Research Article

Microbial load and O157-H7, cp5, cp8 Serotypes assessment of some non-sterile pharmaceutical drug types sold at major outlets in Lafia, Nigeria

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Abstract

Objective: Non-sterile pharmaceutical products are usually subjected to unrestricted handling and are therefore potentially susceptible to postproduction contamination by microorganisms from both handlers and the environment. This study was carried out to determine the microbial quality of some commonly administered non-sterile drug types from hawkers and pharmacy outlets within Lafia Metropolis, Nigeria. **Material and methods**: A total of 240 samples (80 samples each of tablets, syrups and ointments) were purchased from pharmacies and hawkers' outlets and the microbial load determined using the W.H.O Pharmacopoeia and serological methods. **Results and conclusion:** From this study, the mean Total Aerobic Bacterial Counts (TABC) and the Mean Total Yeast and Mold Counts (TYMC) had values higher than the W.H.O recommended levels for some syrups and tablets from both pharmacy and hawkers' outlets. The difference in the values among the various outlets and between the drug types were statistically significant at $p<0.05$. Results of immunoassay indicated that *E. coli* was isolated from 23/120 (19.17%) hawkers' drugs among which 1/23 (4.35%) was the O157:H7serotype. Similarly, 17/120 (14.17%) hawkers' drugs had *Staphylococcus aureus* among which 8/17(47.06%) and 6/17(35.27%) were *cp5* and *cp8* serotypes respectively while 3/17(17.65%) had no capsular antigen (*cp*-). These values were significantly higher ($p<0.05$) than those obtained from pharmacy outlets. The microbial qualities of most of the pharmacy products were in accordance with International Pharmacopoeia while some, especially the tablets and syrups from hawkers, exceeded the recommended acceptance limit and might be of health risks to consumers.

Keywords: Microbial load, Non-sterile pharmaceuticals, hawkers, pharmacies, Nigeria

Introduction

Contamination of pharmaceuticals with microorganisms irrespective of being harmful or not harmful can lead to changes in the characteristics of such drugs. Microbial contamination of pharmaceuticals has been a major problem for researchers as well as pharmaceutical manufacturers worldwide (National Agency for Food and Drugs Administration Control, 2000; Mugoyela and Mwambete, 2010). Microbial contamination can result in the spoilage of the therapeutic formula by breaking down the active ingredients and excipients, affecting the potency, stability and efficacy of such drug (Campana et al., 2006). The presence of high pathogens poses a serious health concern to consumers, especially those who are already in diseased conditions or in a weakened state (Jimenez, 2004; Campana et al., 2006; Ragheb et al., 2012). Several cases of infection due to contaminated pharmaceuticals have been reported in literature (Becks and Lorenzoni, 1995; Reiss et al., 2000; Jimenez, 2004)which could be as a result of either the breakdown of manufacturing procedure or poor storage method and from handlers. Apart from the deteriorating actions of microorganisms on drugs, their effects such as production of toxins and metabolites leads to induction of symptoms when innocently consumed by patients (Nester et al., 2002). Common pharmaceutical contaminants such as Gram-positive bacteria implicates the involvement of human interventions as a major reason for product contamination, while the presence of Gram negative bacteria suggests lack of process control in pharmaceutical

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environments, especially involving water systems and raw materials (Jimenez, 2004).

The microbiological quality of pharmaceutical products mainly depends on the quality of raw materials, manufacture process and environment, hygienic conditions of the personnel involved in the manufacture and the storage conditions (Baird, 2004). Several cases of infection due to contaminated pharmaceuticals have been reported (Becks and Lorenzoni, 1995; Reiss et al., 2000; Jimenez, 2004).

