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Medical Full Length Article

Microbial Contamination of Vended Fever Herbs in different Motor Parks in Akure, South-West Nigeria

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Abstract

Herbs are plant-derived materials used as therapeutic substa email nces or dietary supplements. A cross-sectional study was conducted to determine the microbial contamination of fever herbs vended in Akure motor parks. Fifty (50) samples of fever herbs were purchased from vendors in ten (10) different motor parks in Akure, South-West Nigeria. Microbial loads of the herbs were enumerated using membrane filtration method, isolation and characterization of bacteria, determined using standard microbiological methods. The results revealed that most of the samples (Idanre, Sunshine, Ilesha, Road block, Oloko and Ondo motor parks) had significantly (p<0.05) higher total bacterial counts than the recommended level of ≤ 13cfu/100ml for herbal medicine product intended for human consumption, and all the samples except herb vended at Old garage motor park conform with the set standard of 0.0 cfu/100ml coliform counts herbal medicine product. Age, level of education and job experience of the vendors as well as packaging materials contributed significantly (p<0.05) to microbial contamination of herbs. Bacteria isolated from herbs were; *Salmonella thyphi, Staphylococcus aureus, Shigella dysenteriae, Klebsiella pneumoniae, Serratia marcescens, Escherichia coli, Pseudomonas aeruginosa, Staphylococcus epidermidis, Bacillus subtilis, Lactobacillus plantarum* and *Lactobacillus fermentum* out of which *S. aureus* 10(19.23%) and *L. plantarum* 7(13.46%) were the most occurred. Occurrence of these bacteria in herbs could predispose consumers to the risk of infections with pathogenic bacteria. It is recommended that food scientists, health officers, extension officers and other stakeholders should play their roles of training and enforcing quality assurance through monitoring the production and distribution of herbs to safeguard consumers.

Keywords: Motor Park, fever, herb, contamination, coliform

INTRODUCTION

Fever is a state where normal body temperature is raised over homeostasis conditions and is one of the most common causes of consultations especially in developing countries (Plaza *et al.,* 2016; Guillebaud *et al.,* 2018). Fever is one of the usual clinical features that appear during the course of several infectious diseases in, such as, uncomplicated malaria fever caused by *Plasmodium falciparum* parasite, enteric fever caused by *Salmonella* serovars, adolescents affected by pulmonary tuberculosis due to *Mycobacterium tuberculosis* infection, among other type of infectious diseases which are common in Nigeria (Elhadad *et al.,* 2015; Plaza *et al.,* 2016; Omoya and Ajayi, 2020). Herbs have been used for thousands of years to treat fever (Hines, 2021), in developing countries, many patronize them largely due to cultural acceptability, availability and cost (Abdullahi, 2011).

Herbal medicines (HM) are plant-derived materials or plant extracts which are used as therapeutic substances (Drew and Myers, 1997) and can also be used as dietary supplements. World Health Organization (WHO) (2007) defined HM as botanical medicine or phytomedicine. WHO (2008) describes traditional herbs, herbal materials, herbal preparations, and finished herbal products that contain parts of plants or other plant materials as active ingredients that used for prevention or treatment of different ailments. There exist diverse reasons for the continuing use of herbal medicines for health care in Africa; these include cultural acceptability, easy accessibility and affordability, and in some

instances, non-availability and prohibitive cost of allopathic medicines (Mahomoodally, 2013). Some people also employ herbal medicines under other circumstances, for example, in health conditions that had failed to respond to orthodox treatment or which allopathic medicines are deemed not to treat adequately and less safely (Kofi *et al.,* 2010). Other health conditions believed to have spiritual origins and those thought to need holistic therapies are also managed with herbal medicines (Abdullahi, 20110). Herbs are commonly sold in Nigeria by vendors in motor parks where commercial drivers and motorcyclists have easy access to them (Oshodi, 2007).

Generally, herbal traditional medicines have been considered safe as a result of the long history of use in the treatment of diseases based on knowledge accumulated over several centuries (WHO, 2000) but they are not necessarily free from microbial and other contaminants which are due to factors such as adulteration, substitution, cross contamination, lack of standardization, inappropriate use of antimicrobials and poor handling practices and/or poor storages (Foster, 2002; Lau *et al.,* 2003; Prescott *et al*., 2005). Pathogenic microorganisms commonly isolated from herbs pose a serious threat to human health. Some of these pathogens include *Escherichia coli, Staphylococcus aureus, Salmonella* spp., *Listeria monocytogens, Bacillus* spp., *Mycobacterium* spp*., Campylobacter* spp., *Clostridium* spp., *Pseudomonas aeruginosa* and *Proteus* spp. (Foster, 2002; Prescott *et al*., 2005; Oleyege and Adelabu 2010, Keter and Mutiso, 2011; Meshack *et al*., 2013). This study therefore presents the information on microbial contamination of vended fever herbs in different motor parks in Akure, South-West Nigeria.

MATERIALS AND METHODS

Description of the Study Area

Akure is the largest city and capital of Ondo State, located in south-west Nigeria. Akure lies about 70°15 north of the equator and 50°15 east Meridian. The city has a population of 588,000 which is 0.305% of Nigeria population based on 2006 population census, the people are of Yoruba ethnic group and are situated in the tropic rainforest.

