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Microbial and physical evaluation of selected cough syrups sold at peripheral drug outlets in Bushenyi District, South Western Uganda

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Syrups, being non-sterile liquid pharmaceutical formulations, are prone to microbial contamination. The most common microbial contaminants are bacteria and fungi. Contamination of oral liquid pharmaceuticals makes them hazardous due to potential to cause infections, and may also change their physical, chemical, and organoleptic properties. This study assessed the microbial quality and physical characteristic parameters of 10 samples from each of four different brands of cough syrup sold in Ishaka, Bushenyi district. Microbial quality was assessed on samples of the syrups using spread-plate method via surface spreading of prepared dilutions of the different samples on agar plates. Plates were then incubated and colonies counted and expressed as number of colony forming units per milliliter (CFU/ml). The brand mean pH ranged from 3.04 to 5.02 and density from 1.05 to 1.35 g/cm^3 respectively. All sampled brands were compliant for pH, density, color and taste specifications. Out of the 40 tested samples, 57.5% showed total viable counts within the acceptable British Pharmacopoeia (BP) limit (≤100 CFU/ml), while 42.5% showed results outside the specified limit. All samples complied with BP requirements for *Staphylococcus aureus* while 97.5% samples complied with BP requirements for *Escherichia coli*. Based on mean CFU/ml, all the four brands met the acceptance limits for both *S. aureus* and *E. coli*, but one of the brands had an unacceptably high total viable count. Adequate quality control measures and stringent regulatory monitoring should be enforced in the supply chain to reduce on chances of product contamination.

Key words: Cough syrups, pharmaceutical quality, microbial contamination, community pharmacy, Bushenyi.

INTRODUCTION

Syrup is an oral non-sterile liquid formulation, characterized by sweet taste and viscous consistency,

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which incorporates a medicinal substance in a concentrated aqueous solution of sucrose with or without other additives such as flavors, aromas, and thickeners. Medicated and flavored syrups are the preferred pharmaceutical forms of choice for both children and adults because they are palatable and easily absorbed by the body (Tukur et al., 2012). A common example of syrups is Cough Linctus used for cough treatment.

Syrups are prone to microbial contamination, the most common microbial contaminants being bacteria and fungi. Bacterial contaminants include *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella* spp., *Bacillus subtilis* and *Staphylococcus epidermidis* (Ibezim et al., 2018; Mamun et al., 2014; Mugoyela and Mwambete, 2010; Tukur et al., 2012). On the other hand, fungal contaminants include *Candida albicans*, *Aspergillus niger* and *Aspergillus fumigatus* (Tukur et al., 2012; Na'was et al., 1990). The main sources of such pharmaceutical contaminants are the environment, raw materials, packaging materials, processing machines and cross-contamination from personnel (Gad et al., 2011; Nester et al., 2002). Raw materials such as plant extracts or their derivatives can also deteriorate due to poor harvesting, transportation and storage practices that make them susceptible to microbial attack (Dubey et al., 2014).

The contamination of syrups is predisposed by several factors that interfere with product stability. These factors include temperature, moisture, pH, oxygen content and type of preservative used (Canfield et al., 2005). Variation in these parameters can either encourage or suppress the growth of micro-organisms. The contamination of oral liquid pharmaceuticals makes them hazardous due to potential to cause infections, and also due to possible changes their physical, chemical, and organoleptic properties (Shaikh et al., 1988). Changes in physical and chemical properties of syrups due presence of microbes result in changes in their taste, odor, texture and color which complexes their administration to children (Morgan, 2009). The fermentation of syrups is a typical indicator of contamination. For example, *Klebsiella* spp. and *B. subtilis* metabolize citrate (a component of some cough syrups) thus altering odor of syrups (Korithoski et al., 2005). The changes in pH can further alter the color of syrups, since some colorant excipients are pH-sensitive.