In Nigeria, pharmaceutical industrialization by local and foreign companies have increased substantially and some of their products have been found to be competent and satisfactory (National Agency for Food and Drugs Administration Control, 2000). However, complaints have often originated from different areas concerning the efficacy and quality of some of these products, especially the non-sterile pharmaceutical products. They are usually preserved under different storage conditions and sold mainly by pharmacists, patent and proprietary medicine vendors (PPMV) and hawkers. Though numerous studies on microbial contamination of drugs have been reported, the problems associated with this menace still prevail (Rashed et al., 2015).

Escherichia coli and *Staphylococcus aureus* have been reported to serve as general indicator organisms for the contamination of nonsterile pharmaceutical products (European Directorate for the Quality of Medicine and Healthcare, 2017). Although most of the organisms contaminating non-sterile pharmaceutical products may be nonpathogenic commensals of environmental origin, they pose problems as agents of spoilage and may also become pathogenic in compromised individuals (Takon and Antai, 2006). Some *Staphylococcus aureus* and *Escherichia coli* strains have also been implicated as major contaminants of drugs with public health implications (O'Riordan and Lee, 2004; Obi and Nwannunu, 2010; Adeola and Adeleye, 2012; Mohammed et al., 2019). *Staphylococcus aureus* capsular polysaccharide serotypes cp5 and cp8 have been reported to cause diseases especially nosocomial infections (Mohammed et al., 2019). Studies have shown that cp5 and cp8 are the only serotypes that are clinically relevant and are associated with human diseases hence their presence in drugs could be dangerous to those on medication (Daniyam and Sangodere, 2011). *E coli* 0157:H7 has also been associated with a broad spectrum of diseases ranging from mild diarrhea and haemolytic colitis to more fatal cases of haemolytic uremic syndrome (HUS) (Rahal et al., 2012). This organism though mainly present in faeces of cattle can be spread through the consumption of contaminated produce including drugs (Sebibe and Asfaw, 2020).

Majority of inhabitants in local communities in Nigeria patronize non-sterile and nonprescription (*over-the-counter*) drugs from hawkers due to their friendly disposition and affordability of their prices relative to those from pharmacies. Patent and Proprietary Medicine Vendors (PPMV) and hawkers commonly operate in the informal sector in most communities and are usually seen in public arenas such as markets, motor parks, religious houses and in commercial vehicles (Durowade et al., 2018). Microbial contamination of some nonsterile pharmaceutical products sold in some major towns in Nigeria has been reported (Taylor et al., 2001; Takon and Antai, 2006; Obi and Nwannunu, 2010; Daniyam and Sangodere, 2011; Adeola and Adeleye, 2012; Okpo et al., 2016; Kilani and Olaifa, 2017). Despite these efforts, microbial bioburden in drugs still remains a continuous challenge in the health sector especially in semi-urban and rural communities since most of these studies are limited to major towns and cities. Microbiological assessment of non-sterile products is particularly pertinent in view of the fact that microbial contamination can reduce or even eliminate the therapeutic effect of such products or cause drug-induced infections. It is therefore necessary to perform a microbial health hazard indicator analyses on nonsterile pharmaceutical products from major outlets for standardization purpose. This article was therefore aimed at assessing some microbiological indices that are associated with the quality of some non-sterile drugs from two major outlets (hawkers and pharmacies) in Lafia (a semi-urban community) with additional emphasis on disease implicated serotypes of some notorious nonsterile drug contaminants.

Materials and methods

Samples collection

Pharmacies and hawker outlets were selected at random using systematic random sampling technique. A total of 120 samples each from pharmacy shops and hawkers' outlets were included in the study. The 240 samples comprising 80 samples each of tabletss, syrups and ointments were purchased from outlets within the four geographical regions (Lafia I, Lafia II, Assakio and Shabu) of Lafia Metropolis. Samples were collected among commonly administered nonsterile pharmaceuticals such as antacids, analgesics, antibiotics, antimalarials, vitamins and cough syrups.

