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Assessment of Bacterial Contamination in Herbal Medicine Products Vended in Morogoro Municipality, Tanzania

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Traditional medicines are widely used in Tanzania; however, the microbiological safety of herbal medicine products (HMPs) is unknown. A cross-sectional study was conducted to determine microbial levels and antimicrobial susceptibility of bacteria isolated from HMPs vended in Morogoro Municipality. Fifty samples of HMPs were collected from vendors in six wards in the municipality. Bacterial contamination was determined through total viable count and bacterial isolation while susceptibility to the selected antimicrobials was determined by agar disc diffusion method. About 88% of the tested HMPs significantly ($p<0.05$) had higher total bacterial counts than WHO recommended levels. Ten percent of HMPs were contaminated with the pathogenic *E. coli* and 8% with *S. aureus*. The isolated bacteria were only susceptible to ciprofloxacin but were resistant to the rest of the tested antimicrobials at standard doses. Unhygienic handling practices and limited safety knowledge by the HMPs vendors was also observed. Use of the unregulated but vended HMPs may put the users at risk of acquiring infections with pathogenic and antimicrobial resistant bacteria that portends increased treatment challenges.

Key words: Alternative medicine, herbs, antibacterial resistance

INTRODUCTION

Herbal medicines (HM) are plant-derived materials or plant extracts which are used as therapeutic substances to treat many ailments including chronic conditions [1] and also as dietary supplements. World Health Organization (WHO) describes traditional HM as herbs, herbal materials, herbal preparations, and finished herbal products that contain parts of plants or other plant materials as active ingredients that are used for prevention or treatment of different ailments [2]. In the recent years, HM usage has potentially maintained its popularity worldwide [3].

In Tanzania, over the last few years, there has been a remarkable increase in usage of herbal medicine in the treatments and/or prevention of diseases [4]. The government recognizes HM as it raises income for practitioners and persons who receive HM get healed and become healthy and productive [5]. The contribution of HM cannot be shunned since they are easily accessible healthcare options for many people with limited financial resources [6]. However, the use of HM in the country is not regulated, posing a health risk to the consumers.

On the other hand, a microbial contamination in HMs is a threat to HMPs users. The microbial contamination of HMPs may be indicative of the amount of microbes present, low quality of packaging materials, poor storage and handling practices that may likely cause adverse health effects to consumers [3]. Their adverse effects are of varying severity, including deaths [5]. Since the presence of microbes cannot be detected by organoleptic means and microbial contamination in HMPs is of major public health importance, there is need for product microbiological analysis [7].

Pathogenic microorganisms commonly isolated from HMPs pose a serious threat to human health. Some of pathogenic bacteria reported in HMPs in previous studies include *E. coli*, *S. aureus*, *Salmonella* spp., *Listeria monocytogenes*, *Bacillus* spp., *Mycobacterium*

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spp., *Campylobacter* spp., *Clostridium* spp., *Pseudomonas aeruginosa* and *Proteus* spp. [8-10]. Furthermore, apart from HMPs being potential carriers of pathogens, the responsible microbes may also cause further serious health risk to consumers through the possibility of some carrying antimicrobial resistance genes. Report on global surveillance of antimicrobial resistance (AR) revealed that this phenomenon is no longer a prediction for the future; it is happening right now, across the world, and is putting at risk the ability of currently used antimicrobials to treat common infections in the community and hospitals [11].

The development of AR can be a natural phenomenon [12]; however, certain human actions may accelerate the emergence and spread of AR leading to the public health threat. This includes the inappropriate use of antimicrobial drugs in humans, agriculture and in animal husbandry [13]. Inappropriate control and prevention of bacterial and fungal-borne diseases in plants by using antimicrobials favours the emergence and selection of resistant strains [7, 14]. Presence of antimicrobial residues in HMPs may result from failure by farmers to observe the withdrawal periods before harvesting, incorrect dosage levels and illegal addition of antimicrobials into herbal drugs during production [15]. In addition, poor personal hygiene practices and exposition of products in polluted/contaminated environment during storage and vending are suggested as being sources of antimicrobial residues in HMPs [16]. Antimicrobial resistance can cause significant danger and suffering for children and adults who have common infections which were previously easily treatable by regular antimicrobials [17].

