Microbial contamination of herbs marketed to HIV-infected people in Nairobi (Kenya)

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Dates:
Received: 21 Dec. 2010
Accepted: 10 Apr. 2012
Published: 12 Sept. 2012

How to cite this article:
Kaume L, Foote JC, Gbur EE. Microbial contamination of herbs marketed to HIV-infected people in Nairobi (Kenya). S Afr J Sci. 2012;108(9/10), Art. #563, 4 pages. http://dx.doi.org/10.4102/sajs.v108i9/10.563

Herbal products are used by human immunodeficiency virus (HIV)-infected individuals regardless of safety or efficacy concerns. In this study, we examined the microbiological quality of herbal preparations marketed to HIV-infected individuals. A convenience sample (N = 24) of herbal products was obtained from retailers in Nairobi, Kenya in 2007. Petrifilm plate count methods were used to estimate total aerobic bacteria (APC), coliform, *Escherichia coli*, *Staphylococcus aureus* and yeast and mould counts. APC counts ranged from an estimated 1.5×10^1 colony forming units (CFU)/g to 7.1×10^8 CFU/g. Total and faecal coliform counts ranged from an estimated <10 CFU/g to 3×10^6 CFU/g. *E. coli* load ranged from <10 CFU/g to 5×10^1 CFU/g and *S. aureus* counts ranged from an estimated <10 CFU/g to 2.5×10^3 CFU/g. Yeast and mould counts ranged from an estimated <10 CFU/g to 9×10^4 CFU/g. An evaluation using the World Health Organization limits for medicinal herbs found a percentage of samples to contain microorganisms above allowable limits: 33% (APC), 50% (coliforms) and 33% (yeast and moulds). A total of 67% of samples contained *S. aureus* loads above the United States Pharmacopeia standard. We suggest that the introduction of quality-control measures and safe handling practices for the selling of medicinal herbs and botanicals in Kenya would be beneficial in reducing the potential health risks for immunocompromised consumers of these products.

Introduction

Over 33 million people are infected with the human immunodeficiency virus worldwide.[1] Sub-Saharan Africa and developing countries in general have the highest HIV prevalence and new HIV infections.[1] In addition to costly and inaccessible antiretroviral drugs, sub-Saharan Africa has a high patient to doctor ratio (40 000:1). These factors lead to the increased use of medicinal herbs, especially for HIV-related complications.[2,3] In fact, numerous authors, including the World Health Organization (WHO), report that 30% – 70% of HIV-infected individuals worldwide use herbal products.[4,5,6]

Although consumers consider herbal products to be safe, microbial contamination in medicinal herbs is a concern, especially for HIV-infected individuals as a result of their compromised immunity.[7] Medicinal herbs frequently contain microorganisms indigenous to the soil and plants where they are grown; and insufficient sanitation in primitive harvest and postharvest conditions allows their survival.[8,9] A South African study reported contamination of herbal products with *Bacillus* spp., *Enterobacteriaceae* spp., *Salmonella* spp., *Staphylococcus aureus*, *Penicillium* and *Aspergillus*.[10] Moreover, elevated levels of bacterial and fungal contaminants, such as *Penicillium* spp., *Aspergillus* and *Fusarium*, have been observed in herbs and spices.[9,11]

The purpose of this study was to examine the microbiological quality of herbal products marketed to HIV-infected individuals in Kenya and to evaluate the safety of these products using microbial contamination limits set by the WHO.[12] The United States Pharmacopeia (USP) standard[13] was used for *S. aureus* because there is no applicable WHO standard. Two established WHO microbial load limits were used: Limit I for herbal products to which boiling water is added in order to prepare herbal teas and infusions and Limit II for herbal products prescribed for internal use with no heat treatment.[12] To our knowledge, no other study has investigated the microbiological quality of herbal products used by HIV-infected individuals in Kenya.

Materials and methods

Sample collection and description

Laboratory work was registered and approved by the Institutional Biosafety Committee of the University of Arkansas (Fayetteville, AR, USA). From May to June 2007, a convenience sample of 24 different medicinal herbs was purchased from herbal product retailers in Nairobi, Kenya. Individual samples were not randomly collected because (1) certain parts of Nairobi are

inaccessible for security reasons, (2) some herbalists had only liquid mixtures, concoctions or extracts which were unsuitable for sampling because of shipping restrictions and (3) some retailers refused to sell their products to us for reasons unknown. For 20 of the samples, duplicates were bought at different time points; for the remaining four samples, duplicates were not available because the retailer either ran out of the product or was unavailable to sell a second sample. Samples were purchased from the following markets: Kibera, Kawangware, Shaurimoyo, along Ngong Road, Kamukunji, Ngara, Westlands, Kangemi and downtown Nairobi. To acquire samples for this study, one of us posed as a relative of a HIV-infected person. Herbal retailers or practitioners were requested to recommend herbal products for HIV-infected individuals. Using aseptic techniques, the herbal samples were packaged in labelled sterile bags and shipped to the University of Arkansas; each bag was treated as an experimental unit. Samples were mainly ground dry mixtures or pastes. No botanical identification of the samples was carried out as this was beyond the scope of our experiment.

