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# MICROBIAL ASSESSMENT OF SOME RETAILED COUGH SYRUPS IN MINNA, NIGER STATE

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**Abstract**

Twenty brands of cough syrups were analyzed for microbial assessment from different pharmaceutical and patient medicine stores in Minna. The result revealed the microbial count of $1.6 \times 10^6$ to $7.2 \times 10^6$ cfu/ml bacteria for thirteen samples and $1.4 \times 10^3$ to $8.6 \times 10^3$ cfu/ml for fungi for nine samples. The organisms identified were *Escherichia coli, Bacillus subtilis, Staphylococcus aureus, Salmonella typhi* for bacteria *Aspergillus flavus, Aspergillus fumigatus, Aspegillus niger, Penicillium notatum and Fusarium solani* for fungi. *Staphylococcus aureus* had the highest prevalent bacterial with (65 %) and *Bacillus subtilis* had the least (5 %). *Aspergillus fumigatus* was the most prevalent among the fungi isolated (33 %) while *Penicillium notatum* (11 %) had the least. The presence of these organisms revealed that the syrups that are purchased and administered to children are sometimes a source of complication for the initial ailment. Thus, these organisms could lead to gastrointestinal disorders and as such government regulatory agencies should intensify more effort to see that quality control and assurance are applied regularly.

## 1. Introduction

Cough syrups are liquid forms of pharmaceutical products that are prescribed for patient with upper respiratory infections (Berner, 1986; Albert, 1989). Coughing is physically exhausting and it interferes with rest. Under such circumstance, drugs may be used to suppress this reflex (Holinger, 1991). Cough syrups are prepared from solutions which includes: syrups, elixirs, suspensions and tinctures, all of which are usually prepared by mixing the solutes with a selected solvent in a glass-lined or stainless steel vessel (Zanowiak, 1982). Solutions are then filtered and pumped into storage tanks for quality control inspection prior to packaging in final containers. Suspensions and emulsions are frequently prepared using colloid mills and homogenizers (Zanowiak, 1982). This liquids are prepared with preservatives to prevent mold and bacteria growth, but they do not require sterilization if they are intended for oral or topical use. However, prescriptions and formulations for ophthalmic use must be sterilized and are therefore prepared in a manner similar to non tablet drugs (Zanowiak, 1982).

The quality of pharmaceutical products with different composition and the diversity of contaminating microorganisms set up special problems for each product with its specific field of application. Syrups and solutions contain high sugar concentrations that suppress growth of most microorganisms except osmotolerant microorganisms (Grigo, 1976).

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Given the tremendous health and socioeconomic impact of persistent cough due to respiratory tract infection worldwide, careful re-evaluation of current thinking, as well as adequately performed clinical trials aimed at determining optimal management, are certainly warranted (Couch, 1984).

In two studies of the pattern of morbidity in a cohort of Nigerian children under 5 years of age from a poor urban community, (Osinusi, 1989; Oyedeji *et al*., 1995) reported that symptoms of respiratory tract infections are most common during the harmattan period with nasal discharge having the highest incidence rate, followed by cough with an incidence rate of 12.2 cases per 100 children. (Ijah *et al*., 2003) reported the isolation of coliform group of bacteria, *Streptococcus* species, *Bacillus* species, *Penicillium* species, *Aspergillus* species, *Mucor* species and *Cephalosporium* species from pharmaceutical products sold to the public in Minna (Antai, 1998; Bloomfield, 1990) also isolated *Pseudomonas aeruginosa, Escherichia coli, Micrococcus* and *Bacillus* species, yeast and mold from pharmaceutical products and they suggested that control measure should be taken to prevent their proliferation. The infant mortality rate associated with persistent cough due to severe lower respiratory tract infection in early childhood 84/1,000 more likely to die in Nigeria, making this condition one of the most common causes of infant mortality (UN, 1994).

The objectives of the study are; to examine the different brand of cough syrups sold in Minna for microbial contamination and to isolate, identify and characterize the microbial contaminants.