This study identified, quantified and established associated predisposing factors of contamination by some common bacteria in HMPs. Furthermore, the study determined the susceptibility of the bacterial isolates to commonly used antimicrobials in human health practices. Information gathered from this study will be useful to Municipal Council Authorities and other public health stakeholders in development of strategic plans towards regulating and safe use of HMPs.

MATERIALS AND METHODS

Description of study area

This study was conducted in Morogoro Municipality from November 2014 to March 2015. The municipality lies along 35.6ºE to 39.5ºE Longitudes and 6°S to 10ºS Latitudes. It is located about 195 kilometers to the west of Dar es Salaam, the largest commercial city in Tanzania. The municipal has a population 315 866 persons of which 151 700 are males and 164 166 are females (NBS, 2012). The residents receive health care services from Public Government owned health facilities, Private owned facilities, Faith Based Organizations (FBOs) and HM vendors.

Fifty samples were purchased randomly from 24 vendors in the purposively selected six sites- Morogoro main market, Kihonda, Mafisa, Mazimbu, Mawenzi market and Sabasaba market- based on the number of HMP vendors established during preliminary visits. The vendors were blinded on the actual reasons for purchasing the medicines to avoid bias and encourage cooperation.

Study Design

A cross-sectional descriptive approach was used where HMPs were collected from vendors at the same time as information regarding HMP handling practices, packaging, and water used for preparation and state of HMPs collected using a pre-validated checklist. Demographic and information on training of the vendors on HMP safety were also gathered.

Sample Collection and Preparation for Laboratory Analysis

Lots of approximately 10 g or 10 ml for each product purchased were taken and packed aseptically in sterile zipped polythene bags and plastic bottles in case of powder and liquid samples, respectively. All samples were coded and stored in a cool box with ice packs and shipped to Pest Management Centre (PMC) laboratories at Sokoine University of Agriculture (SUA) for bacteriological analyses on the day of collection.

In the laboratory, sample containers were opened under aseptic conditions and 1 g aliquot(s) or 1 ml of product placed into a sterile container by using a wooden tongue depressor for powders and sterile pipette, respectively, into 90 ml of sterile normal saline. Then, 1 ml of the prepared homogenate inoculum obtained by vortex was transferred into a test tube containing 9 ml of sterile physiological saline. The procedure was repeated up to ten serial dilutions and in the last dilution 1 ml of inoculum was discarded.

Laboratory analysis for bacterial load and antimicrobial susceptibility

Determination of total viable counts

The samples for total viable colony count (TVC) determination were the serially diluted specimens in sterile physiological normal saline in the 10^{-1} to 10^{-10} levels. Using a sterile pipette, 1 ml of each dilution was inoculated into each Nutrient Agar (NA) plate. The plates were incubated at 37°C for 24 hours (±3 hours). Colony counts were done under microscope aided by marker pen and reported as colony forming units (cfu)/g or (cfu)/ ml [18].

Isolation and identification of faecal coliforms

The dilutions for initial suspensions were prepared as described for TVC. Thereafter, about 10 ml of Violet Red Bile Glucose (VRBG) agar at 44ºC- 47ºC was poured into two Petri dishes. Using a sterile pipette, 0.1 ml of the last dilution of test sample was transferred to each of the Petri dishes. The inoculum and the media mixture were carefully mixed by rotating the Petri dishes and allowed to solidify at room temperature. After solidification, a covering layer of about 5 ml of the VRBG agar was added onto each Petri dish to prevent spreading of growth and to achieve semi-anaerobic condition. The contents were then allowed to solidify again. Thereafter, the plates were inverted and incubated at 37ºC for 24 hours. After incubation period, the plates were examined for typical and atypical colonies of coliforms. Typical faecal coliform colonies were pink to red or purple, with or without precipitation haloes or colorless mucoid colonies, with a diameter of 0.5 mm to 3.0 mm. By using a new sterile pipette for each dilution, the procedure was repeated as above with further dilutions up to the first dilution and for the remaining test samples.

Isolation and identification of Escherichia coli

Isolation and identification of *E. coli* was done by sub-culturing of a single colony from the primary faecal coliform culture above. The presumptive isolates of *E. coli* were sub-cultured from the VRBG agar plates and then re-streaked onto MacConkey agar plates to obtain pure colonies for *E. coli*. The plates were inverted and incubated at 44.5ºC for 24 to 48 hours. Presumptive *E. coli* colonies were identified to species level by Gram staining and IMViC tests (indole, methyl red, Voges Proskauer and citrate) [19].

