**ABSTRACT**

Pharmaceutical oral liquid dosage forms are basically formulated as non-sterile products, however, must satisfy the appropriate microbiological purity criteria. Such pharmaceutical products are susceptible to contamination by a variety of microorganisms during manufacturing and use. In this study, sixty seven (67) samples of four (4) different pediatric oral liquid drugs (Ibuprofen, Albendazole, Metronidazole and cotrimoxazole suspensions) were evaluated for the presence of microbial contamination using standard procedures described in the official monograph of the British and United Stated pharmacopoeia. Microbial contamination was observed in 44 (65.7%) of the tested samples with 25 (37.3%) and 17 (25.4%) of the examined products having Total Aerobic Microbial Counts (TAMC) and Total Yeasts Moulds Counts (TYMC) greater than the USP and BP acceptable limit of $10^2$ cfu/ml and $10^3$ cfu/ml respectively. High level of microbial contamination was observed in samples of pediatric oral liquid formulations particularly in samples of Ibuprofen and metronidazole suspensions. Microbial contaminants recovered from the examined products include Bacillus sp., Staphylococcus aureus, Micrococcus sp., Pseudomonas aeruginosa, Enterobacter sp., Escherichia coli, Penicillium sp., Aspergillus sp., and Candida sp. Strict adherence to guidelines of the current good manufacturing practices and good hygienic practices during handling, sales and usage of the products is recommended.

**Keywords:** Microbial contamination, oral liquids, TAMC, TYMC and Acceptance criteria

**INTRODUCTION**

Pharmaceutical products of different forms and dosage are susceptible to contamination by a variety of microorganisms during manufacturing, storage, handling or use by the consumer (Itah et al., 2004; Oviasogie et al., 2015). Oral dosage forms such as aqueous solutions, suspensions, emulsions, syrups and tablets are among the preparations that are at the greatest risk of microbial contamination (Netish et al., 2018). As a result of the lack of good manufacturing practices, liquid dosage forms being non-sterile are likely to contain different types of microbial species. Additionally, pharmaceutical products that contain moderate to high water activity, products containing sweeteners and products in multidose containers are more prone to microbial contamination (Atata et al., 2007). Thus, moisture and a high amount of sugar in the oral liquid drugs in particular can support microbial growth (Khanom et al., 2013). Netish et al. (2018) further emphasized that a high percentage level of sucrose contained in liquid dosage forms especially syrups, makes them more susceptible to microbial growth.

Several studies have reported cases of microbial contamination of liquid dosage forms. Moniruzzaman et al. (2012) reported microbial contamination in antacids and paracetamol samples from different drug stores of Dhaka, Bangladesh, with 75% of the antacid and 60% of the paracetamol samples showing microbial count exceeding the USP limit. In another study that investigated the microbial contamination of brands of multivitamin syrups marketed in Maiduguri metropolis, Borno State, Tukur et al. (2012) reported that one out of the seven different samples contains pathogenic organisms while the other six samples contain non-pathogenic organisms, but were below the limit count set by the microbiological quality of syrups. Similarly, a study by Adeshina et al. (2009) has shown that among some pediatric anti-malarial and cough preparations sold in retail outlets in Ilorin, Nigeria, 14 (70%) were heavily contaminated and exceeded the official tolerance limit (Greater than $10^2$CFU/mL) of permissible microorganisms specified for syrups and suspensions.
Some of the preparations were contaminated with Bacillus subtilis, Escherichia coli, Staphylococcus aureus, and Pseudomonas aeruginosa. Microbial contaminants when present in non-sterile preparations can result in several undesirable consequences that ranged from spoilage resulting to physical and chemical changes, risk of infection to the consumer as well as products recalls and business loss (Calistus et al., 2011; Oviasogie et al., 2015). Such changes in the physical and chemical characteristics of contaminated pharmaceuticals include breaking of emulsions, thinning of creams, and fermentation of syrups, appearance of turbidity or deposit. Likewise, alterations in the organoleptic properties of the drugs such as changes odour, colour, and taste, thus rendering them unacceptable to the patients (Essam et al., 2012).

Furthermore, microbial contaminants when present in non-sterile preparations can possibly reduce or even inactivate the therapeutic activity of the drug (United States Pharmacopoeia, 2009). Use of such kind of products even when the level of contamination is low is also considered to be microbiologically unsafe because it may present potential health hazards to patients (Essam et al., 2012). There have been several published reports describing clinical hazards that are attributable to microbiologically contaminated pharmaceuticals (Mugoyela and Mwambete, 2010).