## 2. Materials and Methods

(a) Collection of Samples

Twenty samples of Cough syrups of different brands were purchased from different pharmacies and patient medicine stores in Minna, Niger State, Nigeria. Their Batch number, manufactured dates and expiry dates were critically considered as found in Table 1 below:

(b) Preparation of Media

The media used are Nutrient agar, Saboraud dextrose agar and MacConkey agar which are prepared for the isolation of various organisms ranging from bacteria to fungi. As described by Fawole and Oso (1998) and Cheesebrough (1984).

(c) Enumeration of Microorganisms

(Total Microbial Counts)

Nine milliliters of distilled water was dispensed into six test-tubes for each syrup sample and sterilized by autoclaving at 121 °C for 15minutes. After sterilization and cooling, 1 ml of each sample (cough syrup) was taken into the first test-tube labeled $10^{-1}$ with a sterile syringe and needle. 1 ml from the first test tube was taken into the second tube ($10^{-2}$) and was mixed up, the same serial dilution process was repeated for the third, fourth, fifth and sixth tubes. One milliliter from the 6th tube was discarded to have equal volume in all the tubes.

(d) Inoculation by Pour Plate Method

Aseptically, 1 ml of the $10^{-3}$ dilution for each sample was plated into 12 petri dishes for each media (Nutrient, Macconkey and Saboraud dextrose agar) prepared respectively. The media were poured at 40-45 °C, swirled and allowed to solidify for the enumeration of total viable, coliform and fungal counts respectively. Nutrient and Macconkey agar plates were incubated at 37 °C for 24 hours in an incubator while the Saboraud's dextrose agar plates was incubated at 28 °C for 72 hours in an inoculation hood. Typical colonies of microbial growth on plates were counted at the end of incubation periods (Fawole and Oso, 1988).

(e) Isolation of the Microorganisms

The bacteria and fungal isolates on the Nutrient agar and Sabraud dextrose agar plates respectively were subcultured on agar slants as pure culture and stored in the refrigerator at 4 °C for further characterization and identification.

(f) Characterization and Identification of Isolates

The bacteria were characterized according to the method described by Fawole and Oso (1988) in which the following biochemical reactions were examined: Gram staining reaction, spore test, motility test, starch hydrolysis test, catalase test, coagulase test, sugar fermentation test, methyl red test and indole test, these were later compared with other known taxas after observation under the microscope. However the fungi were identified by their morphological and microscopic appearance as described by Cheesbrough (1984).

## 3. Results

(a) Microbial Counts

The total viable bacteria varied from $1.6 \times 10^6$ to $7.2 \times 10^6$ cfu/ml for thirteen samples while seven of the samples had no microbial growth. Table 2 below shows the results for total viable bacteria count in cough syrups analyzed.

The bacteria count ranged from ($2.8 \times 10^6$ to $8.0 \times 10^6$ cfu/ml) in five samples while fifteen had no bacteria growth detected. The Table 3 below shows the coliform count for coliform bacteria.

The fungi count for the twenty samples analyzed showed growth in nine samples that ranged from $1.4 \times 10^3$ to $8.6 \times 10^3$. Eleven of the syrups showed no growth for fungi. Table 4 shows the growth of the fungi and its count.

(b) Identification of Fungal Isolates

The colonies on Saboraud's dextrose agar showed distinctive morphology and microscopic appearance which reveal the presence of the following organisms: *Aspergillus flavus*, *Penicillum notatum*, *Aspergillus fumigatus*, *Fusarium solanis*, and *Aspergillus niger*.

(c) Identification of Bacterial Isolates

The results obtained from the biochemical tests revealed the presence of the following organisms, which includes *Bacillus subtilis*, *Escherichia coli*, *Salmonella typhi*, *Staphylococcus aureus*. *Staphylococcus aureus* was the most prevalent (65 %) and *Bacillus subtilis* was the least prevalent (5 %).

## 4. Discussion

Thirteen out of the 20 cough syrups samples analyzed had microbial growth which indicated that the products had been contaminated by these organisms. The isolated fungi include species of *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Penicillium notatum* and *Fusarium solani*. This agrees with Ijah *et al.* (2003) who reported the isolation of *Aspergillus*, *Mucor* and *Cephalosporium* species from pharmaceutical products and Gray (1988) who indicated that species of *Aspergillus* isolated in syrups are known to cause Aspergillosis in children and immunocompromised patients. Some of these fungi are toxin producers, this agrees with the report of Antai (1988) who reported that *Aspergillus* species are capable of producing aflatoxins.