Several studies conducted worldwide have confirmed the occurrence of microbial contaminants in syrups above the British Pharmacopoeia (BP) permitted limits of ≤100 CFU/ml for mean total viable count and *S. aureus* respectively, and ≤0 CFU/ml for *E. coli*. For instance, a study conducted in different drug shops and pharmacies of Benue and Makurdi states, Nigeria, involving 24 cough syrups produced by different pharmaceutical companies revealed high mean colony-forming units (CFU) of 5.9×10^4 CFU/ml and 4.2×10^4 CFU/ml for *S. aureus* and *P. aeruginosa* respectively (Gad et al., 2011). A related study conducted in Ilorin, Nigeria to assess bacteriological quality of 20 different brands of pediatric anti-malarial and cough syrups reported contamination levels in 14 different preparations that exceeded the official tolerance limit of permissible micro-organisms specified for syrups and suspensions. Some of these preparations were contaminated with *B. subtilis*, *E. coli*, *S. aureus*, and *P. aeruginosa*. *B. subtilis* was found to be the most predominant bacterial contaminant (Adeshina et al., 2009). Another microbial contamination study carried out in Dhaka, Bangladesh, on multivitamins and cough syrups found over 50% of the cough syrups noncompliant with United States Pharmacopoeia (USP) official requirements for microbiological quality. The main contaminants were *s. aureus* (75%), *E. coli* (17%) and coliforms (42%) (Mamun et al., 2014).

The administration of contaminated syrups to infants and the elderly particularly poses a great health threat, since these individuals are known for their low immunity. Lack of hygienic handling and inappropriate storage conditions of oral dosage forms has led to deaths of many children in developing countries (Hugbo et al., 2005). As such, routine studies aimed at evaluation of microbial integrity of oral pharmaceuticals are paramount to avert these associated or potential risks. This study, therefore, sought to evaluate microbial and physical parameters of cough syrups sold at peripheral drug outlets in Bushenyi district, South Western Uganda, as an attempt to depict the quality of these products. In turn, this would inform stakeholders on the suitability of existing standards of handling and storage practices for pharmaceuticals in drug outlets in the region.

MATERIALS AND METHODS

Study materials

This experimental study utilized 95% (v/v) ethanol, 40 cough syrups, peptone water, agar plates, test tubes, Petri dishes, brain heart infusion agar, violet red bile dextrose agar (VRBA), and mannitol salt agar (MSA).

Cough syrup samples

A total of 40 physically intact cough syrups that contained neither antibiotic nor preservative as per label claim were collected from 16 licensed drug outlets (8 pharmacies and 8 drug shops) located in Ishaka-Bushenyi Municipality, South Western Uganda. These 40 cough syrups comprised 10 samples for each of the four cough syrup brands code named as DS, P, K, and Z. Brands DS and P were both cough linctus preparations and each had only a primary package, while brands K and Z were herbal cough syrups, with the latter having both primary and secondary packaging. The drug shops were selected using simple random sampling, while universal sampling was used for the pharmacies.

Media preparation

The different media were prepared in accordance with specific protocols supplied by the manufacturer (Sigma Aldrich®). Mannitol salt agar was autoclaved at a temperature of 121°C for 15 min, cooled, and then poured onto Petri dishes and allowed to solidify.

This was then transferred into an incubator for overnight storage. Violet red bile dextrose agar (VRBGA) was prepared on the day of use by suspending 38.5 g of the dehydrated medium in distilled water (1000 ml) and boiled while mixing till it dissolved completely. The medium was then transferred onto the Petri dishes and then cooled to 45°C. Peptone water was prepared on the exact day of culturing by suspending 15.0 grams in distilled water (1000 ml), mixing thoroughly and then transferred to the final containers. This was then followed by autoclaving at 121°C for 15 min. Brain heart infusion (BHI) agar was also prepared on the day of culturing due to its high susceptibility to microbial growth. BHI agar (52 g) were suspended in distilled water (1000 ml) and then heated to boiling till the medium dissolved completely. The medium was autoclaved at 121°C for 15 min, cooled to 45℃, mixed well and then poured into sterile Petri plates.