Preparation of samples

Sample preparation was done following the United States Pharmacopeia Instruction (Hashjin et al., 2019). Each tablet sample was pounded into powder with a sterile mortar and pestle, and 10g dissolved in 90mL of sterile phosphate buffer solution (PBS) (pH 7.2) to obtain 10^{-1} dilution. Further dilutions were performed using same diluent to 10^{-3}. The same procedure and diluent was used for ointments

while 10mL of each syrup sample was diluted serially in 90 mL of the diluent.

Determination of Total Aerobic Bacterial Counts (TABC) from drug samples

One millilitre (1.0 mL) aliquot from 10^{-1} and 10^{-2} sample dilutions were dispensed into petri dishes, pour plated into Casein-peptone Soy Agar (CSA) and incubated at 37°C for 24 hours. Colonies were counted and the results expressed as colony forming units per gram ($cfug^{-1}$) for tablets and ointments and colony forming units per milliliter ($cfumL^{-1}$) for syrups.

Determination of Total Yeast and Mold Counts (TYMC) from drug samples

One millilitre (1.0 mL) from the 10^{-1} dilution of the different drug samples were poured into Sabouraud Dextrose Agar (SDA) and kept for 2 – 5 days at ambient temperature. Colonies were counted using colony counter and recorded as colony forming units per gram ($cfug^{-1}$) for tablets and ointments and colony forming units per milliliter ($cfumL^{-1}$) for syrups.

Isolation and Identification of *Escherichia coli*

Ten milliliter (10.0 mL) from the 10^{-1} diluent of each drug sample was transferred into 100 mL of MacConkey broth and incubated at 42°C for 24 hours. After thoroughly shaken, a loopful of the broth culture was streaked on MacConkey agar and incubated at 35°C for 24 hours. Growth of purple colonies indicated the presence of *E. coli* in the sample.

Isolates were identified using Gram stain and biochemical tests and confirmed with the API 20E identification system. Biochemical tests used for the identification of the isolates include indole test, urease test, oxidase test, citrate utilization test and Triple Sugar Iron agar test (Cheesborough, 2006).

Isolation and Identification of *E. coli* O157 serogroup

Homogenized drug samples (10g mixed with 20ml of sterile normal saline for tablets and ointments and 10mL mixed with 20ml of sterile normal saline for syrups) were enriched by pipetting 10ml of each sample into 90ml of buffered peptone water and incubated at 37°C for 24 hours. Approximately 0.1ml of each enriched sample was subsequently plated on Sorbitol McConkey agar supplemented with cefixime ($0.5mgL^{-1}$) and potassium tellurite ($2.5mgL^{-1}$) (SMAC-CT) and incubated at 45°C for 24 hours. Sorbitol-negative colonies which appeared colorless to gray were considered presumptive *E. coli* 0157 colonies.

Gram staining and other confirmatory biochemical tests typical for *E. coli* such as indole, methyl red, vogesproskauer, citrate and lysine decarboxylase were performed (Cheesborough, 2006).

Growth on 4- Methylumbilliferyl-β-D-Glucoronide (MUG) medium for *E. coli* O157:H7

All positive *E. coli* O157 colonies were further inoculated into test tubes containing *E. coli* with MUG (*E. coli*-MUG) medium and incubated at 42°C for 18 - 24 hours as recommended by Thompson *et al*. (Hashjin et al., 2019). The broth cultures were then observed under ultraviolet (uv) light of long wavelength (650nm) to detect the inability of *E. coli* 0157:H7 to cleave MUG. Positive isolates were considered as those that fermented lactose (yellow broth), produced gas (collected at the tip of the immersed durham tubes) and did not produce any fluorescence.

Serotyping with standard *E. coli* 0157:H7 rabbit antisera

This analysis was performed using standard *E. coli* 0157:H7 rabbit antisera (Difco Laboratories, Detroit, Mich.) preserved with glycerol using 1:2 dilution. Slide agglutination technique was used to test resuscitated colonies directly from sorbitol MacConkey agar (SMAC). A wire loop was used to remove a loopful of the colony and suspended in a drop of normal saline. An equal amount of *E. coli* 0157:H7 antiserum was added and mixed by rocking back and forth for 1min.Colonies that agglutinated rapidly with the *E. coli* 0157:H7 antisera were considered as confirmed positive *E. coli* 0157:H7 colonies.

