina S, et al. Characterization of a staphylococcal food poisoning outbreak in a workplace canteen during the post-earthquake reconstruction of Central Italy. Toxins. 2018;10:523.
24. Aslam A, Okafor CN. Shigella. In: StatPearls [Internet]. StatPearls Publishing; 2022. https://www.ncbi.nlm.nih.gov/books/NBK482337
25. Baker S, The HC. Recent insights into Shigella. Curr Opin Infect Dis. 2018;31:449-454.
26. Mandeel QA. Fungal contamination of some imported spices. Mycopathologia. 2005;159:291-298.