Sterilization of instruments

Spreading rods and test tubes were placed in a hot air oven at a temperature of 170°C for 30 min, will pipette tip were autoclaved at a temperature of 121°C for 15 min.

Sample preparation

Each of the 40 samples was initially disinfected with 95% ethanol at the bottle top and passed through a Bunsen flame three times briskly. An aliquot (1 ml) of each sample was measured using a micropipette and introduced into test tubes containing 9 ml of sterile peptone water to attain a ten-fold dilution.

Microbial analysis by culturing

The spread plate method for the isolation of microbes was adopted for the analysis. Using a micropipette, 0.1 ml of the neat and 100-fold dilution of each of the samples was transferred on the two VRBA Petri dishes labeled VRBA neat and VRBA 10^{-2} and spread using previously sterilized spreading rods and immediately covered. These were transferred into the bacteria incubator at a temperature of 37°C for 24 h. This was done for all the 40 samples and *E. coli* colonies were then counted for each sample. For *Staphylococcus aureus,* the same experiment was repeated except that MSA was used instead of VRBA. The actual microbial population was then obtained by multiplying the plate count by the dilution factor and recorded as the colony-forming unit per ml (CFU/mL) (Al−Kaf et al., 2015).

Evaluation of physical parameters

The pH readings of each sample syrup were measured with a previously calibrated pH meter and then compared with reference values in the quality control certificate of analysis for each brand as specified by the manufacturer (that is pH 2-4 for DS, P and K, and pH of 4.5-6 for Z). The color of each sample was assessed by visual examination and compared with the color specified on the certificate of analysis of each syrup brand. The taste of each sample was evaluated organoleptically and matched to that specified in the quality control certificate of analysis specific to each brand. Density was measured using a pycnometer (density bottle). Weight of empty bottle, net weight of water and net weight of sample being analyzed were measured using a previously calibrated weighing balance. The density was computed using the formula below:

$$Density = [Net\ weight\ of\ sample / net\ weight\ of\ water] \times 0.99602$$

(Herrmann and Bucksch, 2014).

Data handling and analysis

The data was entered into a Microsoft Excel spreadsheet and cleaned by crosschecking its accuracy and consistency. This data was then exported to GraphPad Prism Ver. 7.0. Means ± SD or ±SEM for the microbial counts, pH, and density parameters were computed. One way ANOVA with a follow-up Dunnett's multiple comparison tests was run. Significance was set at $p < 0.05$.

Ethical considerations

Ethical approval was sought from the Faculty Research and Ethics Committee of the School of Pharmacy at Kampala International University (KIU) Western Campus. Strict adherence to biosafety measures was observed in the disposal of used culture plates to safeguard human life.

RESULTS

Cough syrup physical parameters (pH and density)

The four different syrup brands analyzed had mean pH values ranging from 3.04 to 5.02 which were all within their respective acceptable limits. The mean densities of the syrups ranged between 1.05 and 1.35 g per cubic centimeter (Table). The physical parameters of color and taste fitted within the acceptable limits.

Total viable counts

Cough syrup brand K samples had the highest mean total viable counts, followed DS, while cough syrup P has the least (Figure 1). Of the 40 cough syrup samples, 57.5% (23/40) had total viable counts within the acceptable limit (≤100 CFU/mL) while 42.5% (17/40) had total viable counts above the limit.

Staphylococcus aureus and *Escherichia coli* counts

All the four cough syrup brands showed growth of *S. aureus* (Figure 2) while only syrup brand P showed *E. coli* growth (Figure 3). However, all the four cough syrup brands' *S. aureus* counts were within the British Pharmacopeia recommended limit of ≤ 100 CFU/ml, with brand K having the highest *S. aureus* counts and brand P having the lowest.