Isolation and identification of *Staphylococcus aureus*

Aliquots of 0.1mL from 10^{-1} drug dilutions were spread on mannitol salt agar (MSA) plates and incubated at 37°C for 48 hours. Yellow presumptive colonies were further screened using Gram staining, catalase test with 3% H_2O_2 and coagulase test using rabbit plasma. The pure cultures were maintained on Luria broth (LB) agar plates and stored at 4°C.

Serotyping for *Staphylococcus aureus* capsular antigens (cp5 and cp8)

Pure cultures of *S. aureus* isolates were resuscitated on Columbia agar (Difco) supplemented with 2% NaCl for 24 hours at 37°C and the colonies harvested using 1ml of 10mM Phosphate Buffered Saline (PBS) (0.15M NaCl, pH 7.2). The cell suspensions were subsequently autoclaved for 1h at 121°C and the cells pelleted by centrifugation at 10,000 x g. The supernatants containing the cell extracts were filtered through a 0.45um millipore filter and the filtrate stored at -20°C.

Serotyping was performed using the slide agglutination technique incorporating standard *S. aureus* cp5 and cp8 capsular antisera produced from rabbits.

Statistical analyses

Data collected from this study were analyzed using SPSS (version 23; SPSS Inc., Chicago, IL, USA), Chi-square

(x^2) used to test relationships between variables and ANOVA to analyze more than two determining variables. Each test was conducted at 95% confidence interval (p=0.05) at the appropriate degrees of freedom.

Results

Data obtained for total aerobic bacterial counts (TABC) and total mold and yeast counts (TMYC) from drugs from both outlets were compared to standard W.H.O pharmacopoeias values while the frequencies of isolation of the various serotypes were also evaluated based on the drug outlets.

Table 1 showed the mean total aerobic bacterial counts of drugs types from various location. The highest count of 5.0 x 10^4±1.7 was obtained among syrups from hawkers at Assakio while ointment samples had no count from all pharmacy samples. Mean total aerobic bacterial counts (TABC) from all drug types were significantly higher ($p<0.05$) in samples from hawkers compared to those from pharmacies.

The mean total mold and yeast counts (TMYC) of different drug types purchased from different outlets are shown in table 2. The highest mean value of 5.2 x 10^2±1.1 was obtained among tablets from hawkers while all drug types from pharmacies had no value. There were significant differences ($p<0.05$) in the tablets and syrups values from both pharmacies and hawkers while those from ointments were not statistically significant at $p > 0.05$.

The comparison of microbial counts from various drug types and purchase outlets relative to W.H.O standards is shown in table 3. A total of 14/80 (17.50%) syrup and 5/80 (6.25%) tablet samples from hawkers had TABC values above W.H.O recommended acceptance level while all ointment samples had no growth. Also, 5/80 (6.25%) syrup and 2/80 (2.50%) tablet samples from hawkers had TMYC values higher than the W.H.O acceptance level. Drug samples from hawkers with higher values than the recommended were significantly different ($p<0.05$) for both the TABC and TMYC while values from pharmacy drugs were statistically insignificant at $p > 0.05$.

