Microbiological Evaluation of Ten Commercial Cough Syrups during Storage

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ABSTRACT

Fifty samples of 10 different brands of cough syrups were analyzed for microbial quality during storage at ambient temperature (28 ± 2°C) and refrigeration temperature (4 ± 2°C). Physicochemical studies were also conducted to ascertain the stability of their active ingredients. Alcoff® cough syrup recorded the highest average bacterial count of 2.0 × 10⁵ cfu/ml at ambient temperature, and 1.5 × 10⁴ cfu/ml at refrigeration temperature, while Benylin® with 9.0 × 10⁴ cfu/ml at ambient temperature and Tutolin® with 6.4 × 10⁴ cfu/ml at refrigeration temperature were the least. Parkalin® had the highest average fungal count of 5.6 × 10⁴ cfu/ml at ambient temperature while Emzolyn® with 2.2 × 10⁶ cfu/ml was least. The fungal counts at refrigeration temperature showed that Tutolin® and Pirition® with 2.4 × 10⁴ cfu/ml each was the highest, while Coflin® with 4.0 × 10³ cfu/ml was the least. The percentage occurrence of isolates were Staphylococcus aureus (100%), Micrococcus spp. (80%), Bacillus subtilis (90%), Azotobacter spp. (10%), Proteus spp. (20%), Lactobacillus spp. (40%), Pseudomonas aeruginosa (20%), Aspergillus fumigatus (100%), Aspergillus niger (100%), Aspergillus flavus (100%), Fusarium solani (70%) and Penicillium spp. (40%). The antibiotic susceptibility of isolates showed that S. aureus was sensitive to gentamycin (100%) and resistant to cloxacillin (100%), ampicillin (100%), and penicillin (100%). Proteus spp. was sensitive to gentamycin (100%) but resistant to colistin (100%), nitrofurantoin (100%), cotrimoxazole (100%), and ampicillin (100%). P. aeruginosa was resistant to all antibiotics tested. The level of antibiotic resistance of bacterial isolates in cough syrup is a problem to public health. The cough syrups evaluated did not generally meet the standards for microbial limit as specified in official monographs. These products can adversely affect health status of consumers, hence, the need for regular quality control and assurance.

Keywords: antibiotic susceptibility, bacteria, fungi, quality, temperature

Abbreviations: AF, Alcoff®; BN, Benylin®; CN, Coflin®; DK, D-koff®; EN, Emzolyn®; NB, Nichben®; PA, Parkalin®; PN, Pirition®; T, Tutolin®

INTRODUCTION

The warm and humid climatic conditions that are characteristic of tropical countries like Nigeria tend to support the survival and growth of many microorganisms (Hugbo et al. 2003). The climatic condition is responsible for a number of infections and the spoilage of food, cosmetic and pharmaceutical products (Bos et al. 1989; LeChevallier et al. 1996; Ballereau et al. 1997). In a situation whereby a nutritionally-rich pharmaceutical or cosmetic product is contaminated, rapid growth and multiplication of microorganisms would be expected. According to Bos et al. (1989), it could lead to biodegradation and the risk of infection to consumers of the product. Several cases of infection caused by the administration of non sterile medicaments contaminated by microorganisms have been reported (Ringertz and Ringertz 1982; Spooner 1988; de la Rosa et al. 1993). In addition, many of these microorganisms become resistant to one or more antimicrobial agents used in therapy after exposure to these non sterile pharmaceuticals (de la Rosa et al. 1993). Therefore, consumption of products contaminated by these microorganisms may lead to the spread of drug resistance. Product contamination may be from raw materials or water used in formulation or accidentally during use (Hugbo et al. 2003). The unhygienic conditions that prevail usually make these contaminations possible.

In recent years, manufacturers of pharmaceuticals have improved the quality of syrups such that the majority contains a minimal microbial population. Nevertheless, a few rogue products with unacceptable levels and type of contamination do occasionally escape the quality control net and when used contribute to the spread of diseases (Eze and Asogwa 2006). According to Akarele and Ukoh (2003), this is more of concern in tropical countries where pharmaceutical preparations are frequently stored under uncontrolled conditions. Eze and Asogwa (2006) reported that dispensing of most of these medicaments in patent medicine stores take an average of 3-4 weeks under uncontrolled and therefore unhygienic conditions.