DISCUSSION

Microbial contamination of pharmaceuticals can arise from many sources including production environment, raw materials, personnel, pharmaceutical equipment, and poor handling during dispensing, repackaging, and storage among others (Nester et al., 2002; Gad et al., 2011; Jawetz, 1987). Such contamination could affect the physical characteristics if microbial counts reach

Table 1. pH and density values (in grams per cubic centimeter) of the four brands of cough syrup.

Property	pH				Density (g/cm^3)			
Cough brands	DS	P	K	Z	DS	P	K	Z
Mean	3.29	3.04	3.40	5.02	1.05	1.05	1.35	1.32
STDEV	0.0316	0.0843	0.0471	0.0422	0.0057	0.0032	0.0048	0.0048
%RSD	0.96	2.77	1.39	0.84	0.54	0.30	0.36	0.37
Acceptable range	2-4	2-4	2-4	4.5-6	ns	ns	ns	ns

Note: DS, P, K, and Z are cough syrup brands, STDEV—standard deviation, %RSD—percentage relative standard deviation. ns—not specified in the certificate of analysis.

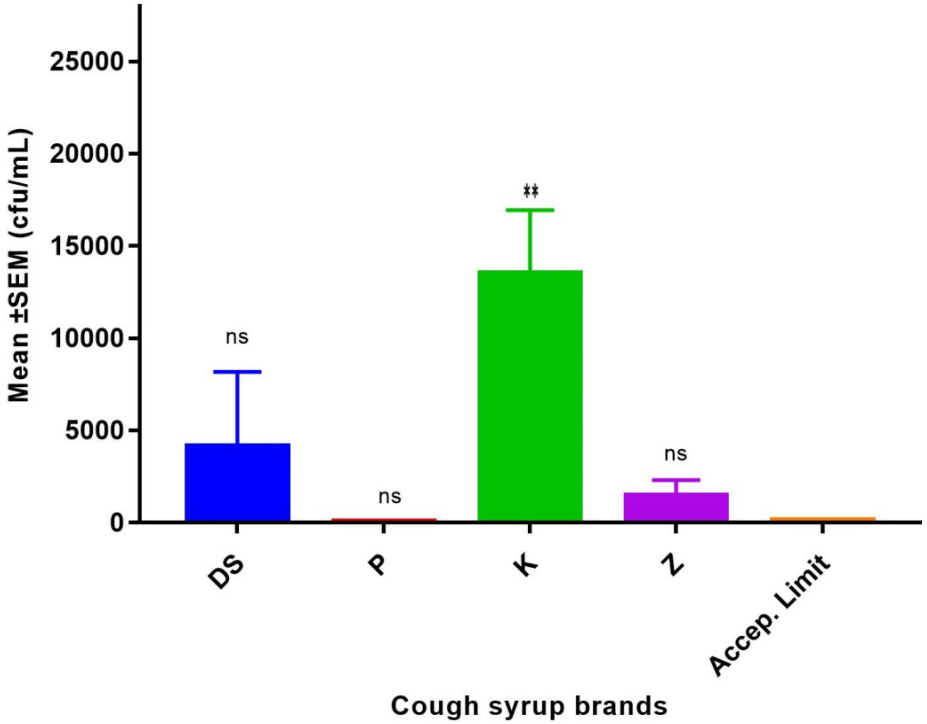

Figure 1. Mean total viable counts in the four cough syrups brands. DS, P, K, and Z are the different cough syrup brands; the acceptable limit is the mean total viable count of ≤ 100 CFU/mL. Mean total viable counts in brands DS, P and Z were not significantly different from the acceptance limit. Only brand K had unacceptable levels of mean total viable counts. ns—not significant ($p > 0.05$) and **—significant ($p < 0.01$) indicate the level of significance of mean total viable counts when compared with the acceptance limit.

significant levels within the products (Shaikh et al., 1988; Lee and Toksoy; 2002). Microbial infections can also originate from the physical presence of such microbial contamination and their metabolites/toxins. Microbial toxins even in minute quantities can cause diarrhea, gastroenteritis and abdominal discomfort when consumed by humans in contaminated pharmaceuticals (Nester et al., 2002).

