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(RESEARCH ARTICLE)

Evaluation of the bacteriological status of cough syrup sold in pharmaceutical stores in Owerri, Imo State, Nigeria

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Abstract

This work was aimed at assessing the level and type of bacterial contaminants in cough syrups sold in Owerri, as well as the associated public health implications. Thirty bottled samples of five different brands of cough syrup were purchased and assessed bacteriologically. Ten out of the thirty samples had a satisfactory microbial load, whereas twenty samples exceeded the tolerance limit of permissible micro-organisms (10^3cfu/ml) specified for syrups. The mean counts obtained ranged from $1x10^3$-$1.15x10^7$ (cfu)/ml. Syrup D had the highest bacterial mean value of $1.15x10^7$ cfu/ml, while C had the lowest bacterial mean value of $1x10^3$ cfu/ml. *Micrococcus luteus, Bacillus subtlis, Serratia macscense, Escherichia coli,* and *Staphylococcus aureus* were identified as the most predominant contaminants. This level of bacterial load reveals contamination which can be traceable contaminated water and other raw materials used for their preparation, personnel, environment and poor manufacturing practice. This portrays the level of health risks to which the consumers can be exposed. From the results, it can be concluded that majority of the samples were heavily contaminated and can serve as source of infection to users, especially those with compromised immunity and children. This therefore calls for good manufacturing and packaging practices, use of treated water and other sterile raw materials, adequate environmental sanitation and personal hygiene, proper handling and storage which will reduce the bacterial loads to permissible limit and eradicate bacteria of public health importance. In other words auditing and quality control measures should be mandatory for all pharmaceutical companies and environment.

Keywords: Cough syrup; Pharmaceutical products; *Serratia macscense*; Bacteriological status

1. Introduction

Microorganisms form an integral part of the environment and the human body. Therefore, it may be common to find that both raw materials and final medicines will contain microorganisms unless specific measures are adopted to exclude them [1]

Pharmaceutical products are generally grouped into two broad groups namely: - Sterile and non-sterile products. Non-sterile products obviously differ from sterile products in that non-sterile product (example the cough syrup), may contain some microorganisms; But the Europeans pharmacopeias specifies the maximum concentration acceptable in different types of products and the species of organisms that are not permitted at all. Sterile products are dosage form of therapeutic agents that are free from viable microorganisms. Principally these include parental ophthalmic and irrigating preparations [2]. Of these parental products are unique among dosage forms of drugs because they are injected into the body compartments. Thus the presence of a single surviving microbial cell is sufficient to render the products non-sterile [3].

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It is now realized that the presence of microorganisms in pharmaceutical preparation may have a variety of consequences, however, the problem arises when there is usually no obvious sign of microbial spoilage or contamination [4]. For example, spore of mold, mucus, may be present in a dormant form and never produce spoilage and harm to the patients who takes the medicine. On the other hand, the presence of *Samonella* in a medicine which although causing little or no visible spoilage, would represent a serious harzard [5].

The knowledge of microbial load of all drugs and medicines, whether they required to be sterile or non-sterile preparations, is essential [6]. In developing countries such as Nigeria, drug-borne infections may have serious debilitating effects on patients because of socio-economic life style. This problem may be compounded by the fact that pharmaceutical preparations are frequently stored under uncontrolled conditions and dispensed from large packs that maybe an average of four to six weeks to exhaust, depending on the demands in hospitals and pharmaceutical stores [7].

Syrups are concentrated solutions of sugar such as sucrose in water or other aqueous liquid. They have unusual opportunities as vehicles in extemporaneous compounding and are readily accepted by both children and adults. Because they contain no or very little alcohol, they are vehicles of choice forming drugs that are prescribed by pediatricians. They possess remarkable masking properties for bitter and saline drugs [8].

Microbial contamination of pharmaceutical environment and steps in processing of drugs highly demands sensitive and accurate procedure due to scale of testing, health risk to consumers, and heterogeneous distribution of microorganisms in a given pharmaceutical products. Since it has been reported that cough syrup can be contaminated by *Candida albicans* and some bacteria, there is need to know the microbiological quality of cough syrup that is being dispatched from pharmaceutical stores. Consequently, the present study is designed to determine and evaluate the microbial status of these cough syrups which are currently sold in Owerri metropolis.