Laboratory procedure

Samples were tested for aerobic plate count (APC), coliform count, *Escherichia coli* count, *S. aureus* count, and yeast and mould count (YM). Standard laboratory methods and procedures, consistent with the American Public Health Association's guidelines, were used.[14,15] Methods for using Petrifilm™ were followed according to instructions from 3M Microbiology Products (3M Microbiology Products, St. Paul, MN, USA). Petrifilm™ methods are reproducible, practical and accurate.[16]

Using aseptic techniques, 1 g of each herbal product sample was mixed with 99 mL Butterfield's phosphate buffer (Weber Scientific Hamilton, NJ, USA) and homogenised for 2 min at 200 rpm (Stomacher® 400, Seward Ltd, London, UK). To obtain a pH between 6.6 and 7.2, either 1 M sodium hydroxide or hydrochloric acid was added before serial dilutions were done. Aliquots of 1 mL for each dilution were plated in duplicate on Petrifilm™ media (3M Company, St. Paul, MN, USA) for APC, *E. coli*, *S. aureus* counts and YM. A Quebec dark field colony counter was used to manually count the colony forming units (CFU) per gram.

The negative control for each sample was Butterfield's phosphate buffer while positive controls were *S. aureus* (ATCC No. 25923), *E. coli* (ATCC No. 25922), *Rhizopus nigricans* (ATCC No. 14038) (all obtained from Presque Isle Cultures, Erie, PA, USA), and *Saccharomyces cerevisiae* (ATCC No. 2601; Quality Technologies LLC, Newbury Park, CA, USA).

Limitations

Liquid samples were not included in this study because of shipping regulations restricting their transport. It is conceivable that liquid samples and dry samples are contaminated with different microorganisms because of the difference in moisture content and preparation methods. Although the samples in this study were not randomly collected, they are representative of herbal products available in different areas of Nairobi at the time, but it is acknowledged that they may not reflect a possibly larger range of contamination issues. However, the difficulty in acquiring samples made the use of a convenience sample a necessity.

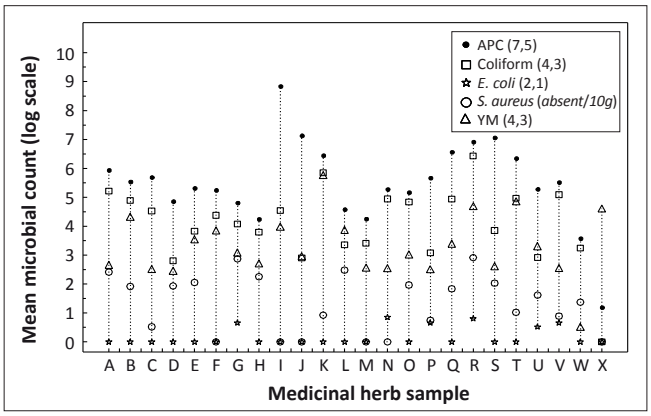
Statistical analysis

Microbial counts were transformed to common logarithmic values for statistical analysis. SAS® was used for all statistical analysis.[17] A significance level of 0.05 was used for all tests. An analysis of variance was used to test for differences between means for each of the microorganisms. A *t*-test was used to find significant differences between USP or WHO limits and sample microbial counts when sample means exceeded the established microbial limits. The degree of variability of microorganisms was evaluated and is presented in box plots; correlation coefficients were calculated to assess combination patterns in the occurrence of the five microorganisms. An overlay plot was constructed to graphically illustrate microorganisms found in each of the 24 samples.

Results

Aerobic bacteria were detected in all 24 samples. APC was the highest count (CFU/g) in 96% of samples, when compared to the other four microorganisms (Figure 1). Further analysis demonstrated that 4% of samples had aerobic bacteria levels above the WHO allowable limits for teas and infusions, and 33% of the samples were above the WHO allowable limit for internal use (Table 1). APC counts ranged from an estimated 1.5×10^1 CFU/g to 7.1×10^8 CFU/g with sample I and sample X containing the lowest and highest counts, respectively (Figure 1).

Coliforms were detected in 96% of the samples (Figure 1). Coliform counts ranged from an estimated <10 CFU/g to 3×10^6 CFU/g and were the second highest in both CFU counts per gram and occurrence (Figure 1). Of the samples,



APC, aerobic plate count; *E. coli*, *Escherichia coli*; *S. aureus*, *Staphylococcus aureus*; YM, yeast and moulds.
The first number in parentheses represents the World Health Organization[12] limit in colony forming units/g (log scale) for teas or infusions and the second number the limit for internal use. The *S. aureus* limit was obtained from the United States Pharmacopeia[13].