Isolation and identification of S. aureus

About 1 g or 1 ml of the sample was suspended into 25 ml of peptone water in sterile McCartney bottle and incubated for 18 hrs at 37°C [20]. Isolation of the *S. aureus* was achieved by streaking the pre-enriched culture from the peptone water onto selective differential agar plates of freshly prepared Mannitol Salt Agar (MSA); a selective and differential medium used for the isolation of pathogenic staphylococci. The plates were incubated at 37°C for 24 hours under aerobic conditions. Colonies showing golden yellow color or colorless were presumed to be *Staphylococcus* spp. On MSA, pathogenic *S. aureus* produces small colonies surrounded by yellow zones as a result of mannitol sugar fermentation. These colonies were sub-cultured onto NA for purification and masking the effect of acid produced during fermentation of MSA. The colonies selected from NA were subjected to Gram stain, to check the morphology and staining characteristics. The gram positive *cocci* organisms were subjected to catalase test to differentiate between *Staphylococcus* spp. and *Streptococcus* spp. Catalase positive colonies were further subjected to slide and tube coagulase test for the confirmation of *S. aureus* [17].

Determination of antimicrobial susceptibility

Evaluation of antimicrobial susceptibility for isolated bacteria was performed on Mueller-Hinton Agar (MHA) by agar disc diffusion

method according to CLSI (2012). In brief, *S. aureus* and *E. coli* were inoculated into MH Broth and incubated at 37ºC for 24 hours. Each isolate from MHB was inoculated in a Petri dish containing MHA and overlaid with antimicrobial discs with amoxicillin (20µg), ciprofloxacin (5µg), gentamycin (10 µg), cefotaxime (30 µg), nalidixic acid (30 µg), oxacillin (1 µg), co-trimoxazole (1.25/23.75 µg) and vancomycin (30 µg). Thereafter, the plates were incubated under aerobic conditions at 37°C for 24 hours. After incubation period, the plates were examined for zones of inhibition around the discs. Diameters of inhibition zones around the discs were measured to the nearest 0.1 mm using a metal caliper, and the results were recorded and classified as resistant (R), intermediate (I) and sensitive (S) according to the standard method approved by the Clinical and Laboratory Standard Institute (CLSI), 2012. The isolates of *E. coli* and *S. aureus* that were resistant to three or more of the eight classes of antimicrobial agent used in this study were defined as having multiple antimicrobial resistances. Standard reference strains of *S. aureus* (ATCC 25922) and *E. coli* (ATCC 25923) were used as positive control organisms in the antibiotic susceptibility determination.

Data Analysis

Descriptive statistics were used to determine distributions and magnitudes of variables. The Student t-test was used to determine statistical differences between the WHO limits and the obtained sample microbial counts. One-way analysis of variance (ANOVA) was adopted to compare differences in means of Total Viable Counts (TVC) across the six sites.

RESULTS

A total of 24 respondents selected from the six sites participated and their social-demographic characteristics were as indicated in Table 1. Unhygienic handling practices and low level of knowledge by vendors on safety of the HMPs was observed across the respondents.

Table 1: Socio-demographic characteristics of HMP vendors

Characteristics assessed	Category	Number of respondents (%)
Gender	Male	15 (62.50)
	Female	9 (37.50)
Age	10–29	6 (25.00)
	30 – 49	4 (16.67)
	50– 69	9 (37.50)
	70+	5 (20.83)
Level of education	No formal education	8 (33.33)
	Standard I – VII	14 (58.31)
	Secondary education	2 (8.33)
	College education	0 (0.00)
Attended training on safety of HM	Yes	2 (8.30)
	No	22 (91.70)

Table 2: Distribution and forms of herbal medicinal product samples

Sites	Number of samples per site	Form of HMP	
		Powder samples	Liquid samples
Kihonda	8	6	2
Msamvu main bus terminal	7	5	2
Morogoro main market	11	9	2
Sabasaba market	11	7	4
Mawenzi Market	6	4	2
Mazimbu	7	6	1
Total	**50**	**37**	**13**

Microbiological Quality assessment of HMPs samples

Total Viable Counts (TVCs)

A total of 44 (88%) of tested samples were found to have microbial contamination. The counts ranged from 9.09×10^4 to 1.64×10^8cfu/g per ml. Mean TVC for liquid and powdered HMPs were 1.4×10^7cfu/ml and 9.26×10^5cfu/g, respectively. The overall results indicated that all contaminated samples had higher TVC than the WHO and BP recommended level of 10^3cfu/ml for herbal medicines intended for human consumption. Comparison of TVC between six sites showed that HMPs from Kihonda had the highest microbial load with a mean of $(2.12 \pm 2.04) \times 10^7$ cfu/ml. However, the variation in bacteriologic parameters between sites was not statistically significant ($p > 0.05$).