There is the possibility that microorganisms present in syrups during the shelf life may contribute to physical deterioration of the product as well as inactivate the therapeutic activity of the product which could affect the health of the patients (European Pharmacopoeia 2007). The risk of this happening is higher in cough syrups which contain additives like plant extracts, sucrose, that could serve as substrate for microorganisms.

Eka et al. (1987) reported the development of diarrhea in children in Western Nigeria after the use of contaminated syrup. Their studies of microbial contamination of oral liquids, tablets, and raw materials in some Nigerian hospitals recorded organisms such as Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus, Micrococcus spp., Bacillus spp., yeasts and moulds. The study suggested raw materials, storage environment, water, containers, air and personnel as possible sources of contamination. Mendie et al. (1993) examined 104 samples of syrups, elixirs, solutions, mixtures, tonics and suspensions and reported that fungi accounted for 72.2% of isolates, and, the rest were mainly Staphylococcus aureus (5.9%), Micrococcus spp. (4.9%), Klebsiella spp. (4.0%), Escherichia coli (8.0%) and Pseudomonas spp. (5.0%). In a similar study by Oycleke et
al. (2005), 20 brands of cough syrup were analyzed for micr-
obial contamination, and result revealed the microbial count of $1.6 \times 10^5$ to $7.2 \times 10^5$ cfu/ml bacteria for 13 samples and $1.4 \times 10^8$ to $8.6 \times 10^8$ cfu/ml fungi for nine samples. This study therefore, is aimed at assessing the microbial quality of cough syrup during storage at ambient tempera-
ture ($28 \pm 2^\circ$C) and refrigeration temperature ($4 \pm 2^\circ$C), as well as to determine the antibiotic susceptibility of bacterial isolates in the cough syrup samples.

**MATERIALS AND METHODS**

**Sample collection**

A total of 50 samples (5 each of 10 commercial cough syrups) pur-
chased from registered pharmaceutical stores in Umuahia metro-
polis were used in this study. They were all non-expired cough 
syrups and manufactured in different states in Nigeria. The cough 
syrups were: Tuxil-D® (TD), Emzolyn® (EN), D-Koff® (DK), Pirin-
ton® (PN), Benylin® (BN), Tutolin® (TN), Parkalin® (PA), Alcoff® 
(AF), Nichben® (NB) and Collin® (CN). The container labels were 
examined for the date of manufacture and expiry, active ingredi-
ents and other compositions were noted. The samples purchased 
aged between two and six months. All the products were approved 
by the National Agency for Food and Drug Administration and 
Control (NAFDAC), as they all had the NAFDAC registration 
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**Microbiological analysis**

The pour plate method of isolation of microbes from pharmaceuti-
cal products as described by United States Pharmacopoeia (1980) 
was adopted for the analysis. This involved serial dilution of sam-
ple. An aliquot of 1ml of each sample was measured and intro-
duced into test tubes containing 9 ml sterile water. Exactly 1 ml of 
the appropriate dilution was placed into each of two sterile Petri 
dishes. Thereafter, approximately 20 ml of each of the sterile Nut-
rient agar, Sabouraud Dextrose agar and MacConkey agar previ-
ously cooled to 45°C was added into the Petri dishes for the enumer-
ation or isolation of bacteria, fungi and coliform counts respec-
tively. The Petri dishes were covered immediately and the sample 
mixed with the agar by tilting or rotating the dishes. The agar was 
allowed to solidify at room temperature. Afterwards, the Petri dishes 
were inverted and incubated for 24-48 hrs at 35-37°C for Nutrient agar and MacConkey agar while Sabouraud Dextrose agar Petri dishes were incubated for 3-5 days at 26-30°C. Each proced-
ure was repeated independently in 3 replicates. After incubation, 
acceptable colonies, (within 30-300) were counted. Actual micro-
bial population was obtained by multiplying the plate count by the 
dilution factor and recorded as the colony forming unit per ml 
(cfu/ml).

