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Microbial assessment of Chloroquine syrup sold in Minna

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ABSTRACT: Ten Chloroquine phosphate syrups brand retailed to the public were randomly purchased from different pharmacies and patient medicine stores in Minna, Niger State. These were examined for microbial contamination. The brands that were analyzed include: PC, AC, MK, LQ, GC, MC, EZ, NQ, BF and CP. The results revealed that EZ, MC and PC had a mean bacterial count of $1.0x10^3$cfu/ml and GC had a bacterial count of $2.0x10^3$cfu/ml. NQ, BF, LQ and CP had fungal counts of $1.0x10^3$cfu/ml and PC had fungal counts of $2.0x10^3$cfu/ml. The pH of the samples ranged from 2.3 to 3.9 with the exception of NQ, MC and CP which had a pH range of 4.3 - 6.0. The organisms isolated include *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus* for bacteria and *Aspergillus niger*, *Penicillium notatum*, *Aspergillus flavus* and *Aspergillus fumigatus* for fungi. The presence of these organisms pose a threat to human health particularly children.

Key Words: Chloroquine syrup, Microbial assessment, Minna

Introduction

Chloroquine phosphate syrup is a pleasantly flavored and moderately sweetened non-viscous preparation, which is very much acceptable to children. It is a 4-aminoquinolin derivative drug active against the parasite responsible for malaria (WHO, 1970). Like hydroxychloroquine, chloroquine phosphate is a blood schizontical agent and is active against the asexual erythrocyte forms of most strains of *Plasmodium malariae*, *Plasmodium ovale*, *Plasmodium vivax* and many strains of *Plasmodium falciparium.* (Westman, 1994).

Microorganisms are extremely diverse and found everywhere thus; most pharmaceutical products are susceptible to microbial contamination. However, contamination tends to arise during manufacture rather than during use.(Antai, 1988). Many sugars and other sweetening agents used in the pharmacy are ready substrate for microbial attack, however, very concentrated stock solution of sugar (syrups) with low water activities are more resistant to attack, although spoilage by growth of osmophilic yeast in them has been reported (Bloomfield, 1990).

Pseudomonas, *Enterobacter*, *Flavobacterium* species and other species of *Klebsiella*, *Staphylococcus*, *Bacillus* and *Serratia* are opportunistic pathogens that exist in syrups. (Denya,1988). *Clostridium* and *Salmonella* species are some of the pathogenic bacteria that have been reported (Bloomfields,1990) and *Aspergillus flavus* and *Penicillium notatum* among the fungi (Antai,1988).

The aims of this work are to examine Chloroquine phosphate syrups sold in Minna for microbial contamination and to isolate and identify the microbial contaminants in Chloroquine phosphate syrups.

Materials and Methods

Collection of Samples

Ten samples of Chloroquine phosphate syrups of different brands were purchased from different pharmacies and patient medicine stores in Minna. These includes: LQ, MK, AC, MC, EZ, GC, BF, PC, NQ and CP.

The manufacturer's date, Batch number and expiry date of the syrups used for the work are as shown below:

Table 1: *Batch number of sampled Chloroquine phosphate syrups*

S NO	SAMPLES	BATCH NUMBER	MANUFACTURE DATE	EXPIRY DATE
1	PC	PCQ009	January 2004	Dec. 2007
2	EC	7045F	November 2003	Nov. 2006
3	AC	AC802	January 2001	Dec. 2003
4	BF	26017	August 2001	August2004
5	GC	TR001H	November 2003	October 2006
6	MC	O14252	September 2003	August 2006
7	CP	CQO3123B	December 2003	Dec. 2006
8	NQ	FF181	April 2003	April 2006
9	MK	BO101	October 2002	Sept. 2006
10	LQ	Q506P	July 2002	June2005

*sample names have been coded.

Determination of pH for Chloroquine Syrup

The pH of Chloroquine syrup was determined using a pH meter (Micro pH 2000, Crimson instruments S.A Barcelona).

Enumeration of Microorganisms

The microorganisms were enumerated by methods described by Fawole and Oso, (1988) and compared with the standard microbiological specifications for certification of syrups (NAFDAC Handbook (2000)).

Total microbial counts

Nine milliliters of distilled water was dispensed into 6 test tubes of each syrup and sterilized by autoclaving at 121°C for 15minutes. After sterilization and cooling, 1ml of each sample (chloroquine phosphate syrup) was pipetted into the first test tube labeled $10^{-1}$with a sterile syringe and needle aseptically and mixed homogeneously. 1ml of the diluents from the first test tube was transferred into the second tube (10^{-2}) and mixed homogeneously. 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. The process for serial dilution was done aseptically, and then tubes prepared for inoculation.