Prevalence and intensity of coliform bacteria

A total of 44 HMP samples were cultured for coliforms count, identification and isolation of *E. coli*. Out of those 21 (47.73%) had positive growth of coliforms with a mean growth $2.15 \times 10^5 \pm 3.9 \times 10^4$ cfu/ ml with a range between 0.00 and 1.0×10^6 cfu/ml. The mean coliform established was significantly higher than international standards for HM ($p < 0.05$) [6, 21].

Specific bacteria isolated in HMP samples

A prevalence rate of 10% and 8 %, respectively for *E. coli* and *S. aureus* was recorded for the 50 samples collected. When focusing on the 44 infected samples, an isolation rate of 11.36% and 9.1 % for *E. coli* and *S. aureus*, respectively was observed. The results showed that the liquid HMPs were more contaminated with *E. coli* and *S. aureus* compared to the powdered products. The variation in contamination between these two forms was statistically significant ($P < 0.05$). Other bacteria isolated were *Enterobacter* spp. *Bacillus* spp. *Staphylococcus epidermidis*, *Klebsseilla pneumonia* and *Pseudomonas aeuroginosa*.

Antimicrobial susceptibility of isolated E. coli and S. aureus

Five *E. coli* isolates from HMP samples tested for susceptibility to different antimicrobials, showed a resistance pattern as detailed in Figure 1. Higher resistance was observed in Oxacillin (OX), Vancomycin (VA) and Cefotaxime (CTX). There was no resistance to CIP and NA while low resistance was observed in AMC, CN and SXT. All the four *S. aureus* isolates in this study were found resistant to NA while two were resistant to VA and one to CTX. On the other hand, all the isolates were susceptible to the rest of antimicrobials tested (Figure 1). Generally, it was observed that the isolated *E. coli* and *S. aureus* were susceptible to Ciprofloxacin but resistant to Nalidixic acid, Cefotaxime, Oxacillin, Co-trimoxazole and Vancomycin with resistance pattern ranging from (25%) to (100%) (Figure 1).

DISCUSSION

The study established high levels of microbial contaminations in HMPs vended in Morogoro Municipality. The presence of microbes in HMPs could be due to several factors such as exposition of products in a polluted environment, poor handling practices of raw materials, use of untreated water, poor packaging materials, use of contaminated containers and harvest of materials from contaminated environment as reported elsewhere [8, 10, 22, 23]. The present study further found that most of the HMPs had higher bacterial levels (TVC) than what is acceptable from the public health point of view [6]. This implies that most HMPs sold in the study area were not safe for human use.

The presence of Coliforms such as *E. coli* in some samples may indicate faecal contamination suggestive of poor personal hygiene and handling practices by HMPs vendors. According to WHO, no coliform organism is acceptable in any product intended for human consumption [6]. In addition, the HMPs vended were not certified and hence not regulated. The vendors had low education and

lacked training in hygienic practices related to good handling of HMPs (Table 1). These observations suggest that there is need for necessary training for HMPs vendors to safeguard consumers of these products.

Presence of *S. aureus* has previously been reported to occur in HMPs circulating in various countries including Tanzania [5], Kenya [10, 24], Nigeria [8,20] and India [22]. *S. aureus* is an important cause of food poisoning following ingestion of preformed heat-resistant toxins that results to severe gastroenteritis [7,17]. Isolation of these contagious pathogens in HMPs especially in underdeveloped countries is highly related to use of unhygienic equipment and poor personal hygiene practices in handling of HMPs by most vendors which may be due lack of knowledge on good manufacturing practices [25].