Pharmaceutical formulations of Syrups and suspensions contain sweetening agents and other excipients that make them susceptible to microbial contamination even when stored at particular conditions (Sudeshika et al., 2014). Increased attention is needed on the microbiological quality of liquid oral formulations because these represent the most common category of drugs that are administered to pediatric patients whose immune system is yet matured. This study therefore aimed at investigating the presence of microbial contamination in oral liquid drugs obtained from hospitals and patent medicine stores in Katsina State, Nigeria.

**MATERIALS AND METHODS**

**Collection and processing of drug samples**

A total of sixty seven (67) samples of four (4) different pediatric oral liquid drugs were obtained from the hospital, private pharmacies and patent medicine stores randomly selected within the state. The samples included liquid formulations of Ibuprofen, Albendazole, Metronidazole and cotrimoxazole suspensions.

Prior to opening of the samples, the outside surfaces of sample containers such head cap of the oral suspensions were wiped with 70% v/v ethanol as described by Shaqra et al., (2014). Ten (10 ml) of the products to be examined were diluted in 90ml of Trypticase Soy Broth (TSB) and serially diluted to get ten-fold dilutions of $10^{-1}$ to $10^{-5}$.

**Assessment of microbial contamination**

**Microbial Enumeration Tests:** One (1ml) of the first three ($10^{-1}$ to $10^{-5}$) dilutions of each test samples was inoculated onto plates of casein soya bean digest agar (CSBDA) and incubated at $37^\circ C$ for 3–5 days, and Sabouraud-dextrose agar at $25^\circ C$ for 5–7 days. After the incubation period, colonies were counted and the results were expressed as colony forming units per milliliter (cfu/ ml) of the sample (United States Pharmacopoeia, 2018).

**Isolation and identification of bacterial contaminants**

After the incubation period, colonies were sub cultured on the MacConkey agar, Salmonella Shigella agar, Mannitol salt agar and Nutrient agar plate to get pure culture for identification. Gram staining and microscopy, an array of biochemical tests which include catalase, coagulase, oxidase, indole, citrate utilization, Voges prauskeur test, methyl red, urease production, motility was carried out as described by Cheesbrough (2006).

**Identification of fungal contaminants**

Fungal growths from the SDA plate were identified using colonial morphology such as growth pattern, pigmentation and size of colonies. Further identification was achieved by lactophenol blue stain and microscopically (Davise, 2012).

**RESULTS**

The results of the assessment of the microbial contamination of the analyzed oral drug samples as depicted on Figure 1 revealed the presence of microbial contamination in 44 (65.7%) of the tested samples, of which 12 (27.3%) samples even though were contaminated, complied with the USP and BP acceptance criteria of non-sterile oral preparations while 32 samples were heavily contaminated above the acceptable limit. Microbial loads in the tested samples, as shown in Table 1, showed that 25 (37.3%) of the examined products had total aerobic microbial count greater than $10^2$ cfu/ml. Ibuprofen suspension had the highest percentage of samples ($45.0\%$) with total aerobic microbial count greater than $10^2$ cfu/ml, followed by samples of metronidazole suspensions of which $41.2\%$ had total aerobic microbial count greater than $10^2$ cfu/ml.
On the other hand, the proportions of the tested samples having total yeast and mould count greater than $10^1$ cfu/ml was 17 (25.4%). Similarly, samples of ibuprofen suspension had the highest percentage of samples (35.0%) with total yeast and mould count greater than $10^1$ cfu/ml, followed by samples of metronidazole suspensions of which 29.4% had total aerobic microbial count greater than $10^1$ cfu/ml.