**Antibiotic susceptibility testing**

Antibiotic susceptibility was determined by the agar diffusion 
technique as described by Baker and Breach (1980). A sterile cotton 
swab dipped into the broth culture of isolate was streaked 
evenly all over the surface of Mueller Hinton’s agar (Oxoid), and, 
antibiotic disk was placed aseptically on the inoculated plates 
using sterile forceps. The plates were then incubated for 24 hrs at 
35-37°C. Isolates were considered as sensitive or resistant to an 
antibiotic according to the diameter of inhibition zone size inter-
pretative chart (Clinical Laboratory Standards Institute 2006). 
The antibiotics (Oxoid) tested and their standard concentra-
tions were as follows: ampicillin (10 μg), chloramphenicol (10 μg), 
cloxacillin (5 μg), erythromycin (5 μg), gentamycin (10 μg), peni-
cillin (1 μg), streptomycin (10 μg) and tetracycline (10 μg) for 
Gram positive organisms, while that of Gram negatives are ampi-
cillin (25 μg), gentamycin (25 μg), tetracycline (25 μg), colistin 
(25 μg), streptomycin (25 μg), nalidixic acid (25 μg), nitrofuranto-
in (200 μg) and cotrimoxazole (25 μg).

**Identification of fungal isolates**

Moulds were identified according to Barnett and Hunter (1972). 
The morphological characteristics of colony both surface and 
reverse, rate of growth, colour and pigmentation in the medium 
were considered. The wet mount technique explained by Chees-
brough (1984) was employed. Two drops of lactophenol was placed 
on a clean grease free slide. Then, a sterile inoculating needle was 
used to transfer a small portion of mycelia growth to the lacto-
phenol. The fungal growth was then teased, covered with a cover 
slip and observed using ×40 objective lens.

**Statistical analysis**

The student’s $t$-test was used to compare the values of the samples 
at ambient and refrigeration temperature.

**RESULTS**

Microbiological analyses showed that the cough syrups 
were generally contaminated to a varying degree. Figs. 1 
and 2 showed the total count of bacteria and fungi at am-
bient temperature (At) and refrigeration temperature (Rt) 
for the different brands of cough syrup samples for thirty 
five days of storage. From the result, Product AF had the 
highest average bacterial count of $2.0 \times 10^5$ cfu/ml at am-
bient temperature while product BN with $9.6 \times 10^5$ cfu/ml 
had the least. At refrigeration temperature, product AF had the 
highest with $1.5 \times 10^5$ cfu/ml, while product TN with 
$6.4 \times 10^5$ cfu/ml was the least. For fungi, product PA with 
$5.6 \times 10^5$ cfu/ml had the highest average fungi count at am-
bient temperature, while product EN with $2.2 \times 10^5$ cfu/ml 
had the least, but at refrigeration temperature, product TN 
and PN with $2.4 \times 10^5$ cfu/ml each had the highest count, 
while product CN with $4.0 \times 10^5$ cfu/ml had the least aver-
age fungi count. Generally there were a significant dif-
f erences observed between microbial counts in samples 
stored at ambient and refrigeration temperatures.

Based on their cultural morphology, microscopic, bio-
chemical and physiological characteristic the isolates in-
clude: *Staphylococcus aureus*, *Bacillus subtilis*, *Micrococcus 
spp.*, *Lactobacillus spp.*, *Pseudomonas aeruginosa*, 
*Azolobacter spp.* and *Proteus spp.* for bacteria. Moulds 
were *Aspergillus flavus*, *Aspergillus Niger*, *Aspergillus fumi-
gatus*, *Fusarium solani* and *Penicillium spp*. The percen-
tage occurrences of these microorganisms were presented in 
Figs. 3 and 4 for bacteria and fungi respectively.

The antibiotic sensitivity patterns of the isolates are 
shown in Tables 1 and 2. All the *Staphylococcus aureus* iso-
lates were resistant to ampicillin, cloxacillin and penicillin, 
while they were all sensitive to gentamycin. All the *Pseu-
donas aeruginosa* isolates were resistant to all the anti-
biotics tested. Also, *Proteus spp.* was resistant to colistin, 
nitrofurantoin, cotrimoxazole, tetracycline and ampicillin, 
while it showed sensitivity to gentamycin.

**DISCUSSION**

Microbiological properties of cough syrup samples during 
storage were investigated at both ambient and refrigeration 
temperature. However, higher bacteria count was recorded 
at ambient temperature whereas lower fungi count was re-
corded at both temperatures compared to that of bacteria.