Collection of herb samples

Herb samples were collected from different vendors (five (5) vendors in a park) three times a week between October, 2019 and December, 2019 from randomly selected 10 motor parks (Idanre, Ado, Sunshine, FUTA North gate, Ilesha, Road block, Oloko, Old garage, Shasha and Ondo) in Akure, Ondo State, Nigeria. Multiple choice structured questionnaires were used to obtain the sociodemographic characteristics of the vendors as well as mode of preparation of the herb, type of the herb (root, leaf, stem, liquid, paste, powdery, aqueous extract or other solvent, single plant herb or multiple plant based). Herbs (100 ml) were purchased and collected using pre-sterilized plastic materials and transported to the laboratory in Ice Park with 30 minutes of collection for further microbiological analysis.

Determination of the microbial contamination of the herbs and identification of microorganisms

Microbiological examinations of herbs were carried out using membrane filtration method as described by Kumar *et al*. (2012) and WHO (2016), on nutrient agar, some selective and differential media (*Salmonella Shigella* agar, Eosin Methylene Blue agar, MacConkey agar and Manitol Salt agar), were used for isolation of bacteria while Potato Dextrose agar was used for isolation of mould and yeast. One hundred (100 ml) milliliter of herb was gently shake and filtered with 0.45 µm membrane filter and the filter was aseptically placed on molten agar. Morphological and biochemical characterisation of bacteria and cultural and microscopic characteristics of fungal isolates were used for identification (Hunter and Bamett, 2000; Fawole and Oso, 2004; Cheesbrough, 2014).

Incubation was carried out at 37 ^{o}C for 24 hours for bacteria growth and 25 ^{o}C for 2-4 days for fungi growth. After incubation, individual colonies were counted and isolated in pure culture using streak plate technique (Eze *et al*., 2010). Bacterial isolates were then characterized using standard microbiological method as described by Fawole and Oso (2004) while cultural, morphological and microscopic examination fungal spores were carried out using the method of Hunter and Bamett (2000).

Data were statistically analysed using SPSS version 20, mean microbial loads were separated using new Duncan's Multiple Range test and significant difference was value at $p \leq 0.05$.

RESULTS

Socio-demographic characteristics of the herb vendors at different motor parks in Akure

Socio-demographic characteristics of herb vendors is shown in Table 1. The results revealed that all the vendors are female, higher number of the vendors are between age range 21 to 30 years (28(56%), had primary level of education 37(74%), Muslim religion 28(56%), household of 5 people 25(50%) and married 45(90%).

Microbial contamination of herb vended at different motor parks in Akure

Microbiological examination of herb vended at different motor parks is shown in Table 2. It was noted

that there were variations in microbial loads in different locations, however, the total bacterial, Staphylococcal, lactic acid bacterial, coliform and fungal counts ranged from 2.09±0.86 (Old garage) to 98.71±3.91 (Ondo) cfu/100ml, 0.00±0.00 (Shasha) to 42.97±1.08 (Ilesha) cfu/100ml, 2.49±1.38 (Sunshine) to 88.91±0.42 (Ondo) cfu/100ml, 0.00±0.00 (Old garage) to 28.34±0.38 (Oloko) cfu/100ml and 10.55±1.06 (Shasha) to 32.92±0.81 (Ondo) sfu/100ml respectively.

Microbial contaminations were related with vendors' age range as shown in Figure 1. It was observed that there was no significant ($p<0.05$) difference in staphylococcal load of herb based on different age range, but the age range of the vendors contributed significantly to total bacterial, lactic acid, coliform and fungal counts which were higher in 11 to 20 (38.44±1.22 cfu/100ml), 21 to 30 (24.51±0.76 cfu/100m), 11 to 20 (20.04±1.32 sfu/100m) and 21 to 30 (22.32±0.63 sfu/100m) respectively. Level of education also contributed significantly ($p<0.05$) to staphylococcal and lactic acid bacteria counts in which vendors with primary (23.11±0.55 cfu/100m) and secondary (23.13±0.13 cfu/100m) level of education had higher counts respectively (Figure 2). Also, the Figure 3 revealed that number of household had significant ($p<0.05$) effect on total bacterial (vendors with 5 household size 32.43±0.63 cfu/100m) and coliform (vendors with 5 household size 12.16±0.63 cfu/100m) counts.

Figure 4 showed the effect of packaging materials on microbial contamination of herbs vended at different motor parks in Akure. It was noted that there was no significant ($p<0.05$) difference in total bacterial, staphylococcal and fungal counts of the herbs based on the packaging materials used, however, packaging materials contributed significantly significant ($p<0.05$) to lactic acid bacteria (LAB) and coliform counts. The herb packed in warmer had higher LAB counts (26.41±0.11 cfu/100m) than those packaged in plastic (16.24±0.32 cfu/100m) and nylon (9.86±0.62 cfu/100m) while the coliform counts of herb packaged in warmer had reduced coliform counts (5.31±0.43 cfu/100m).

Vendors' year of job experience revealed that the herbs vended by those with more than 9 years of practicing experience had a significant reduction in total bacterial (2.01±0.02 cfu/100m), staphylococcal (2.59±0.07 cfu/100m), coliform (1.13±0.16 cfu/100m) and fungal (12.11±0.13 sfu/100m) counts compared with those with lesser year of practicing (Figure 5).