FIGURE 1: A plot showing the number of microorganisms contained in 24 samples, labelled A–X, of medicinal herbs marketed to HIV-infected individuals in Kenya.

TABLE 1: Summary of results showing the distribution of microorganisms (colony forming units/g) in 24 samples of medicinal herbs marketed to HIV-infected individuals in Kenya.

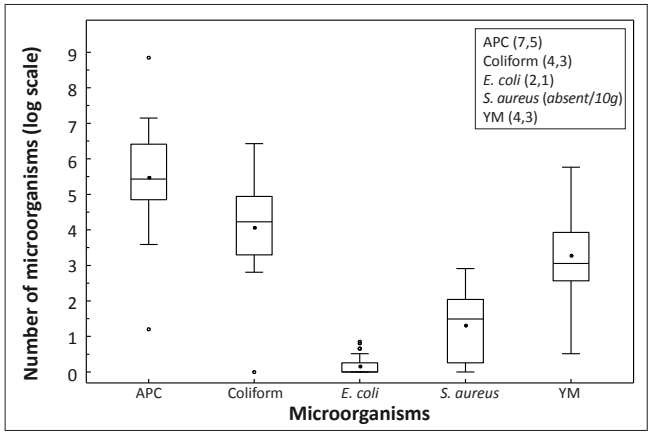
Microorganism	25th percentile	Median	75th percentile	Mean	WHO Limit I†	Samples above Limit I (%)	WHO Limit II†	Samples above Limit II (%)
Aerobic plate count	7.21×10^4	2.68×10^5	2.43×10^6	3.09×10^5	$\leq 10^7$	4	$\leq 10^5$	33
Coliforms	2.13×10^3	1.70×10^4	8.73×10^4	1.20×10^4	$\leq 10^4$	13	$\leq 10^3$	50
Escherichia coli	1.00×10^0	1.00×10^0	1.35×10^0	1.49×10^0	$\leq 10^2$	0	≤ 10	0
Staphylococcus aureus	2.46×10^0	3.14×10^1	1.09×10^2	2.11×10^1	absent/10g*	67	absent/10g*	67
Yeasts and moulds	3.66×10^2	1.14×10^3	1.14×10^3	1.96×10^3	$\leq 10^4$	13	$\leq 10^3$	33

†, Limit I is the World Health Organization (WHO) limit for herbal teas and infusions and Limit II is the WHO limit for internal use,[12] except for *S. aureus*, for which the limit was obtained from the *, United States Pharmacopeia[13].

13% had coliform levels above the WHO allowable limits for teas and infusions, and 50% had counts above the WHO allowable limit for internal use (Table 1). *E. coli* was detected in 25% of the samples (Figure 1), ranging from <10 CFU/g to 5×10^1 CFU/g; all *E. coli* counts were lower than the WHO limits (Table 1). *S. aureus* counts ranged from an estimated <10 CFU/g to 2.5×10^3 CFU/g, with 67% of samples containing *S. aureus* in quantities above that recommended by USP (Table 1). Yeast and mould counts were detected in all 24 samples tested (Figure 1) and ranged from an estimated <10 CFU/g to 9×10^4 CFU/g. Further analysis showed that 13% of samples had YM levels above WHO allowable limits for teas and infusions and 33% of the samples contained levels above the WHO allowable limit for internal use (Table 1). No attempt was made to identify yeasts and moulds to the species level, as this was beyond the scope of this study.

Side-by-side box plots show the distribution of microorganisms within the 24 samples (Figure 2). The distributions of *E. coli* and *S. aureus* are highly skewed to the right and left, respectively. Several outliers are shown for APC, coliforms and *E. coli* (Figure 2).

Correlation coefficients were calculated to investigate patterns in the occurrence of the different microorganisms within samples. Counts of APC in each sample were positively correlated to counts of coliforms (r = 0.65; 95% confidence interval = 0.33–0.83). No other significant correlations were observed between the numbers of the microorganisms in the samples (data not shown).



APC, aerobic plate count; *E. coli, Escherichia coli*; *S. aureus, Staphylococcus aureus*; YM, yeast and moulds.
The first number in parentheses represents the World Health Organization[12] limit in colony forming units/g (log scale) for teas or infusions and the second number the limit for internal use. The *S. aureus* limit was obtained from the United States Pharmacopeia[13].

FIGURE 2: Distribution of microorganisms in 24 samples of medicinal herbs marketed to HIV-infected individuals in Kenya.