Table 1: Manufacturers and expiry date

| S/No | Brand of cough syrup | Batch Number | Date of Manufacture | Expiry Date |
|---|---|---|---|---|
| 1 | BC | D145 | February 2004 | February 2007 |
| 2 | BE | D160 | March 2004 | March 2007 |
| 3 | BR | L069 | November 2003 | November 2006 |
| 4 | CL | 12584 | May 2004 | May 2007 |
| 5 | CO | 31203 | December 2003 | November 2006 |
| 6 | CA | 4A130001 | January 2004 | December 2007 |
| 7 | DC | DC05R | April 2004 | March 2007 |
| 8 | DE | DA04R | January 2004 | December 2006 |
| 9 | EC | 1897G | April 2004 | April 2007 |
| 10 | LE | XO44YL | October 2003 | October 2006 |
| 11 | NO | 281315 | December 2003 | November 2006 |
| 12 | PC | 054 | January 2004 | January 2007 |
| 13 | PH | 17691 | October 2003 | September 2006 |
| 14 | PE | 3H514004 | August 2003 | August 2006 |
| 15 | TC | 17490 | August 2003 | August 2006 |
| 16 | TS | 3054 | March 2003 | March 2006 |
| 17 | TU | TCC027 | May 2004 | February 2007 |
| 18 | TE | TCE029 | June 2004 | February 2007 |
| 19 | TN | LA78005 | November 2003 | October 2006 |
| 20 | ZC | 031 | August 2003 | July 2006 |

*Brand names are coded

Table 2: Total viable count of bacteria in cough syrups analyzed

| Brands of syrup | Batch Number | Total viable counts (cfu/ml) | Manufacture Date | Expiry Date |
|---|---|---|---|---|
| BC | D145 | $3.6 \times 10^6$ | February 2004 | February 2007 |
| BE | D160 | No growth | March 2004 | March 2007 |
| BR | L069 | $4.4 \times 10^6$ | November 2003 | November 2006 |
| CL | 12584 | $3.2 \times 10^6$ | May 2004 | May 2007 |
| CO | 31203 | No growth | December 2003 | November 2006 |
| CA | 4A130001 | $2.0 \times 10^6$ | January 2004 | December 2007 |
| DC | DC05R | $4.8 \times 10^6$ | April 2004 | March 2007 |
| DE | DA04R | $4.4 \times 10^6$ | January 2004 | December 2006 |
| EC | 1897G | $3.2 \times 10^6$ | April 2004 | April 2007 |
| LE | XO44YL | $4.7 \times 10^6$ | October 2003 | October 2006 |
| NO | 281315 | No growth | December 2003 | November 2006 |
| PC | 054 | $4.0 \times 10^6$ | January 2004 | January 2007 |
| PH | 17691 | No growth | October 2003 | September 2006 |
| PE | 3H514004 | $1.6 \times 10^6$ | August 2003 | August 2006 |
| TC | 17490 | $4.8 \times 10^6$ | August 2003 | August 2006 |
| TS | 3054 | No growth | March 2003 | March 2006 |
| TU | TCC027 | $2.0 \times 10^6$ | May 2004 | February 2007 |
| TE | TCE029 | No growth | June 2004 | February 2007 |
| TN | LA78005 | $7.2 \times 10^6$ | November 2003 | October 2006 |
| ZC | 31 | No growth | August 2003 | July 2006 |

Cfu/ml: colony forming units per milliliter

Table 3: Total coliform count in cough syrups analyzed.