Innoculation by pour plate method

Aseptically, 1ml of the 10^{-3}dilution for each sample was plated into 10 petri dishes for each media (Nutrient, MacConkey, and Sabouraud 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 for 24 – 48 hours in an incubator at 37°C while the Saboraud dextrose agar plates were incubated at 20 - 28°C for 3-5 days in an inoculation hood. Typical colonies of microbial growth on plates were counted at the end of incubation period. (Fawole and Oso 1988). NAFDAC HANDBOOK (2000) state that the standard microbiological specifications for the certification of syrups. - Typical viable and fungal counts for bacteria and yeast plate (cfu/ml) respectively must not exceed 1.0×10^{-3}cfu/ml. The chloroquine phosphate syrup was assessed with this standard.

Isolation Of The Microorganisms

The bacteria and fungal colonies developed on the Nutrient agar and Saboruad 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.

Characterization and identification of isolates

The bacteria were characterized according to the method described by Fawole and Oso (1988) in which the following 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. However the fungi were identified using the methods described by Cheesbrough (1984).

Results

Table 2 shows the total viable bacteria counts, the fungal counts and the pH values of some of the brands of chloroquine syrup.

Table 2: The Total Viable Bacteria Counts, Fungal Counts And Ph Values Of Brands Of Chloroquine Phosphate Syrups

S/NO	BRANDS OF SYRUPS	TOTAL/VIABLE *COUNTS(cfu/ml)	FUNGAL *COUNTS(cfu/ml)	pH values
1	EZ	$1.0x10^3$	No growth detected	3.90-3.94
2	NQ	No growth detected	$1.0x10^3$	5.95-5.96
3	BF	No growth detected	$1.0x10^3$	2.31-2.34
4	MC	$1.0x10^3$	No growth detected	5.72-5.72
5	PC	$1.0x10^3$	$2.0x10^3$	3.90-3.94
6	LQ	No growth detected	No growth detected	3.20-3.22
7	MK	No growth detected	No growth detected	2.82-2.84
8	AC	No growth detected	No growth detected	2.80-2.81
9	GC	$2.0x10^3$	$1.0x10^3$	3.31-3.33
10	CP	No growth detected	$1.0x10^3$	4.34-4.36

*Cfu/ml: colony forming units per millilitre.

Identification of microorganisms.

The results revealed that the products have been contaminated by various bacteria. The isolates were characterized and identified as *Bacillus subtilis*, *Escherichia coli* and *Staphylococcus aureus* while the fungi species isolated include *Aspergillus niger*, *Aspergillus flavus*, *Penicillium notatum* and *Aspergillus fumigatus*.

Discussion

Two brands of the chloroquine phosphate syrups MK and AC had no microbial growth indicating that the products may not have been contaminated. Four brands and five brands of the samples had viable bacteria and fungal growth respectively but within the acceptable range for syrup, NAFDAC Handbook (2000) reported that a total count of $1.0x10^3$cfu/ml for bacteria and $1.0x10^2$cfu/ml for mould/yeast are microbiological specifications allowed for syrups. The isolated fungi growths include *Aspergillus niger, A. flavus, A. fumigatus* and *Penicillium notatum.* Some of the fungi isolated are possible toxin producers. This agrees with the report of Antai (1988) who reported that *Aspergillus* species are capable of producing aflatoxins.

The microbes identified include *Escherichia coli*, *Staphylococcus aureus* and *Bacillus subtilis*. *Escherichia coli* are a very good indicator microorganism for faecal pollution. This has been reported to cause gastroenteritis in children (Antai, 1998). *Staphylococcus aureus* is a gram-positive organism that secretes toxin which contributes to gastrointestinal distress (Denya, 1988). The presence of *Bacillus subtilis* in PC and GC syrups could be due to contamination by soil. *Bacillus subtilis* is often associated with food poisoning as reported by Antai (1988).

The presence of these microorganisms in the syrups may be due to improper techniques by the manufacturer, the processing of raw materials or dirty equipments used by the factory workers. The pH of the samples analyzed was within acceptable limits (4.43-5.94). Some samples were acidic which may affect the quality of the products. Low pH of syrups hinders microbial growth and also affects the activities of the preservatives (Booth, 2002).

The results reveal the susceptibility of chloroquine syrups to microbial attack which could aid in the deterioration of the drug. This microbial contamination can be minimized by proper quality control and quality assurance method.

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