The level of microbial contamination and the isolation of 
*Staphylococcus aureus* and *Pseudomonas aeruginosa* 
showed that the products did not comply with standard 
(United States Pharmacopoeia 1980; European Pharmacopoeia 
2007), indicating a defect in production. This finding
Fig. 1 Total viable count of bacteria at ambient temperature (At) and refrigeration temperature (Rt) in different brands of cough syrup samples for 35 days of storage. TD = Tuxil-D®; EN = Emzolyn®; DK = D-Koff®; PN = Piriton®; BN = Benylin®; TN = Tutolin®; PA = Parkalin®; AF = Alcoff®; NB = Nichben®; CN = Coflin®.

Fig. 2 Total viable count of fungi at ambient temperature (At) and refrigeration temperature (Rt) in different brands of cough syrup samples for 35 days of storage. TD = Tuxil-D®; EN = Emzolyn®; DK = D-Koff®; PN = Piriton®; BN = Benylin®; TN = Tutolin®; PA = Parkalin®; AF = Alcoff®; NB = Nichben®; CN = Coflin®.

Fig. 3 Percentage occurrence of bacteria in cough syrup samples.

Fig. 4 Percentage occurrence of fungi in cough syrup samples.
is in agreement with the studies conducted by other workers (Mendie et al. 1993) who reported high microbial counts and organisms such as Micrococcus spp., Bacillus spp., Azotobacter spp., Pseudomonas spp., Klebsiella spp., and Escherichia coli. The yeast isolates were Saccharomyces spp., and Candida spp., while the mould isolated include Rhizopus spp., Penicillium spp., and Aspergillus spp. Charneck (2004) reported the presence of Gram positive endospore forming rods and Gram negative organisms. Similarly, Oyeleke et al. (2005) identified organisms such as Escherichia coli, Bacillus subtilis, Salmonella typhi and Staphylococcus aureus for bacteria; Aspergillus flavus, Aspergillus fumigatus, Aspergillus niger, Penicillium notatum and Fusarium solani for fungi. They identified Staphylococcus aureus as having the highest prevalence (65%) and Bacillus subtilis the least (5%) for bacteria; Aspergillus fumigatus (33%) was the most prevalent among the fungi isolates while Penicillium notatum (11%) had the least. The microflora of the cough syrup was found to consist mainly of Gram positive bacteria and mould. However, the microbial quality of the cough syrups examined in this study was relatively lower than the products described in the report of Oyeleke et al. (2005), where microbial counts above 10^5 and Salmonella typhi and Escherichia coli was not recorded. The isolation of Staphylococcus aureus, Pseudomonas aeruginosa, Proteus spp. and Aspergillus spp. is of public health concern because of the pathogenic potential of these organisms.

The likely source of contamination of these products are raw materials or water used in formulation, personnel as well as the condition prevalent in the environment in which the products are manufactured and packed (Mendie et al. 1993; Okolo and Lamikanra 2001). In addition, most products were observed to be loosely coated and not firmly closed, and it could serve as a source of contamination. The European Agency for the Evaluation of Medicinal Products (2003) stated that orally administered aeous solutions, suspensions and emulsion are among the preparations at greatest risk of contamination. Since most of these liquid preparations are administered to children or infants who are highly prone to infection, it may pose a real danger even when the level of contamination is low. According to Oyeleke et al. (2005), it could lead to gastrointestinal disorders and possible complication for the initial ailment.

The sensitivity tests (Tables 1, 2) indicated that the isolates were resistant to one or more antibiotics, although generally, a low percentage of the isolates were sensitive to the antibiotics tested. The result of the sensitivity test indicates that S. aureus and Proteus spp. were very sensitive to gentamycin. The Gram positive and Gram negative isolates showed high resistance to commonly available antibiotic such as ampicillin, penicillin, cloxacillin, cotrimoxazole, tetracycline, colistin and nitrofurantoin. This implies that treatment of possible infection due to these organisms may not be feasible and would require a new antibiotic which are not commonly available. Of particular concern is the sensitivity pattern of P. aeruginosa isolates to the antibiotics, although the organism is known to be resistant to many chemcal antimicrobial agents or antibiotics. The level of antibiotic resistance recorded in this study is similar to the report of Charnock (2004), who reported an unusual antibiotic resistance among isolates from pharmaceuticals and allied products.