Microbial contamination of herb based on the marital status of the vendors is shown in Figure 6. It was observed that there was no significant ($p<0.05$) difference in the total bacteria, coliform, staphylococcal, LAB and fungal counts of the herb among the vendors that are single and those that are married.

Bacterial isolated from the vended herbs were identified and the occurrence is shown in Table 3. The following bacteria comprising of Gram negative; *Salmonella thyphi, Shigella dysenteriae, Klebsiella pneumoniae, Serratia marcescens, Escherichia coli* and *Pseudomonas aeruginosa;* and Gram positive; *Staphylococcus epidermidis, Bacillus subtilis, Staphylococcus aureus, Lactobacillus plantarum* and *Lactobacillus fermentum* were isolated. It was noted all the herbs vended at different location had the presence of *S. aureus* 10(19.23%). The frequency of *Lactobacillus plantarum* 7(13.46%)*, Bacillus subtilis* 6(11.54%) and *Shigella dysenteriae* 6(11.54%) were higher while the least frequent bacterial isolates were *Pseudomonas aeruginosa* 1(1.92%) and *Staphylococcus epidermidis* 1(1.92%).

Table 1: Socio-demographic characteristics of the herb vendors at different motor parks in Akure

Socio-demographic characteristics		Names of motor parks										Total (%)
		Idanre	Ado	Sunshine	FUTA North gate	Ilesha	Road block	Oloko	Old garage	Shasha	Ondo	
Gender	Female	5	5	5	5	5	5	5	5	5	5	50(100)
Age (year)	11 – 20	0	0	0	0	0	3	0	0	0	0	3(6)
	21 – 30	5	0	5	5	2	2	2	2	0	5	28(56)
	31 – 40	0	5	0	0	3	0	3	3	5	0	19(38)
Level of education												
	Primary	5	0	3	0	2	0	3	0	0	0	13(26)
	Secondary	0	5	2	5	3	5	2	5	5	5	37(74)
Religion	Christianity	2	3	3	0	0	2	0	2	5	5	22(44)
	Muslim	3	2	2	5	5	3	5	3	0	0	28(56)
Household size	4	3	0	0	3	0	0	0	0	5	2	13(26)
	5	2	3	3	2	2	3	5	2	0	3	25(50)
	6	0	2	2	0	3	2	0	3	0	0	12(24)
Marital status	Single	0	0	0	0	0	3	0	0	2	0	5(10)
	Married	5	5	5	5	5	2	5	5	3	5	45(90)

Table 2: Microbial contamination of herb vended at different motor parks in Akure

Motor park locations	Total viable bacterial counts (cfu/100ml)	Total Staphylococcal counts (cfu/100ml)	Total lactic acid bacterial counts (cfu/100ml)	Total coliform counts (cfu/100ml)	Total fungal counts (sfu/100ml)
Idanre	25.00 ± 0.77^{c}	17.05 ± 2.90^{c}	8.82 ± 0.58^{b}	4.82 ± 0.11^{b}	24.63 ± 0.33^{b}
Ado	6.44 ± 1.54^{a}	4.56 ± 2.06^{b}	3.44 ± 0.74^{a}	4.11 ± 0.09^{b}	10.98 ± 0.27^{a}
Sunshine	22.31 ± 2.87^{c}	8.33 ± 1.44^{b}	2.49 ± 1.38^{a}	8.06 ± 0.63^{b}	12.04 ± 0.82^{a}
FUTA North gate	8.07 ± 1.93^{b}	24.41 ± 6.06^{d}	4.02 ± 0.77^{a}	2.93 ± 0.06^{b}	22.37 ± 0.66^{b}
Ilesha	14.16 ± 3.90^{b}	42.97 ± 1.08^{f}	5.11 ± 0.68^{a}	10.52 ± 0.11^{b}	16.44 ± 1.38^{a}
Road block	38.09 ± 3.05^{c}	12.82 ± 1.00^{c}	15.93 ± 0.91^{c}	20.41 ± 0.20^{c}	14.92 ± 0.38^{a}
Oloko	41.99 ± 2.44^{d}	33.43 ± 3.05^{e}	6.92 ± 0.38^{a}	28.34 ± 0.38^{c}	28.05 ± 0.95^{b}
Old garage	2.09 ± 0.86^{a}	3.71 ± 0.55^{b}	44.73 ± 0.41^{d}	0.00 ± 0.00^{a}	15.38 ± 0.44^{a}
Shasha	10.76 ± 3.01^{b}	0.00 ± 0.00^{a}	5.55 ± 0.33^{a}	8.90 ± 0.93^{b}	10.55 ± 1.06^{a}
Ondo	98.71 ± 3.91^{e}	2.34 ± 0.91^{ab}	88.91 ± 0.42^{e}	5.58 ± 0.42^{b}	32.92 ± 0.81^{b}

Values are presented as mean±SE, values in the same column carrying same superscript are not different significantly according to new Duncan's Multiple Range test at $p<0.05$