2. Material and methods

2.1. Preparation and Sterilization of Materials

All materials use in this research work were kept under strick sterility to avoid the introduction of external microorganisms into the test samples. Media were prepared and sterilized by autoclaving at 121^{0c} (15psi) for 15 minutes. While glass wares, wire loops, forceps and other metals were sterilized by dry heat using hot air oven and burnsen burner flame respectively, working benches were kept sterile by chemical method using disinfectant [9, 10, 11].

2.2. Collection of Samples

A total of thirty (30) cough syrup samples of five different brands were purchased from pharmaceutical stores in Owerri west, North and central. The different brands were designated A,B,C,D and E. Six (6) samples of each brand were collected and labeled 1-6 accordingly. These samples were transported to the microbiology Laboratory unit of the Department of Medical Laboratory Science, Imo State University, Owerri for processing and analysis.

2.3. Laboratory Processing and analysis of Samples; viable Bacterial counts

Ten –fold serial dilution of the original samples were made in sterile distilled water 10-5 and mixed by vigorous shaking. An aliqnote of 1.0ml of the dilution was asceptically transferred to sterile petri dishes in duplicate for each sample.

About 20ml of sterile cooled molten Nutrient, MacConkey and Manmtol salt agar was poured into the different plates for the different samples for viable bacteria coliform and *Staphylococcal* counts respectively. The contents of the plates were properly mixed by rotational movements in tro and fro, clockwise and anticlockwise direction, allowed to solidify, inverted and incubated at 37^{0c} for 24-48 hours. Heterotrophic bacterial, Coliform and Staphylococcal counts were made and mean values determined for each samples and dilution in the different brands [11]. The discrete colonies were isolated and identified based on their colonial and cellular morphology, as well as biochemical characteristics. Isolates identity was placed by comparison with the standard in Bergey's Manual of determinative bacteriology.

The data from this analysis were evaluated using randomized block design Analysis of Variance (ANOVA) and the differences were separated by the least significant different (LSD).

3. Results

The results from this research are presented in Tables 1-14. While the heterotrophic viable bacterial counts were shown in tables 1-5, -coliform and staphylococcal counts were displayed in Tables 6-10 and 11-15 respectively. Identified bacteria and specific sample of occurrence are in Table 16.

Ten (10) out of the 30 samples examined recorded no bacterial growth. The level of bacterial contamination in the remaining 20 samples was higher above standard ($x10^3$) cfu/ml. The highest mean value, $1.5x10^7$cfu/ml was observed in sample "D" (Table 4), while the lowest $0.1x10^4$ cfu/ml was recorded in sample C (Table 3).

Table 1 viable heterotrophic bacterial count for D-Koff (A)

S/NO	DF	Total Bacterial Count	Mean Count	Bacterial Cell Count/Ml
A1	-4	40	20	$2.0X10^5$
A2	-3	74	37	$3.7X10^4$
A3	-3	200	100	$1.0X10^5$
A4	-4	280	140	$1.4X10^6$
A5	-3	12	6	$0.6X10^4$
A6	--4	100	50	$1.0X10^6$

Table 2 viable heterotrophic bacterial count for conf mix(B)

S/NO	DF	Total Bacterial Count	Mean Count	Bacterial Cell Count/Ml
B1	-5	4	2	$0.2X10^6$
B2	-3	12	6	$0.6X10^4$
B3	-3	263	131	$1.31X10^5$
B4	-3	206	103	$1.3X10^5$
B5	-4	36	18	$1.8X10^5$
B6	-5	0	0	$0.0X10^5$

Table 3 viable heterotrophic bacterial count for emzolyn(C)

S/NO	DF	Total Bacterial Count	Mean Count	Bacterial Cell Count/Ml
C1	-4	30	15	$1.5X10^5$
C2	-3	6	3	$0.3X10^4$
C3	-4	180	90	$9.0X10^5$
C4	-3	298	149	$1.49X10^5$
C5	-3	2	1	$0.1X10^4$
C6	-4	2	1	$0.1X10^5$

Table 4 Viable heterotrophic bacterial count for broncholyte (D)

S/NO	DF	Total Bacterial Count	Mean Count	Bacterial Cell Count/Ml
D1	-5	60	30	$3.0X10^{6}$
D2	-3	6	3	$0.3X10^{4}$
D3	-3	124	62	$6.2X10^{4}$
D4	-3	230	115	$1.15X10^{7}$
D5	-4	0	0	$0.0X10^{3}$
D6	-5	0	0	$0.0X10^{5}$