The possible explanation of the antibiotic resistance pattern among the isolates may be due to pre-existing factors in the microorganisms or synthesis of excess enzymes over the amount that can be inactivated by the antibiotic, or, inabilty of the drug to penetrate the cell due to some alteration of the cell membrane (Pelczar et al. 1993). Possibly, some biochemical changes in the product may contribute to antibiotic resistance. de la Rosa et al. (1993) reported that organisms become resistant to one or more antimicrobial agents after exposure to non sterile pharmaceuticals. The level of antibiotic resistance observed in this study is surprising and is a very serious public health problem, and brings to light the need for good manufacturing protocol and use of effective preservative system to prevent possible contamination by these microbes.

According to Lamikanra (1999), Booth (2000) and European Pharmacopoeia (2007), the presence of certain microorganisms in non sterile preparations may have the potential to reduce or even inactivate the therapeutic activity of the product and has the potential to adversely affect the health of the patient. The microbial quality of pharmaceutical products is influenced by the environment and quality of the raw materials used during formulation (Mendie et al. 1993; Hugbo et al. 2003). Hence, manufacturers should prevent the contamination of raw materials, finished products and the packaging components so as to maintain appropriate quality, safety and efficacy of the product. Sufficient quantity of a suitable preservative should be included to prevent or reduce contamination of the product (Lamikanra 1999).

This study revealed that cough syrups are susceptible to microbial contamination. Organisms like Staphylococcus aureus and Pseudomonas aeruginosa known as opportunistic pathogens and are considered as objectionable by competent authorities were recovered in the products. Colony forming units per ml (cfu/ml) recovered was higher than that stipulated by same authority; and a worrisome level of antibiotic resistance of the isolates. Therefore, consumption of these cough syrup by children or infants and the critically ill who are highly prone to infection may present a potential hazard.

This study also indicated the possible proliferation of microorganisms even in the presence of a preservative. Higher microbial counts were observed at ambient temperature than refrigeration temperature; hence, cough syrups may be stored in refrigerator or preferably at temperature below ambient temperature. More research should be focussed on cough syrups as potential source of infection.

### Table 1 Antibiotic sensitivity pattern of Staphylococcus aureus isolates

<table>
<thead>
<tr>
<th>Antibiotic (conc.)</th>
<th>Number (%) of sensitive isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin (10 μg)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Chloramphenicol (10 μg)</td>
<td>5 (50)</td>
</tr>
<tr>
<td>Cloxacillin (5 μg)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Erythromycin (5 μg)</td>
<td>4 (40)</td>
</tr>
<tr>
<td>Gentamycin (10 μg)</td>
<td>10 (100)</td>
</tr>
<tr>
<td>Penicillin (1 μu)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Streptomycin (10 μg)</td>
<td>3 (30)</td>
</tr>
<tr>
<td>Tetracycline (10 μg)</td>
<td>2 (20)</td>
</tr>
</tbody>
</table>

n = Number of organisms tested.
Conc. = Concentration of antibiotic disk in microgram.

### Table 2 Antibiotic sensitivity pattern of Pseudomonas aeruginosa and Proteus sp isolates

<table>
<thead>
<tr>
<th>Antibiotic (conc.)</th>
<th>Number (%) of sensitive isolates</th>
<th>P. aeruginosa</th>
<th>Proteus sp.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colistin (25 μg)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>Gentamycin (10 μg)</td>
<td>0 (0)</td>
<td>2 (100)</td>
<td></td>
</tr>
<tr>
<td>Nalidixic acid (30 μg)</td>
<td>0 (0)</td>
<td>1 (50)</td>
<td></td>
</tr>
<tr>
<td>Nitrofurantoin (200 μg)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>Cotrimoxazole (25 μg)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>Streptomycin (25 μg)</td>
<td>0 (0)</td>
<td>1 (50)</td>
<td></td>
</tr>
<tr>
<td>Tetracycline (25 μg)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>Ampicillin (25 μg)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td></td>
</tr>
</tbody>
</table>

n = Number of organisms tested.
Conc. = Concentration of antibiotic disk in microgram.

### REFERENCES