In this study, 43% (17 out of 40 samples) of the cough syrup samples had total viable counts exceeding the BP limits of ≤ 100 CFU/ml. Consumption of microbiologically contaminated pharmaceuticals exposes one to various drug-borne infections (Parker, 2000). Although *S. aureus* and *E. coli* is normal flora, they can cause serious or fatal diseases. For instance, *S. aureus* which inhabits the skin and nose in healthy individuals can cause bacteremia (sepsis), pneumonia, endocarditis and osteomyelitis in patients with chronic conditions, weakened immune systems and those undergoing procedures in hospital settings (Tong et al., 2015). On the other hand, although most *E. coli* strains live harmlessly in the ileum of humans, some foodborne ones like enterohemorrhagic *E*.

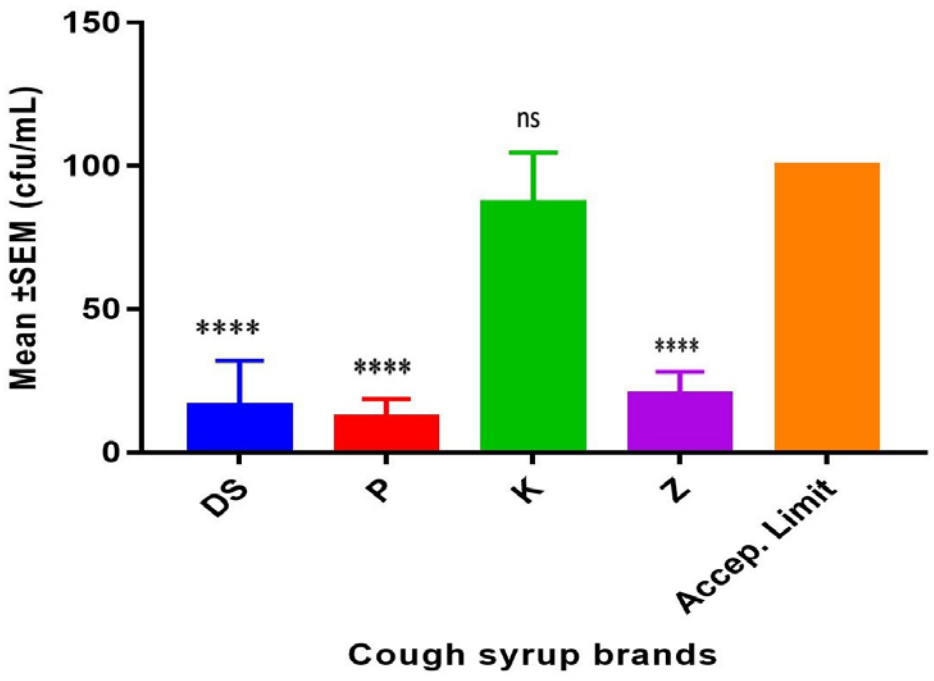

Figure 2. Mean CFU/ml of *Staphylococcus aureus* in the different cough syrup brands. The mean CFU/ml of the tested brands were either significantly below the required upper limit (DS, P and Z) or equal to it (brand K), implying that all brands were compliant for *S. aureus.* Ns,not significant ($p > 0.05$) and ****significant ($p < 0.0001$) indicate the level of significance of the differences in mean CFU/mL of *S. aureus* of the different cough syrup brands when compared with the acceptable limit of ≤ 100 mean CFU/ml.