Table 5 viable heterotrophic bacterial count for benylin (E)

S/NO	DF	Total Bacterial Count	Mean Count	Bacterial Cell Count/Ml
E1	-4	196	98	$9.8X10^{5}$
E2	-2	450	225	$2.25X10^{4}$
E3	-3	26	13	$1.3X10^{4}$
E4	-4	246	123	$1.23X10^{6}$
E5	-3	16	8	$0.8X10^{4}$
E6	-4	6	3	$0.3X10^{5}$

Table 6 Viable coliform count from D-KOFF (A)

S/NO	DF	Total Bacterial Count	Mean Count	Bacterial Cell Count/Ml
A1	-2	10	5	$0.5X10^{3}$
A2	-1	0	0	$0.0X10^{1}$
A3	-1	0	0	$0.0X10^{1}$
A4	-1	0	0	$0.0X10^{1}$
A5	-2	2	1	$0.1X10^{3}$
A6	-1	4	2	$0.2X10^{2}$

Table 7 Viable coliform count from Cofmix (B)

S/NO	DF	Total Bacterial Count	Mean Count	Bacterial Cell Count/Ml
B1	-2	4	2	$0.2X10^{3}$
B2	-1	4	2	$0.2X10^{2}$
B3	-2	0	0	$0.0X10^{2}$
B4	-1	0	0	$0.0X10^{1}$
B5	-1	2	1	$0.1X10^{2}$
B6	-1	4	2	$0.2X10^{2}$

Five species bacteria which include; *Micrococcus lulous, Staphylococcus aureus, Serratia macscense, Escherichia coli* and *Bacillus subitilis* were identified. This level of bacterial load indicates contamination on the higher side of the permissible limit for syrups (10^3cfu/ml). Samples from Owerri central presented higher contamination than those from Owerri West and North pharmaceutical stores, although they were associated with more number of bacteria species (Table 16). *Staphylococcus aureus* predominated especially among syrups from Owerri west stores, whereas *Serratia macscense* was observed among sample from Owerri central . *Bacillus subtlis* occurred in one sample from Owerri west and North areas respectively, whereas *E.coli* was isolated from only a sample from Owerri west area (Table 16).

Table 8 Viable coliform count from emzolyn (C)

S/NO	DF	Total Bacterial Count	Mean Count	Bacterial Cell Count/Ml
C1	-2	0	0	0.0X102
C2	-1	12	6	0.6X102
C3	-1	0	0	0.0X101
C4	-2	0	0	0.0X102
C5	-3	12	6	0.6X104
C6	-2	6	3	0.3X103

Table 9 Viable coliform count from broncholyte (D)

S/NO	DF	Total Bacterial Count	Mean Count	Bacterial Cell Count/Ml
D1	-2	4	2	$0.2X10^3$
D2	-1	2	1	$0.2X10^2$
D3	-1	4	2	$0.2X10^2$
D4	-1	0	0	$0.0X10^1$
D5	-3	8	4	$0.4X10^4$
D6	-1	6	3	$0.3X10^2$

Table 10 Viable Coliform Count from Benylin (E)

S/NO	DF	Total Bacterial Count	Mean Count	Bacterial Cell Count/Ml
E1	-2	0	0	$0.0X10^2$
E2	-3	2	1	$0.0X10^4$
E3	-2	0	0	$0.0X10^2$
E4	-1	4	2	$0.0X10^2$
E5	-3	4	2	$0.1X10^4$
E6	-2	0	0	$0.2X10^2$

Table 11 Viable *staphylococcus* count from D-KOFF (A)

S/NO	DF	Total Bacterial Count	Mean Count	Bacterial Cell Count/Ml
A1	-2	2	1	$0.1X10^3$
A2	-4	2	1	$0.1X10^5$
A3	-2	2	1	$0.1X10^3$
A4	-2	2	1	$0.1X10^3$
A5	-2	0	0	$0.0X10^2$
A6	-3	4	2	$0.2X10^4$

Table 12 Viable staphylococcus count from cofmix (B)

S/NO	DF	Total Bacterial Count	Mean Count	Bacterial Cell Count/Ml
B1	-2	2	1	$0.1X10^3$
B2	-2	2	1	$0.1X10^3$
B3	-2	4	2	$0.2X10^3$
B4	-4	2	1	$0.1X10^5$
B5	-3	10	5	$0.5X10^4$
B6	-4	2	1	$0.1X10^5$