Table 1. Mean total aerobic bacterial counts (TABC) of different drug types from various locations

Location	Drug Type					
	Tablets ($CFUg^{-1}$) ≤ 2×10^{3a}		Syrups ($CFUml^{-1}$) ≤ 2×10^{2a}		Ointments ($CFUg^{-1}$) ≤ 2×10^{2a}	
	PHR	HAW	PHR	HAW	PHR	HAW
Lafia I	0.00	1.6×10^2±1.5	3.6×10^1±1.2	7.5×10^1±2.6	0.00	1.5×10^2±2.0
Lafia II	5.0×10^1±1.3	3.1×10^4±1.2^b	1.2×10^1±3.0	1.6×10^4±1.0b	0.00	0.00
Assakio	2.7×10^3±1.0^b	3.0×10^3±0.7^b	6.0×10^1±1.8	5.0×10^4±1.8b	0.00	1.8×10^1±1.0
Shabu	0.00	1.0×10^3±1.0	7.0×10^3±1.3^b	2.5×10^3±2.1b	0.00	0.00
	p<0.05	p>0.05	p>0.05	p>0.05	p>0.05	p<0.05

a=W.H.O recommended acceptance criteria; b=values higher than W.H.O standard; PHR= Samples from pharmacies; HAW= samples from hawkers

Table 2. Mean total mold and yeast counts (TMYC) of different drug types from various locations

Location	Drug Type					
	Tablets ($CFUg^{-1}$) ≤$2\times10^{2\,a}$		Syrups ($CFUml^{-1}$) ≤ 2×10^{1a}		Ointments ($CFUg^{-1}$) ≤2×10^{1a}	
	PHR	HAW	PHR	HAW	PHR	HAW
Lafia I	0.00	5.2×10^2±1.1^b	0.00	3.2×10^2±1.5^b	0.00	0.00
Lafia II	0.00	0.00	0.00	0.00	0.00	0.00
Assakio	0.00	3.7×10^1±1.2	0.00	8.3×10^1±1.5^b	0.00	0.00
Shabu	0.00	1.0×10^1±0.2	0.00	1.6×10^1±1.0	0.00	1.1×101±0.1
	p>0.05	p<0.05	p>0.05	p<0.05	p>0.05	p>0.05

a= W.H.O recommended acceptance criteria; b= Values higher than W.H.O standard; PHR= samples from pharmacies; HAW= samples from hawkers

Out of the 120 samples analyzed from hawkers, 23(19.17%) had *Escherichia coli* with highest value of 13/40(32.50%) obtained among the syrups. *E. coli* O157:H7 serotype was detected in 3 (13.04%) of the 23 *E. coli* isolates. Values for *E. coli* (6.67%) and 0157:H7 serotype (0.00%) isolated from pharmacy drugs were significantly lower ($p<0.05$) compared to those from hawkers (table 4). Similarly, the frequency values of *E. coli* isolation among the various drug types from hawkers were significantly different at $p<0.05$ while all parameters from pharmacies had no significant difference ($p > 0.05$).

Out of 17/120 (14.17%) *Staphylococcus aureus* isolates obtained in hawkers' drugs, capsular antigens *cp5* and *cp8* serotypes were obtained from 8/17(47.06%) and 6/17(35.27%) respectively while 3/17(17.65%) had no capsular antigen (*cp*-) as shown in table 5. *Staphylococcus aureus* was also isolated from 5/120 (4.17%) of the pharmacy drugs among which 2/5(40.00%) each were cp5 and cp8 while 1/20 (20.00%) had no capsular antigen (cp-). There was significant difference ($p<0.05$) in the isolation of the organism among the various drug types from the hawkers while all parameters from pharmacies and serotypes frequencies from hawkers had no significant difference ($p > 0.05$).

Table 3. Distribution of microbial counts from various drug types and sources based on W.H.O recommended standards

Drug type			TABC			TMYC	
	Total no. of samples	W.H.O std (cfu/g/ml)	PHR n (%)	HAW n (%)	W.H.O std	PHR n(%)	HAW n (%)
Tablets	80	$\leq 2.0x10^3$	2(2.50)	5(6.25)	$\leq 2\times10^2$cfu/g	0(0.00)	2(2.50)
Syrups	80	$\leq 2.0x10^2$	3(3.75)	14(17.50)	$\leq 2\times10^1$cfu/ml	0(0.00)	5(6.25)
Ointments	80	$\leq 2.0x10^2$	0(0.00)	0(0.00)	$\leq 2\times10^1$cfu/g	0(0.00)	0(0.00)
			p>0.05	p<0.05		p>0.05	p<0.05

n =No. of samples with values above W.H.O recommended acceptance level; TABC= Total Aerobic Bacterial counts; TMYC= Total Mold and Yeast Counts; PHR=Samples from Pharmacies; HAW=Samples from Hawkers.