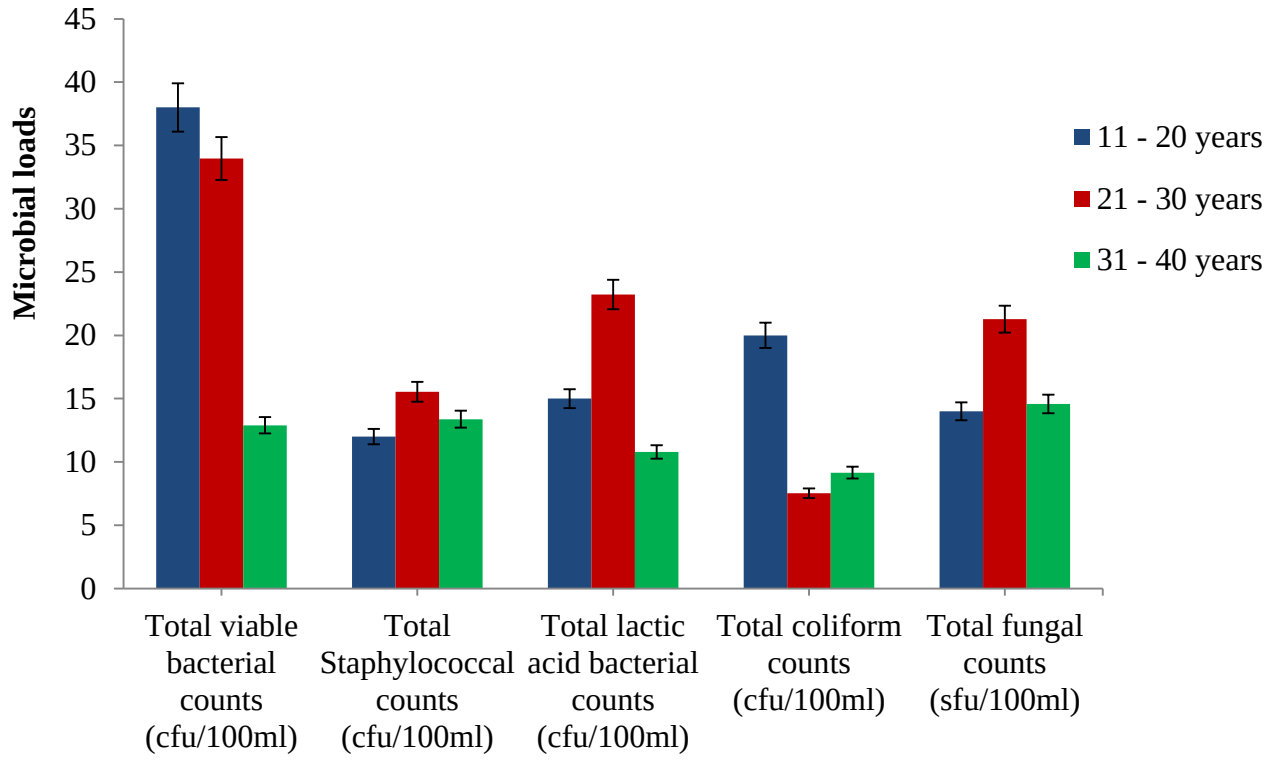

Figure 1: Effect of vendors' Age on Microbial contamination of herbs vended at different motor parks in Akure

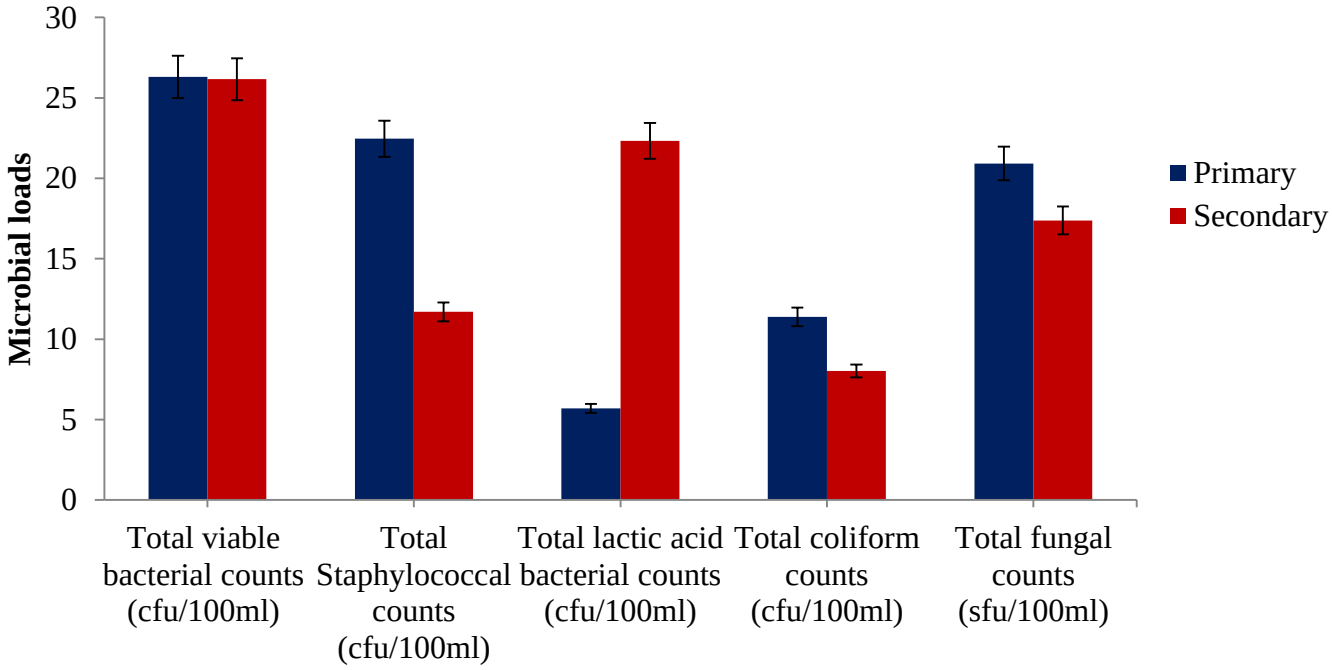

Figure 2: Effect of vendors' level of education on Microbial contamination of herbs vended at different motor parks in Akure