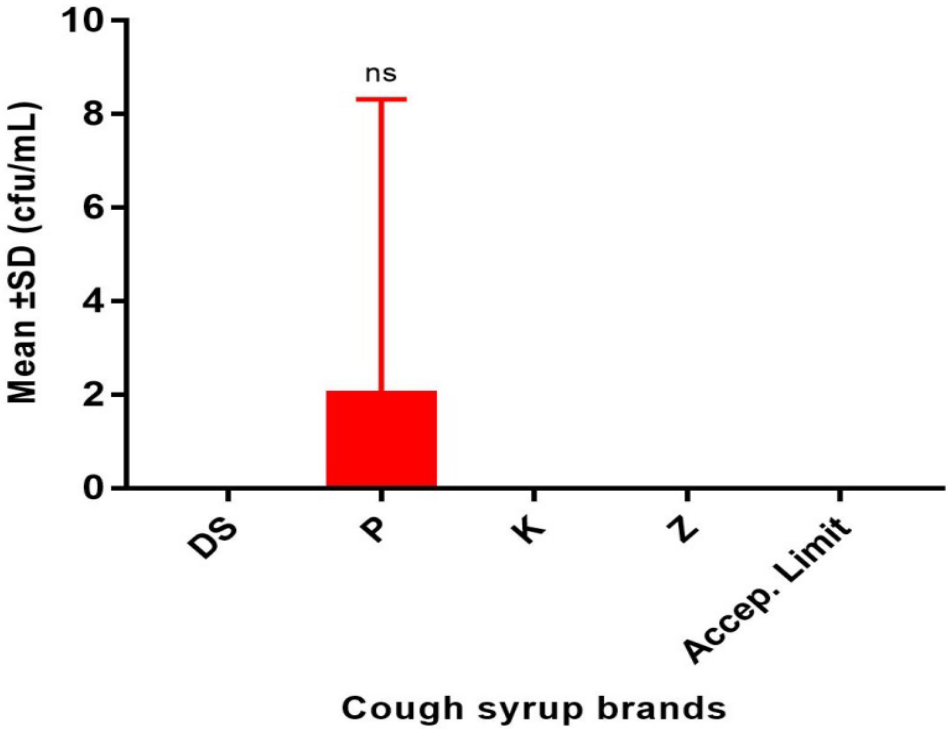

Figure 3. Mean CFU/ml of *E. coli* in the different cough syrup brands. Only brand P showed detectable levels of *E. coli* although not significantly different from the acceptance limit of ≤ 0 CFU/ml. ns—not significant ($p > 0.05$).

coli (EHEC) is pathogenic and can cause serious diarrheal diseases (Croxen et al., 2013). Infants and children who consume most of the syrups have increased susceptibility to pathogenic and opportunistic infections since their immune response is still below optimum (Ruckwardt et al., 2014). Thus it is crucial that the syrups being offered on the market are free of microbes. The occurrence of total viable microbial counts above the recommended limits may be attributed to cross-contamination (Ibezim et al., 2018; Mugoyela and Mwambete, 2010) which could have been exacerbated by the lack of preservatives in the formulations. Deficiencies in the use of preservatives increase proneness to deterioration of syrups (Bassat et al., 2015). Furthermore, Bushenyi, South Western Uganda, is located in the tropics and as such experiences humid conditions that are known to favor the growth of various microbes (Mugoyela and Mwambete, 2010).

On the whole, total viable counts in 3 of 4 cough syrup brands fitted within BP acceptable limits, indicating a 25% non-compliance rate. Brand K had the highest mean total viable count of 1.3465×10^4 CFU/mL. The mean total viable count in brand K was significantly higher than all the other brands and this could indicate non-compliance with current good manufacturing practices (cGMP) during production. Related studies conducted in Bangladesh and Ghana respectively reported over 75 and 17.78% of the samples as having the mean total microbial counts exceeding the BP acceptable limits (Opoku and Nyanor, 2019; El-Houssieny et al., 2013). Overall, there was no significant difference ($p > 0.05$) between the mean total microbial counts of cough linctus preparations versus herbal cough syrup preparations. Herbal preparations are more prone to microbial contamination majorly introduced by handling processes of medicinal product raw materials such as collection, drying, and storage (de Freitas Araújo and Bauab, 2012). Brand Z had significantly lower mean total viable counts ($p = 0.0097$) when compared to brand K which was also an herbal cough syrup. However, there were no significant differences in mean total viable counts between brand Z and the cough linctus preparations DS and P. Brands DS, P, and K were all packed in only the primary package. This could imply that being packed in both a primary and secondary package has no significant protection towards microbial contamination or that the contamination of these syrups occurred largely during the production process and not because of unhygienic handling at the pharmaceutical outlets.