Table 4. Occurrence of *Escherichia coli* and 0157;H7 serotypes in major drug types from pharmacies and hawkers' outlets

Drug Type	PHARMACIES (N=40)		HAWKERS (N=40)	
	***nEC*/*N* (%)**	***nECO*/*nEC* (%)**	***nEC*/*N* (%)**	***nECO*/*nEC* (%)**
Tablets	3/40(7.50)	0/3(0.00)	8/40(20.00)	0/8(0.00)
Syrups	5/40(12.50)	0/5(00.00)	13/40(32.50)	1/13(7.70)
Ointments	0/40(0.00)	0/0(0.00)	2/40(5.00)	0/2(0.00)
Total	8/120(6.67)	0/8(0.00)	23/120(19.17)	1/23(4.35)

N=Total no. of analysed samples per drug type; nEC= No. of *E. coli* positive samples nECO = No. of *E. coli* 0157:H7 isolates

Table 5. Occurrence of *Staphylococcus aureus* and serotypes cp5 and cp8 in major drugs types from pharmacies and hawkers' outlets

Drug Type	PHARMACIES (N=40)				HAWKERS (N=40)			
	***nSA*/N(%)**	***ncp*5/nSA(%)**	***ncp*8/nSA(%)**	***ncp*/*nSA*(%)**	***nSA*/N(%)**	***ncp*5/nSA(%)**	***ncp*8/nSA(%)**	***ncp*/*nSA*(%)**
Tablets	3/40(7.50)	2/3(66.67)	1/3(33.33)	0/3(0.00)	6/40(15.00)	3/6(50.00)	2/6(33.33)	1/6(16.67)
Syrups	2/40(5.00)	0/2(0.00)	1/2(50.00)	1/2(50.00)	9/40(22.50)	3/9(33.33)	4/9(44.44)	2/9(22.22)
Ointments	0/40(0.00)	0/0(0.00)	0/0(0.00)	0/0(0.00)	2/40(5.00)	2/2(100.00)	0/2(0.00)	0/2(0.00)
Total	5/120(4.17)	1/5(20.00)	2/5(40.00)	1/5(20.00)	17/120(14.17)	8/17(47.06)	6/17(35.29)	3/17(17.65)

N=Total no. of samples per drug type; nSA = No. of *Staphylococcus aureus* positive samples; ncp5 = No. of cp5 isolates; ncp8 = No of cp8 isolates; ncp- = No. of cp- isolates

Discussion

Non-sterile drugs regardless of their dosages, forms and routes of administration must meet with the microbiological purity criteria set out by regulatory authorities. The quality of drugs available in some underdeveloped countries is usually poor due to widespread counterfeit pharmaceuticals. Excessive decomposition of active ingredients occurs as a result of high temperature, humidity, redox potential and poor quality assurance during the manufacturing process which create favourable conditions for microbial growth (Taylor et al., 2001; Murtaza et al., 2021).

In this study, high mean TABC of $5.0x10^4 \pm 2.1 cfumL^{-1}$ and $3.1x10^4 \pm 1.2$ $cfug^{-1}$ were obtained from hawkers' syrups and tablets respectively. Generally, syrups from hawkers were shown to have higher TABC values compare to other drug types with statistically significant difference at $p<0.05$. Similarly, mean bacterial count range between 1.0×10^3 and 3.0×10^3 $cfumL^{-1}$ in chloroquine syrups sold in Calabar, Nigeria has been reported (Okpo et al., 2016). In this study, syrups were found to be the most common drug forms with higher level of contamination by bacteria most of which were samples obtained from hawkers. This may be as a result of its physical state as microorganisms require moisture as favourable condition to grow and multiply. A similar result was reported in Egypt (Salem et al., 2021) and in Minna, Nigeria (Daniyam and Sangodere, 2011). The low level of contamination of tablets in this study is in agreement with the study carried out in Jordan where 10.6% contamination of tablets tested was reported (Qasem et al., 2014).