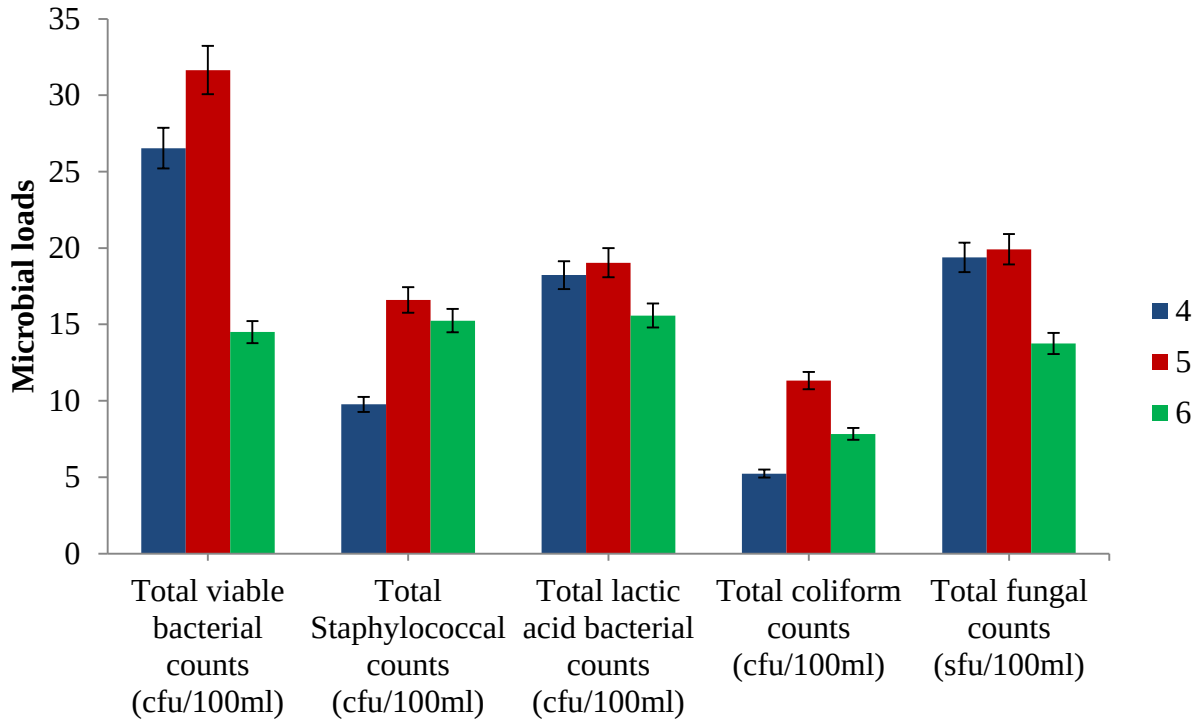

Figure 3: Effect of vendors' number of household on Microbial contamination of herbs vended at different motor parks in Akure