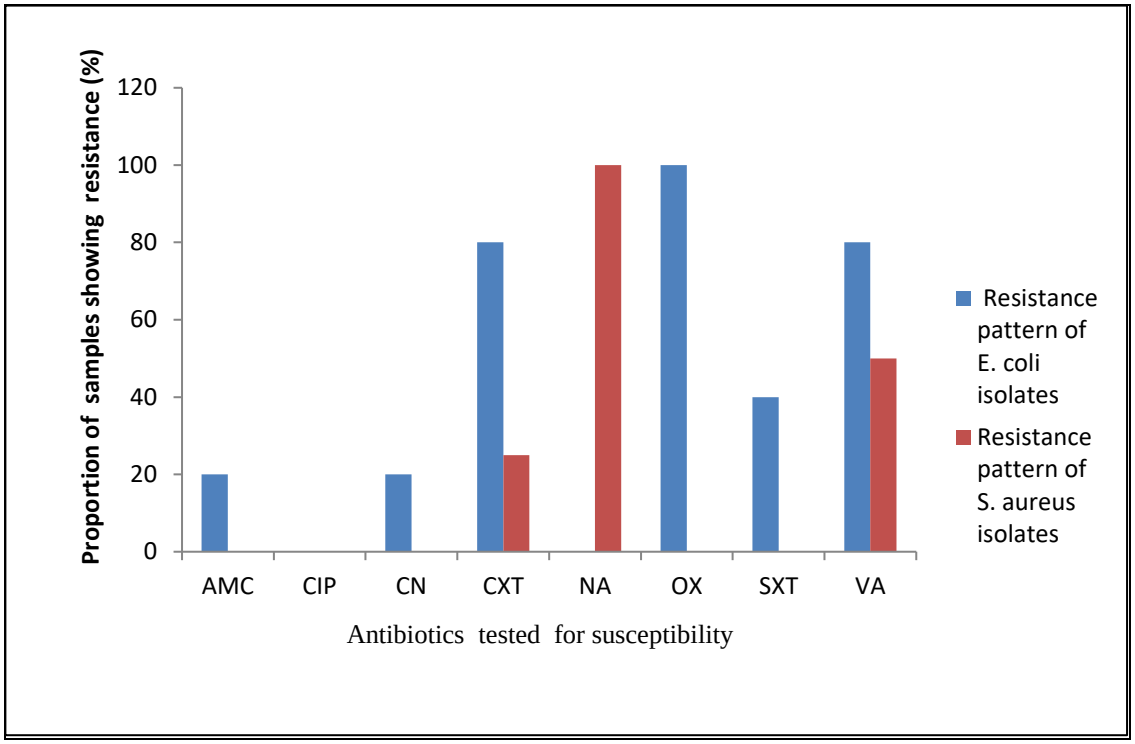

Figure 1: Resistance pattern for *E. coli* and *S. aureus* bacteria isolated from HMP samples.
AMC= Amoxicillin-Clavulanic, CIP = Ciprofloxacin, CN = Gentamycin, CXT = Cefotaxime, NA= Nalidixic acid, OX= Oxacillin, SXT = Co-trimoxazole and VA = Vancomycin.

Bacteria isolated in this study were resistant to some of the commonly used antimicrobials (Figure 1). Studies conducted elsewhere reported similar prevalence of bacterial resistance to commonly used antimicrobials [12, 26]. This observation is possibly due to indiscriminate use of antimicrobials in human health and livestock practices for prevention and control of bacterial diseases [14, 16, 27]. Besides, the presence of any introduced anti-bacteria activity in some of HMPs in suboptimal amounts may increase the possibilities of bacteria to develop resistance mechanism against specific antimicrobials with similar mechanism of action [28, 29].

The presence of antibacterial resistant pathogens has important public health implications particularly in developing countries like Tanzania where there is widespread and uncontrolled use of antimicrobials in treatment of bacterial diseases in livestock and humans [12, 30]. The present study did not evaluate the association between antimicrobial resistance and the intended use of the HMPs. There is therefore need for further studies to determine possible active ingredients of the HMPs and the intended uses as well as their association with antimicrobial resistance.

CONCLUSION

This study showed that the unregulated HMPs marketed in Morogoro Municipality are highly contaminated with microbes, some of which are potentially pathogenic bacteria such as *E. coli*

and *S. aureus*. On the other hand, some of the commonly used antimicrobials have lost their potency against the isolated bacteria. The unregulated vending of HMPs may therefore facilitate transmissions of bacterial infections with increased treatment challenges.

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