![Figure 1: Percentage contamination of some pediatric oral liquid formulations sold in Katsina State, Nigeria.](image)

<table>
<thead>
<tr>
<th>Drug type</th>
<th>No. of Samples Tested</th>
<th>Total Aerobic Microbial Count &gt; $10^2$ cfu/ml</th>
<th>Total Yeast and Mould Count &gt; $10^1$ cfu/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>%</td>
<td>N</td>
</tr>
<tr>
<td>Albendazole suspension</td>
<td>13</td>
<td>03 23.1</td>
<td>02 15.4</td>
</tr>
<tr>
<td>Ibuprofen suspension</td>
<td>20</td>
<td>09 45.0</td>
<td>07 35.0</td>
</tr>
<tr>
<td>Cotrimoxazole suspension</td>
<td>17</td>
<td>06 35.3</td>
<td>03 17.6</td>
</tr>
<tr>
<td>Metronidazole suspension</td>
<td>17</td>
<td>07 41.2</td>
<td>05 29.4</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>67</strong></td>
<td><strong>25 37.3</strong></td>
<td><strong>17 25.4</strong></td>
</tr>
</tbody>
</table>

**Distribution of microbial contaminants from the tested products**

The different oral liquid preparations were found to be contaminated with various microbial species of bacteria and fungi. In general, 63 microbial contaminants were successfully identified, of which 42 (66.7%) were bacteria and the remaining, 21 (33.3%) were fungi. Table 2 presents the distribution of the identified bacterial contaminants recovered from the tested preparations. The most frequently found bacterial contaminants were *Bacillus species*, 12 (28.6%) followed by *Staphylococcus* sp. Other bacterial contaminants recovered include *Micrococcus* sp., *Pseudomonas aeruginosa*, *Enterobacter* sp., *Escherichia coli* and *Klebsiella* sp. While table 3 present the percentage occurrence of the fungal isolates, *Candida* sp., were the predominant fungal contaminants, 09 (42.9%) followed by *Penicillium* sp. and *Aspergillus flavus.*
**Table 2: Distribution of the bacterial contaminants recovered for the tested preparations**

<table>
<thead>
<tr>
<th>Bacterial isolate</th>
<th>Number</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacillus sp.</td>
<td>12</td>
<td>28.6.0</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>05</td>
<td>11.9</td>
</tr>
<tr>
<td>Other Staphylococcus sp.</td>
<td>07</td>
<td>16.7</td>
</tr>
<tr>
<td>Micrococcus sp.</td>
<td>03</td>
<td>7.1</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>04</td>
<td>9.5</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>02</td>
<td>4.8</td>
</tr>
<tr>
<td>Enterobacter sp.</td>
<td>04</td>
<td>9.5</td>
</tr>
<tr>
<td>Klebsiella sp.</td>
<td>03</td>
<td>7.1</td>
</tr>
<tr>
<td>Citrobacter sp.</td>
<td>02</td>
<td>4.8</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>42</strong></td>
<td><strong>100</strong></td>
</tr>
</tbody>
</table>

**Table 3: Distribution of fungal contaminants recovered for the tested preparations**

<table>
<thead>
<tr>
<th>Fungal isolate</th>
<th>Number</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspergillus flavus</td>
<td>04</td>
<td>19.0%</td>
</tr>
<tr>
<td>Aspergillus niger</td>
<td>03</td>
<td>14.3%</td>
</tr>
<tr>
<td>Penicillium sp.</td>
<td>05</td>
<td>23.8%</td>
</tr>
<tr>
<td>Candida sp.</td>
<td>09</td>
<td>42.9%</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>21</strong></td>
<td><strong>100%</strong></td>
</tr>
</tbody>
</table>

**DISCUSSION**

The occurrence of microbial contamination of 65.7% in the tested non-sterile pharmaceutical oral drug formulations in this study is comparable to the results of other studies: Calistus et al. (2011) reported microbial contamination in 62.5% and 55.5% of metronidazole and co-trimoxazole suspensions sourced from different locations in South-Eastern Nigeria.

The prevalence of microbial contamination observed in our study is much lower than the report of other studies. In related work, Khanom et al. (2013) evaluated the microbial contaminations of liquid oral drugs available in Bangladesh, comprising 26 syrups and 14 suspensions. It was reported that all the samples, except one syrup were found to be contaminated with bacteria and fungi. Similarly, in another study by Fatema, (2014) on the assessment of microbiological quality of the pediatric oral liquid drugs in Dhaka metropolis in Bangladesh reported that all the 20 (100%) samples were found to be highly contaminated with bacteria. Moreover, Daniyan and Sangodere (2011) reported that 72.2% of pediatric syrup preparations sold in patent medicine stores in Minna metropolis, Nigeria, were heavily contaminated with bacteria and fungi. On the other hand, lower incidence of microbial contamination has been reported, Tamalli et al. (2013) reported microbial contamination in 21.6% of the tested syrups and 26.7% in tablets commonly used at Alkhoms Market, Libya.