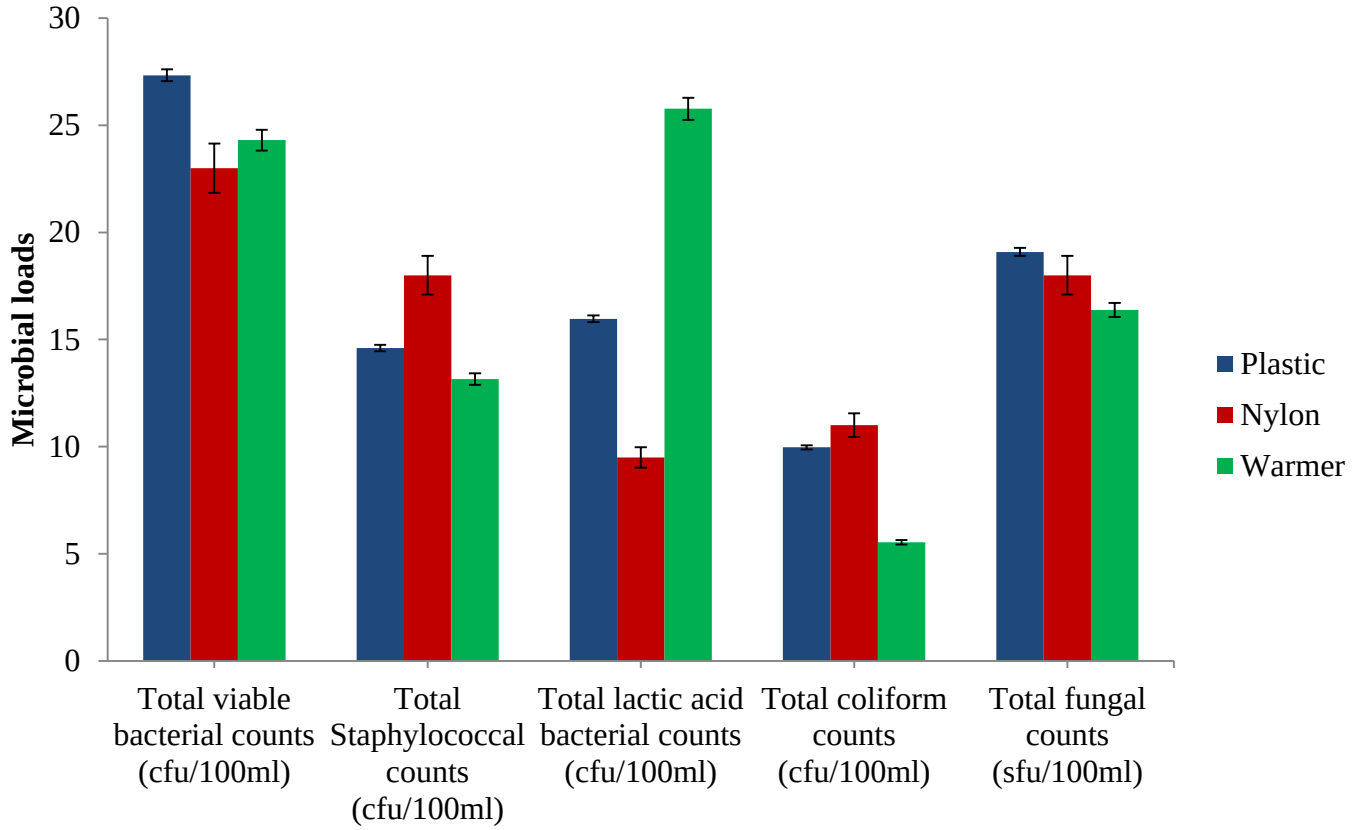

Figure 4: Effect of herbs packaging materials on Microbial contamination of herbs vended at different motor parks in Akure

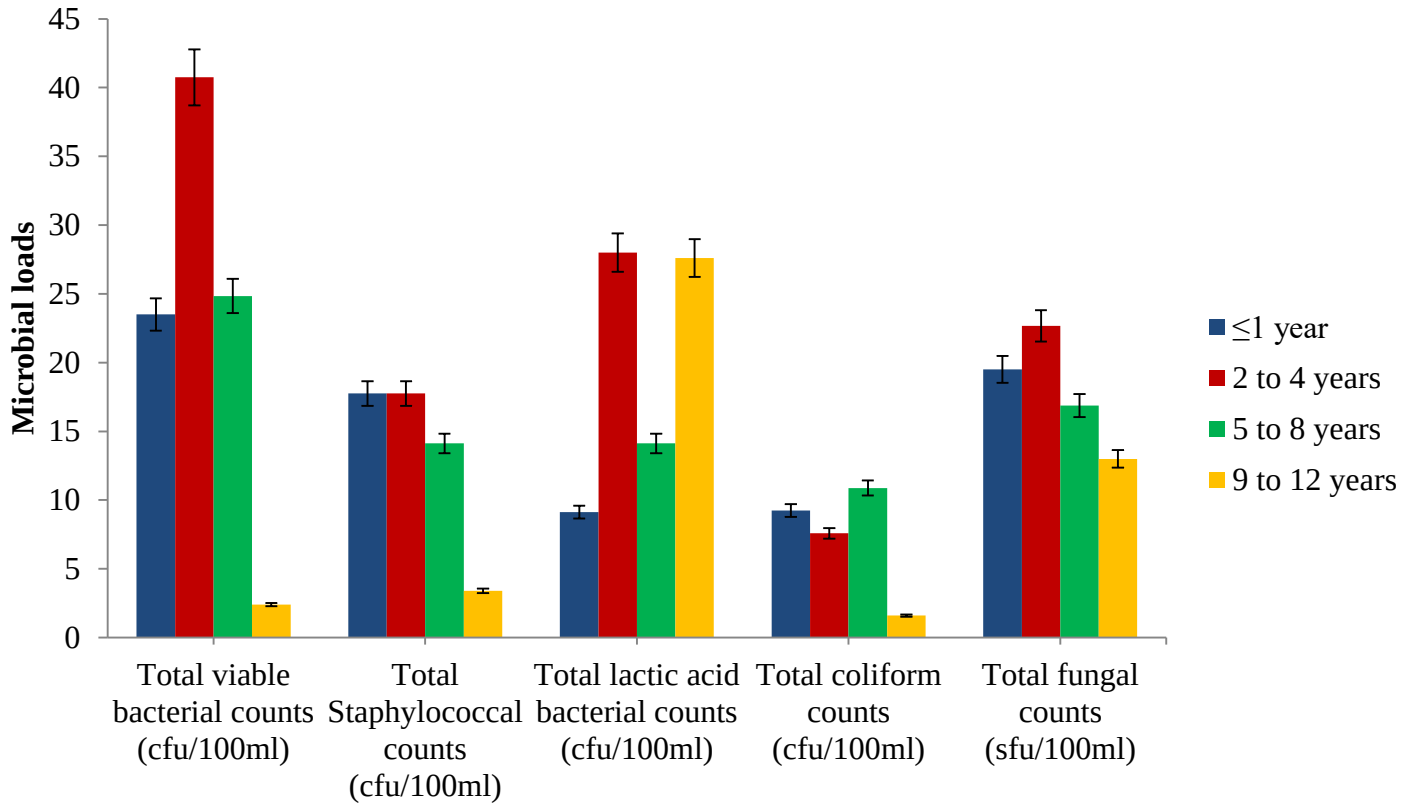

Figure 5: Effect of vendors' year of job experience on Microbial contamination of herbs vended at different motor parks in Akure