High bacterial contamination of some cough syrups and multivitamins has been reported in Pokhara, Nepal (Gurung and Rai, 2021). Also, moisture content (water activity) has been shown to be a favorable condition for the growth of microorganisms in nonsterile pharmaceutical products (Murtaza et al., 2021). Bacteria may also be introduced through contaminated water used by the pharmaceutical industries for drug preparations. However, a study of non-sterile drugs manufactured by different pharmaceutical plants in Polish observed that bacterial counts for non-aqueous preparations range between $8.2x10^3$ and $1.6x10^5$ $cfug^{-1}$ while aqueous preparation had a lower value of $4.2x10^4 cfumL^{-1}$ (Ratajczak et al., 2015). Maximum total bacterial count value of $9.0 \times 10^2 cfug^{-1}$ was obtained from retailed tablets in Lagos (Adeola and Adeleye, 2012) while higher incidence of 55% microbial contamination of paracetamol tablets in Dutsinma Metropolis, Nigeria has also been reported (Kilani and Olaifa, 2017). These findings show higher values of bacterial count from non-aqueous preparations contrary to the findings of this study. This is also corroborated by the study on the new biological quality of syrups and water used in pharmaceutical industries in Kano State, Nigeria which was shown that all plate counts did not exceed the U.S pharmacopoeia acceptable criteria (Olaitan and Muhammad, 2018). These findings therefore suggest that other factors (not limited to water availability) might have contributed to the higher levels of bacterial count obtained in this study. Handling, packaging and storage processes as well as preservatives have been suggested to be a source of contamination for capsules, tablets and dry powder suspensions (Murtaza et al., 2021). The involvement these factors in drugs contamination may likely explain why pharmacy drugs tend to have lower bacteria counts than those from hawkers. Pharmacy drugs were observed to have fewer TABC values which were higher than the W.H.O recommended acceptance criteria compared to those from hawkers. In most cases, drugs sold by hawkers in the study community were usually handled and packaged under unhygienic conditions while most are manufactured by unlicensed companies. Their counterparts from most pharmacies were handled following strict regulations and manufactured by licensed companies.

In this study, *E. coli* was isolated from 23/120 (19.17%) samples from hawkers and 8/120(6.67%) from pharmacies with the frequency values having statistically significant differences ($p<0.05$) between both outlets and among the hawkers' drug samples. Result from hawkers' samples is similar to 16.66% obtained for expired drugs sold in Calabar (Takon and Antai, 2006). However, higher value of 38% was obtained from same environment (Okpo et al., 2016) and 47.6% working on non-sterile pharmaceutical preparations in Egypt (Gamal et al., 2011). This suggests that mode of handling by brand producers and marketers may alter results obtained from same location. This may also explain the significant differences obtained from values between the drug outlets and also among the various drug types from the hawkers. Packaging and handling of the drugs by hawkers were observed to be subjected to high procedural discrepancies with some (especially the tablets) completely exposed to atmospheric contamination and spoilage. The presence of *E. coli* isolates serves both as an indication of possible contamination of the drugs and as a possible health threat especially with orally administered drugs. *E. coli* must be absent in 1g or 1ml of non-aqueous and aqueous preparations for oral use based on the European Directorate for the Quality of Medicine and Healthcare regulation (European Directorate for the Quality of Medicine and Healthcare, 2017). Although the isolation of the O157:H7 serotype among the *E. coli* isolates was 1/23(4.35%) in hawkers' drugs and none was found from the pharmacies, the results is still of serious health concern. *E coli* O157:H7 has been associated with enteric hemorrhagic diarrhea and colitis whose virulence is attributed to plasmid-induced (pO157) haemolysin and the locus of enterocyte effacement (LEE) associated with A/E