In this study, Bacillus species accounted for the majority of microbial contaminant in the tested samples. Bacillus species have been reported as the predominant contaminant of non-sterile pharmaceutical products by other studies conducted in different areas of the world such as; Adeshina et al. (2009) reported Bacillus subtilis as the most predominant contaminants in different brands of paediatric anti-malaria and cough preparations sold in Ilorin, Nigeria. Similarly, Mugoyela and Mwambete, (2010) in Tanzania reported that the majority of microbial contaminants isolated from non-sterile pharmaceuticals were Bacillus spp. In a related work from Benin, Nigeria, Akerele and Godwin (2002) reported the isolation of Bacillus species as the major contaminant of pharmaceutical products. Their presence in a product suggests poor environmental hygiene during manufacturing, distribution and storage (Willey et al., 2008) or as heavily contaminated or adulterated raw materials (Mugoyela and Mwambete, 2010).

The implication of microbial contamination of non-sterile pharmaceutical products with Bacillus species is that even though most species are non-pathogenic, several studies have shown that their presence in non-sterile pharmaceutical products is undesirable because of their spoilage potential, it can reduce the potency and therapeutic activity of the products with their potential adverse effects to the patients taking such medicaments (Mugoyela and Mwambete, 2010; Adeola et al., 2012).

In this study, Staphylococcus aureus, other coagulase negative Staphylococcal sp. and Micrococcus sp. accounted for about 28.0% of the bacterial species recovered from the tested dosage forms. Agbulu et al, (2016) reported the isolation of Staphylococcus aureus in addition to Pseudomonas sp. in cough syrups produced by different pharmaceutical companies sourced...
from pharmacy shop in Benue State, Nigeria. In a related study in Bangladesh, Fatema (2014), reported *Staphylococcus* species as the most predominant microbial contaminant in paediatric oral liquid drugs. The presence of *Staphylococcus* species in non-sterile oral pharmaceutical products could be attributed to unhygienic handling of the products, or possibly contamination from the equipment and/or raw materials (Gad et al. 2011). *Pseudomonas aeruginosa* was recovered from three samples of syrups and suspensions. These involved two samples Ibuprofen and one samples of Cotrimoxazole suspensions. Contamination of non-sterile pharmaceutical products with *Pseudomonas* species has been reported worldwide. Jimenez (2007) had reported *Pseudomonas aeruginosa* as the most frequently found contaminant in pharmaceutical products from all over the world. The presence of *Pseudomonas aeruginosa* in non-sterile pharmaceutical products possibly reflects contamination of the raw materials used as well as the conditions of the manufacturing environment in which the products are manufactured and packaged (Rania et al., 2013).

The presence of *Escherichia coli*, *Enterobacter* species and other members of the enterobacteriaceae in some of the tested preparations is a good indicator of faecal contamination which may be principally from production personnel or from water supply (Adeshina et al., 2009). In this study, one sample each of Cotrimoxazole and Metronidazole suspensions showed the presence of *Escherichia coli* and therefore failed the qualitative test for the absence of objectionable microorganism in non-sterile oral dosage forms. The presence of *Escherichia coli* is objectionable in oral dosage forms as clearly stated by the pharmacopoeias worldwide (BP, USP, European pharmacopoeia and the International pharmacopoeia) because its presence is an indication of pathogenic contamination of the products. In this study, the identified fungal contaminants were mainly moulds which comprised of *Penicillium* sp., *Aspergillus flavus* and *Aspergillus niger* while *Candida* sp. was the only identified yeast. Fungal contamination of pharmaceutical products is undesirable because they can produce metabolites/toxins that may be harmful to the consumers and additionally can cause deterioration of the products due to the biodegradation of the various components that constitute the formulation, and thus interfere with the therapeutic function of the product (Gad et al., 2011).

**CONCLUSION AND RECOMMENDATION**

High level of microbial contamination was observed in samples of pediatric oral liquid formulations particularly in samples of Ibuprofen and metronidazole suspensions. Moreover, bacterial contamination was the most common among the tested samples. Thus stringent quality control processes and compliance with the guidelines of the current good manufacturing practices during the manufacture, storage, distribution of these products is recommended. Also there should be good hygienic practices during handling, sales and usage of the products.

**REFERENCES**