| Brands of syrup | Batch Number | Total viable count (cfu/ml) | Manufacture Date | Expiry Date |
|---|---|---|---|---|
| BC | D145 | $2.9 \times 10^6$ | February 2004 | February 2007 |
| BE | D160 | No growth | March 2004 | March 2007 |
| BR | L069 | $5.2 \times 10^6$ | November 2003 | November 2006 |
| CL | 12584 | $8.0 \times 10^6$ | May 2004 | May 2007 |
| CO | 31203 | No growth | December 2003 | November 2006 |
| CA | 4A130001 | No growth | January 2004 | December 2007 |
| DC | DC05R | No growth | April 2004 | March 2007 |
| DE | DA04R | No growth | January 2004 | December 2006 |
| EC | 1897G | No growth | April 2004 | April 2007 |
| LE | XO44YL | $2.8 \times 10^6$ | October 2003 | October 2006 |
| NE | 281315 | No growth | December 2003 | November 2006 |
| PC | 054 | No growth | January 2004 | January 2007 |
| PH | 17691 | No growth | October 2003 | September 2006 |
| PE | 3H514004 | No growth | August 2003 | August 2006 |
| TC | 17490 | No growth | August 2003 | August 2006 |
| TS | 3054 | No growth | March 2003 | March 2006 |
| TU | TCC027 | $8.0 \times 10^6$ | May 2004 | February 2007 |
| TE | TCE029 | No growth | June 2004 | February 2007 |
| TN | LA78005 | No growth | November 2003 | October 2006 |
| ZC | 031 | No growth | August 2003 | July 2006 |

Cfu/ml: colony forming units per milliliter.

Table 4: Fungal counts in cough syrups analyzed.

| Brands of syrup | Batch Number | Fungal counts (cfu/ml) | Manufacture Date | Expiry Date |
|---|---|---|---|---|
| BC | D145 | No growth | February 2004 | February 2007 |
| BE | D160 | No growth | March 2004 | March 2007 |
| BR | L069 | No growth | November 2003 | November 2006 |
| CL | 12584 | $5.1 \times 10^3$ | May 2004 | May 2007 |
| CO | 31203 | $1.9 \times 10^3$ | December 2003 | November 2006 |
| CA | 4A130001 | No growth | January 2004 | December 2007 |
| DC | DC05R | $3.1 \times 10^3$ | April 2004 | March 2007 |
| DE | DA04R | No growth | January 2004 | December 2006 |
| EZ | 1897G | $6.9 \times 10^3$ | April 2004 | April 2007 |
| LE | XO44YL | No growth | October 2003 | October 2006 |
| NE | 281315 | No growth | December 2003 | November 2006 |
| PC | 054 | No growth | January 2004 | January 2007 |
| PH | 17691 | $4.2 \times 10^3$ | October 2003 | September 2006 |
| PE | 3H514004 | No growth | August 2003 | August 2006 |
| TC | 17490 | $2.2 \times 10^3$ | August 2003 | August 2006 |
| TS | 3054 | $1.4 \times 10^3$ | March 2003 | March 2006 |
| TU | TCC027 | No growth | May 2004 | February 2007 |
| TE | TCE029 | $3.5 \times 10^3$ | June 2004 | February 2007 |
| TN | LA78005 | $8.6 \times 10^3$ | November 2003 | October 2006 |
| ZC | 031 | No growth | August 2003 | July 2006 |

Cfu/ml: colony forming units per milliliter.

The bacteria identified include *Escherichia coli, Staphylococcus aureus, Salmonella typhi, and Bacillus subtilis*. This is in accordance with the reports from Antai (1998) and Bloomfield (1990) that isolated species of *Pseudomonas* and *E. coli* from pharmaceutical products. The presence of *Escherichia coli* signifies faecal contamination of the samples and this can lead to gastroenteritis in children as revealed by Miller (1987). *Staphylococcus aureus* is an important human pathogen because its ability to cause disease is not diminished with the introduction of antibiotics as described by Sheagren (1984). Besides, Denya (1988) indicated that *S. aureus* is a gram positive organism capable of secreting toxins which contribute to gastrointestinal distress. *Bacillus subtilis* is associated with food poisoning and *Salmonella* species are known to cause gastroenteritis and enteric fever as revealed by Miller (1987).

The presence of these microorganisms in the syrups may be due to improper sanitary conditions of the factory, contaminated raw materials and improper packaging and storage facilities. This suggests that these microorganisms may have the ability to degrade the active components of the syrup, thereby rendering the syrup inactive. The government regulatory agencies responsible for quality assurance and control should be alive to their civic responsibility by conducting a regular analysis on the retailed products sold to the public.

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