Table 13 Viable staphylococcus count from emzolyn (C)

S/NO	DF	Total Bacterial Count	Mean Count	Bacterial Cell Count/Ml
C1	-2	2	1	$0.1X10^3$
C2	-3	2	1	$0.1X10^4$
C3	-2	0	0	$0.0X10^2$
C4	-3	2	1	$0.1X10^4$
C5	-2	8	4	$0.4X10^3$
C6	-2	4	2	$0.2X10^3$

Table 14 Viable staphylococcus count from broncholyte (D)

S/NO	DF	Total Bacterial Count	Mean Count	Bacterial Cell Count/Ml
D1	-2	2	1	$0.1X10^3$
D2	-4	2	1	$0.1X10^5$
D3	-2	0	0	$0.1X10^2$
D4	-3	0	0	$0.0X10^3$
D5	-2	4	2	$0.2X10^3$
D6	-3	6	3	$0.3X10^4$

Table 15 Viable staphylococcus count from benylyn (E)

S/NO	DF	Total Bacterial Count	Mean Count	Bacterial Cell Count/Ml
E1	-2	2	1	$0.1X10^3$
E2	-4	8	4	$0.4X10^5$
E3	-3	0	0	$0.0X10^3$
E4	-2	2	1	$0.1X10^3$
E5	-2	2	1	$0.1X10^3$
E6	-2	2	1	$0.1X10^3$

Table 16 Identified bacteria and specific sample of occurrence

Isolate	A	B	C	D	E
No. Name	1 2 3 4 5 6	1 2 3 4 5 6	1 2 3 4 5 6	1 2 3 4 5 6	1 2 3 4 5 6
1 *Micrococcus spp*	+ + - - - -	- - - - - -	- - - - - -	+ - - - - -	+ - - - - -
2 *Staphylococcus Aureus*	+ + - - - +	+ + - - - -	+ + - - - +	- + - - - -	- + - - + +
3 *Seratia spp.*	- - + + - -	- - + + - -	- - - + - -	- - - + - -	- - + + + -
4 *Bacillus spp.*	- - - - - +	- + - - - -	- - - - - -	- - - - - -	- - - - + +
5 *E.coli*	- - - - - -	- - - - - -	- - - - - -	+ - - - - -	- - - - - -

4. Discussion

The results from this study revealed that 10 of the 30 samples were bacteriologically satisfactory. The bacterial levels complied with the British pharmacopoeia permissible limit specification of $x10^3$cfu/ml. On the other hand the remaining 20 samples gave loads on the higher side of this standard, potraying the potential health hazard to the public.

Identification of *Micrococcus lecteus, Staphylococcus aureus, serratia, Bacillus subtilis and Escherichia coli* cann be traceable to contamination from water, personnel and environment and agrees with the reports of Mendie and Hugbo, [12] and Ibezim *et al.,* [13]. The heavy contamination observed from the 30 samples can be specifically attributed to poor manufacturing practices, raw materials, air, and water, poor personal hygiene among workers; packaging procedures , containers and equipments.

Incrimination of *Bacillus subtillis* in some of products is an indicator of air, soil, water and animal products contamination. Also from recent studies, *Bacillus subtilis* has been revealed as predominant contaminant in non-sterile pharmaceuticals [13]. Identification of *E. coli* in finished product signifies feacal contamination which may be traceable handlers and water used for the production. Also *serratia marcescens* being an environmental organism in consumable cough syrup is a concern since it can lead to urinary tract infection. The heavy contamination observed in Ekeonunwa market samples can be attributed to poor personal hygiene among personnel and inadequate environmental sanitation.

5. Conclusion

With this understanding that cough syrups meant for remediation of health problem can serve as sources of threat to life due to the presence of these organisms of public health importance. It is therefore inferred that precautious measures should be taken in the manufacturing and processing of this drugs. Appropriate authorities or body for food safety like NAFDAC should organized enlightenment training campaign target at the producers and other workers in these pharmaceutical companies and monitor these products on regular bases. These will check the level of contaminants present in these products. This will reduce if not eradicate the risk associated with the consumption especially to the immunocompromized individuals and children who use these syrups regularly.

Compliance with ethical standards

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Disclosure of conflict of interest

There is no conflict of interest.

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