lesions (Rahal et al., 2012). The persistence of *E. coli* and its O157:H7 serotype may also be attributed to the high multidrug resistance exhibited by the organisms especially to 2nd generation antibiotics which are commonly sold by hawkers and patent medicine vendors in most communities (Sebibe and Asfaw, 2020).

Staphylococcus aureus was isolated from 17/120 (14.17%) hawkers' drug samples among which 8/17(47.06%) and 6/17(35.27%) were cp5 and cp8 serotypes respectively while 3/17(17.65%) was cp-. The presence of *S. aureus* in this study confirms other studies that isolated the organism from drugs (Obi and Nwannunu, 2010; Daniyam and Sangodere, 2011; Qasem et al., 2014; Okpo et al., 2016). *Staphylococcus* spp are normal flora of the skin and are usually incriminated as contaminants of production equipment as well as raw materials. Therefore, their probable presence in the drug samples could have been one or a combination of these sources including handling. The presence of *S. aureus* in the drugs analyzed is of great concern, as the organism have been reported to secrete enterotoxin which causes gastrointestinal distressed. According to W.H.O regulations, *S aureus* must be absent in drugs for oro-mucosal, gingival, cutaneous, nasal, auricular vaginal, transdermal patches and inhalation therapies (W.H.O, 2019).

The isolation of *cp5* and *cp8* serotypes of *S. aureus* from the drug samples is of serious health concern. *S. aureus* isolates that express capsular polysaccharide types 5 (cp5) and 8 (cp8) have been associated with diseases especially nosocomial infections (Mohammed et al., 2019). They also reported that these serotypes are produced by 75 – 80% of *S. aureus* clinical isolates which agrees with the cumulative values of 82.35% and 80.00% obtained from hawkers and pharmacy samples respectively in this study. These capsular antigens have been shown to impede phagocytosis resulting in persistence of the organism in the bloodstream. They also assist *S. aureus* to adhere to endothelial surfaces and promotes colonization and persistence on mucosal surfaces (O'Riordan and Lee, 2004).

The presence of microbial bioburden higher than the W.H.O pharmacopoeia standards and isolation of pathogenic contaminants from some of the drug samples serves as an indication of a possible health hazard in the study community, if necessary, measures are not taken to prevent further contamination. The drugs will certainly lose their potency due to contamination and deterioration by these microorganisms except continuous and effective surveillance mechanism is being enforced.

Conclusion

The result of this study revealed that some of the drugs sold in Lafia Metropolis are contaminated with microorganisms with microbial load above acceptable specifications by W.H.O. The organisms isolated have pathogenic records and may serve as possible sources of unsuspected infection to consumers. The level of drugs contamination tends to be significantly higher among those from hawkers than those from pharmacy outlets while the syrups remain highly contaminated relative to the other drug types. Microbiological quality evaluation of drugs and sensitization of personnel involved in the various stages in drugs production, packaging and sales on the need to observe standard operational procedures remain sacrosanct. Current Good Manufacturing Practice (CGMP) regulations must be enforced by concerned regulatory bodies to prevent possible disease outbreak.

Authors' Contributions

This research was carried out with the total collaboration of all the authors. Author JFN conceived, designed and wrote the protocols and first draft of this study. Author DDO and ASK compiled the necessary literature and statistical analyses while authors HDA, AA and FAK contributed to the laboratory analyses. All authors reviewed and approved the first manuscript.

Conflicts of Interest

All items used in this research were obtained locally and mainly used in our area of research. There is therefore no conflict of interest between the authors and producers of such items. This research was also completely funded by the authors without assistance from any institution or organization.

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