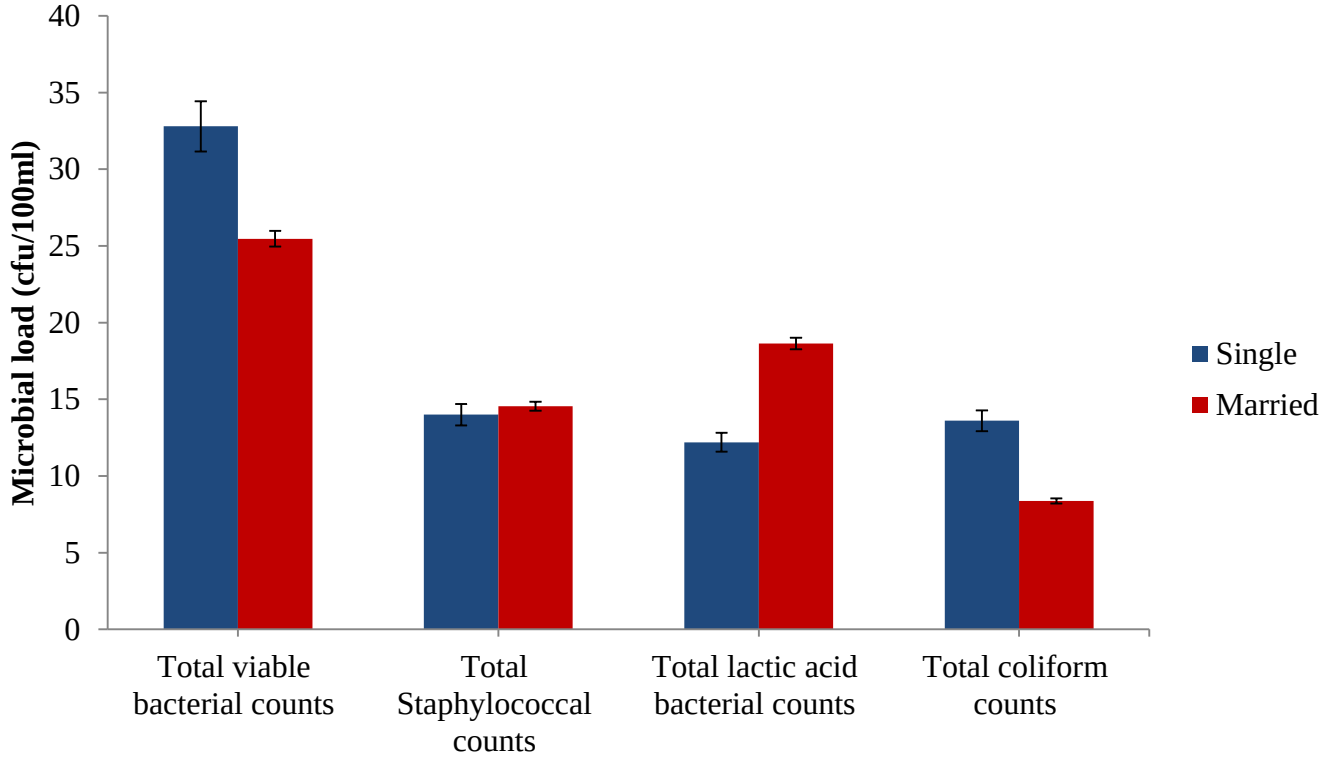

Figure 6: Effect of vendors' marital status on Microbial contamination of herbs vended at different motor parks in Akur

Table 3: Occurrence of bacterial in herbs vended at different motor parks in Akure

Bacterial isolates	Location of motor parks										Total (%)
	Idanre	Ado	Sunshine	North FUTA gate	Ilesha	South FUTA gate	Oloko	Old garage	Shasha	Ondo	
Salmonella thyphi	+	+	+	-	-	-	-	+	+	-	5(9.62)
Staphylococcus aureus	+	+	+	+	+	+	+	+	+	+	10(19.23)
Shigella dysenteriae	-	+	-	+	+	-	-	+	+	+	6(11.54)
Klebsiella pneumoniae	-	-	+	+	+	+	+	-	-	-	5(9.62)
Serratia marcescens	-	-	-	+	-	+	+	+	-	-	4(7.69)
Escherichia coli	-	-	-	-	-	+	+	-	-	+	3(5.77)
Pseudomonas aeruginosa	-	-	-	-	-	+	-	-	-	-	1(1.92)
Staphylococcus epidermidis	-	-	-	-	-	-	-	-	+	-	1(1.92)
Bacillus subtilis	+	-	-	+	+	-	+	-	+	+	6(11.54)
Lactobacillus plantarum	+	-	+	+	+	+	+	-	-	+	7(13.46)
Lactobacillus fermentum	-	+	-	-	+	-	-	+	+	-	4(7.69)
Total	4	4	4	6	6	6	6	5	6	5	52

Key: + = present, - = absent

DISCUSSION

This study detected high microbial contamination in herbs vended at different motor parks in Akure. Only female genders were encountered among the vendors during this study, and higher percentage of them had secondary education, this could be because most women demonstrated a good skill in food preparation than men; and women in Africa get involved in the preparation of herbs as a means of livelihood (Klontz *et al*., 2015). However, the findings of this study do not corroborate the study of Temu *et al*. (2009) in Tanzania.