Discussion

Aerobic bacteria occur naturally in plant-based products and are the most commonly used quality parameter for assessing the hygiene status of food and drug samples.[18] The WHO APC limit provided for herbal products made for internal use is 5 log units/g, and over one third of the samples in this study had APCs higher than this limit. This finding implies that conditions during harvest or postharvest were unsanitary. Storage tends to decrease APC counts in dried foods[19]; therefore variation in counts between samples may be as a result of differences in storage times.

Coliforms are found mainly in soil, vegetation and the faeces of warm-blooded animals.[9] Previous studies have reported high coliform counts, up to 3.4×10^4 CFU/g, in other botanical products such as chamomile.[20] In this study, we observed that 50% and 13% of the samples failed to meet the WHO requirement of less than 3 log units/g for teas and 4 log units/g for internal use. High coliform counts are an indication of poor hygiene and a lack of sanitation measures. Contamination results from several sources, including improper cleaning procedures and open air drying of plant materials which results in contamination with soils and dust.[21]

Although none of the samples in this study contained *E. coli* counts above the WHO standards of 2 log units/g for teas or infusions and 1 log unit/g for internal use, 25% of the samples were contaminated with *E. coli* at low levels. Detection of *E. coli* confirms faecal contamination, which is directly associated with unsanitary conditions. Because heat treatment of herbal products results in a considerable reduction in the viable counts of *E. coli*,[19] consumer education is critical to prevent foodborne illnesses among consumers.

The presence of *S. aureus* in food and herbal products is associated with unsanitary human handling. Botanicals frequently undergo human handling as they are collected, cleaned, pooled, dried, packed and dispensed. *S. aureus* was detected in 67% of samples, at levels that were above 1 log unit/g. Other studies in Nigeria and South Africa have reported similar *S. aureus* loads.[10,22]

Fungal contamination of herbal products chiefly occurs during a slow drying process, because of inadequate drying or during postharvest storage if relative humidity is high and temperatures are favourable.[21] In this study, while all samples were contaminated with fungi at different concentrations, over one third of the products did not fall within acceptable microbial limits for internal use and 13% failed to fall within the limit for teas or infusions. Such contaminated products may cause fungal infections or other serious health

complications as a result of mycotoxin accumulation from toxin-producing fungi such as *Aspergillus parasiticus* and *Aspergillus flavus*.[21] In fact, several studies have reported the presence of mycotoxins in botanical preparations.[9,23]

Recommendations

Although *S. aureus* is infrequently found in medicinal herbs, our and previous findings suggest that there is a need to develop microbial limits for *S. aureus* for countries that rely on WHO quality standards for herbal products. Moreover, there is a need to re-evaluate quality-control standards for herbal and traditional medicines to reduce the health risks associated with microbial contamination in developing countries.

Training in food safety procedures may improve the safety of the products and reduce health risks to consumers. It should be noted that during sample collection in this study, several retailers recommended not using boiling water and encouraged the addition of the botanical to food or its use as a 'paste' by adding it to water or milk. Although boiling water will not kill all the microorganisms or spores in a botanical mixture, it may significantly reduce the microbial load.

As a safety precaution, botanicals are not recommended for infants because of the risk of infant botulism, which is caused by *Clostridium botulinum* spores. Although *Clostridium* spp. were not tested in this study, given the microbial numbers seen in this project, and the prevalence of *C. botulinum* in medicinal herbs,[15] the possibility of infant botulism is a concern. *Clostridium* spp., *Listeria* spp., *Shigella* spp., and *Bacillus cereus* should be tested for in future studies, as these are common contaminants of herbs and spices.

In addition, dates of manufacture and expiration were unknown in 98% of the samples in this study. Consequently it was not possible to estimate the effects of storage on microbial loads in the samples, although storage is known to influence microbial activity and counts.[19]

Conclusion

The use of herbal or botanical products is part of the health-care culture in Kenya. When standard pharmaceuticals are not readily available or affordable, consumers use botanical products as their only option. Microorganism contamination of these products was found to frequently exceed the accepted limits. However, given the environmental conditions and how they were dispensed, the levels of contamination could have been greater. We recommend that quality-control measures and safe handling practices be established for medicinal herbs or botanicals sold in Kenya. Health-care providers and public health professionals should be aware that these products are being sold to HIV-infected individuals and that they may serve as a source of infection and cause potential health risks to these immunocompromised individuals.

Acknowledgements

L.K. thanks the School of Human Environmental Sciences, University of Arkansas, and the American Association of University Women (AAUW) for the financial support that enabled her to complete her MS degree.

Competing interests

We declare that we have no financial or personal relationships which may have inappropriately influenced us in writing this paper.

Authors' contributions

L.K. performed the experiments and contributed to the writing of the manuscript. J.C.F. was the project leader and was in charge of the project design and contributed to the writing and editing of the manuscript. E.E.G. was responsible for the experimental design, the statistical analysis and the editing of the manuscript.

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