About 97.5% of the analyzed syrup samples complied with British Pharmacopoeia (BP) requirements of zero CFU/mL for *E. coli*. Three brands; DS, K, and Z were easily complaint with BP requirements for microbiological quality for *E. coli* while brand P was barely compliant as the CFU/mL were at the upper acceptance limit. The presence of *E. coli* in cough syrups has also been reported in other studies carried out in Bangladesh (Mamun et al., 2014) and Nigeria (Mamun et al., 2014; Ibezim et al., 2018) where levels of 17% and 22% respectively were observed in the samples. However, the prevalence of *E. coli* contamination in this study was lower than that reported by both Mamun et al. in Dhaka and Ibezim et al. in Port Harcourt. Non-aqueous and aqueous pharmaceutical preparations for oral use should not contain *E. coli* as per the BP microbiological quality requirements for pharmaceuticals. Exposure to pathogenic strains of *E. coli* such as EHEC can result in severe diarrhea and other diseases (Croxen et al., 2013).

The *S. aureus* mean CFU/ml value for all brands was within the BP acceptable limit (≤ 100 CFU/ml) with brand K having the highest mean CFU/mL value in comparison with the other brands. *S. aureus* thrives best in a sugared environment since glucose is its main source of carbon (Onyango and Alreshidi, 2018). A relatively higher *S. aureus* count in brand K could be explained by the possibility of having higher sugar concentrations compared to other brands. This study results are consistent with related studies carried out in Nigeria, Jordan, Ghana, and Tanzania which also reported *S. aureus* mean CFU/mL within acceptable limits (Na'was et al., 1990; Tukur et al., 2012; Mugoyela and Mwambete, 2010; Opoku and Nyanor, 2019). A very high *S. aureus* contamination prevalence of 75% was reported by a study by Mamun et al. in Dhaka city, Bangladesh, on cough syrups (Mamun et al., 2014). This could indicate possible extensive cross-contamination during the manufacture and storage of the syrups considered in the Dhaka study.

The physical parameters of color, pH, density and taste for the different cough syrup brands were within the acceptable BP limits. According to the International Commission on Microbiological Specifications for Foods (ICMSF), *E. coli* thrives best at pH 7-8 (Kim et al., 2018), while *S. aureus* thrives best at pH 7-8, but can grow over the range of pH 4-10 (ICMSF, 1996). The pH range for the products studied was 3.04 and 5.02 and so could allow for the growth of *S. aureus* in the absence of good hygienic practices and when inadequate preservatives are used. This could explain the presence of *S. aureus* in all the cough syrup samples evaluated, although within the acceptable BP limits. Despite the presence of microbial contamination in most samples, all syrups retained their physical characteristics within the acceptable BP limits. However, overtime during the shelf life of the product, the quality of physical parameters of such pharmaceuticals is bound to deteriorate.

Conclusions

From the above results it can be concluded that out of the 40 syrup samples, only 57.5% passed the official requirement for microbiological quality of syrups that is total viable counts ≤100 CFU/ml as specified in the BP.

The above figure relates to inadequate quality control standards for raw-materials, air, packaging material, and equipment in some pharmaceutical plants as possibly being the source of contamination. Stringent quality assurance processes are needed during production in order to eliminate cross contamination of syrups and thus ensure their safety and efficacy throughout their shelf life.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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