The presence of microbes in herb could be due to several factors such as traditional handling practices and exposition of products in a polluted environment, contaminated raw materials, untreated water during preparations and packaging in unsterilized materials. Similar findings were reported in Nigeria by Oleyege and Adelabu (2010), Tanzania by Temu *et al*. (2009) and elsewhere by Foster (2002), Surekha (2011), Meshack *et al*. (2013) and Ruth (2013).

Microbial contamination of herbs has been documented and is likely to cause adverse effects on the consumers (WHO, 2011; Merlin *et al.,* 2019). Coliforms bacteria were detected in the herbs except the herb vended at old garage, the presence of coliforms suggests faecal contamination, which is normally associated with uses of untreated water for preparations and poor hygienic practices in handling (WHO, 2020). The present study further found that the vended herb had higher microbial contaminations than acceptable levels from the public health point (WHO, 2007; British Pharmacopoeia, 2007) except those sold at Old garage that has zero coliform counts. This implies that herbs sold in Akure motor parks are not safe for human consumption from microbiological point of view.

This study also showed a high relationship between vendors' socio-demographic characteristics and herbs microbial contaminations. Vendors with age range 11 to 20 years and 5 household size contributed to high total bacterial counts (TBC). The high TBC indicates possible contamination from various factors, the higher number of household could play a significant role in microbial contamination.

Those with primary level of education contributed significantly to high staphylococcal counts of the herb, the presence of *Staphylococcus* in herb corroborated other findings from Nigeria (Abba *et al*., 2011; Oleyege and Adelabu, 2010), Kenya (Kaume *et al*., 2012; Meshack *et al*., 2013), and India by Surekha (2011). This could be that those with primary level of education had poor personal hygiene practices because occurrence of this bacterium in herb especially in developing countries is highly related to use of unhygienic equipment and poor personal hygiene practices in handling of herb by most vendors. Education on the importance of GACP, GMP and personal hygiene should be provided to producers, vendors and consumers (Rocha *et al*., 2011).

High number of vendors' household size, herbs packaged with plastics and nylon contributed significantly to high coliform counts. The presence of coliforms suggests faecal contamination, which is normally associated with uses of untreated water for preparations, poor hygienic practices in handling herb and use of

unsterilized plastic and nylon used for packaging of herb. No coliform is allowed in any product intended for human consumption (British Pharmacopoeia, 2007; WHO, 2020). Furthermore, vendors' year of job experience also contributed to microbial contamination of herb, those with less than 9 years job experience had higher microbial contamination.

The presence of Gram negative and positive pathogenic bacterial isolated from herb in this study has been reported in other studies by Foster (2002), Temu *et al*. (2009), Oleyege and Adelabu (2010), Surekha (2011), Meshack *et al*. (2013) and Ruth (2013), they reported the presence of the following bacteria in herb *B. cereus*, *Aeromonas hydrophila, Shigella* spp., *Enterobacter agglomerans*, *E. cloacae*, *Vibrio fluvialis*, *Pasteurella multocida*, *S. epidermidis*, *Acinetobacter iwoffii*, *Klebsiella* spp., *B. subtilis, S. aureus* and *Pseudomonas aeruginosa* .

Higher occurrence of *S. aureus* in vended herbs could pose a greater health risk to the consumers. This bacterium species is normal skin flora, they live as a commensal organism on the in the nose and throat of humans and animals (Varshney *et al.,* 2018), it is an important cause of food poisoning following ingestion of preformed heat-resistant toxins. It has been demonstrated that ingestion of *Staphylococcus* enterotoxins (SEs) within food cause food poisoning, which is characterized by severe vomiting and diarrhea. Those symptoms occur within hours after eating of SE-contaminated food (Marrack and Kappler, 2014).

The presence of *Lactobacillus plantarum* a lactic acid bacterium in some of the herbs suggest that the herb could have been fermented, lactic acid bacteria could increase the bioavailability of nutrients and increase in shelf life of herb (Ojokoh, 2014).

CONCLUSION

This study has showed that the herbs vended in different motor parks in Akure are highly contaminated with microbes, some of which are potentially pathogenic and could therefore facilitate the transmissions of communicable diseases. In this study, vendors, age, level of education, household number, year of working experience and herbs packaging materials are the major contributing factors to microbial contamination of herb.

Based on the findings of this study, it is recommended that: authorised food and drug agents such as NAFDAC should perform routine assessment of the quality of herb vended at motor parks in order to safeguard the health of general public from bacterial infections and other contaminants which might be caused by consumption of unsafe herbal products; strict hygienic measures should be applied during herb preparation and handling, achievable through formal training of producers especially farmers and vendors on good agriculture and collection practices and good manufacturing practices.

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