World Journal of Pharmaceutical and Life Sciences
WJPLS
www.wjpls.org
SJIF Impact Factor: 5.088

ASSESMENT OF MICROBIAL QUALITY OF SOME PHARMACEUTICAL SYRUPS AVAILABLE IN UYO LGA OF AKWA IBOM STATE

Akeem Agboke* and Nneoma Igbokwe

Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmacy, University of Uyo, Uyo Nigeria.

***Corresponding Author: Akeem Agboke**
Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmacy, University of Uyo, Uyo Nigeria.

Article Received on 09/05/2018 **Article Revised on 30/05/2018** **Article Accepted on 20/06/2018**

ABSTRACT

This study was designed to evaluate the microbial quality of some pharmaceutical syrups available in Uyo LGA, Akwa Ibom. The microbiological quality of 10 different syrup samples of 10 pharmaceutical companies was assessed. The total aerobic microbial count (TAMC) varied between 1.0×10^3 CFU/ml and 6.0×10^3 CFU/ml.The total yeast and mold count (TYMC) ranged from 1.0×10^3 CFU/ml to 2×10^3 CFU/ml. In general, the microbiological qualities of six (6) which is 60% of the syrups tested have viable bacterial load within USP acceptable limits while four (4) which is 40% fell above stipulated standards. None of the syrups tested contained objectionable microbe (*E.coli*). Three samples of the syrups did not contain any mould while five samples of the syrups contained moulds which were within acceptable limits of USP. Two samples of the syrups contained moulds well above the stipulated standard. This work showed that quality of syrups that conformed to the standards was well prepared according to Current Good Manufacturing Practice (CGMP), while some might be contaminated during distribution and storage.

KEYWORDS: Evaluate, Microbial, Quality, Pharmaceutical, CGMP, Syrups, Uyo.

INTRODUCTION

The microbial quality of pharmaceutical products primarily depends on the quality of raw materials, production process, production environment, hygiene of the personnel involved in manufacture and the storage conditions. Not only the presence of pathogenic microorganisms but the presence of relatively high number nonpathogenic microorganisms is also objectionable in pharmaceutical products. The presence of high number of non-pathogenic microorganisms in pharmaceutical products is objectionable for two reasons: firstly, these microorganisms can deteriorate active ingredients and can interfere with the desired activity of the product; and secondly, they can produce some metabolites that may be toxic to the consumer [Black, 2012].

Drugs and other pharmaceutical products are manufactured based on stipulated standards. These standards are regulated by the regulatory authorities. The standards are achieved through well-articulated current good manufacturing practice (CGMP).

Maintaining current good manufacturing practice will ensure the formulation of products of acceptable standards in contents of active ingredients, good physical and chemical stability and acceptable microbial quality. For oral dosage formulations, official standards required that viable microbial count should not exceed 1×10^3 CFU/ml and the products should not contain enteric organisms (Nwakile et al., 2011). Deviations from these standards attract serious sanctions from the regulatory authorities (Nwakile et al., 2011).

Substandard pharmaceutical products which pose serious challenge to good health are often produced in compromised environment which predispose the products to risks of contamination (Clement et al., 2010).

Microbial contamination of pharmaceutical dosage forms can adversely affect the consumer, the preparation or the manufacturer in various ways (Clement et al., 2010). On the part of the product, microbial contamination can lead to spoilage resulting in physical and chemical changes. It can also lead to destruction of active ingredients and loss of activity.

On the part of the consumer, syrups are mostly consumed by paediatrics, hence, microbial contamination of pathogenic bacteria can cause an infection. On the part of the manufacturer, established presence of a microbial contaminant in a product will cause a drop in demand of the product, causing economic losses for the manufacturer (Clement et al., 2010).

Pharmaceutical syrups are concentrated solutions of sucrose or other sugars to which medicaments or flavourings are often added. They can contain up to 80% of sugars and are prepared in order to mask the unpleasant tastes of many drugs. Their high demand especially for paediatrics makes them targets for adulteration and faking by dubious manufacturers.

Need For Pharmaceutical Syrups

As liquids, pharmaceutical syrups are easier to swallow than solids and are therefore particularly acceptable for paediatric and geriatric uses. Syrups are homogeneous systems and therefore the drug will be uniformly distributed throughout the preparation.

A drug must be in solution before it can be absorbed. Formulations of drugs as syrups make the drugs immediately available for absorption. Therefore, the therapeutic response is faster than when using a solid dosage form. Syrups are also formulated in order to mask the unpleasant tastes of many drugs. [(Clement et al., 2010].

Challenges of pharmaceutical syrups

Crystallization of the sugar within the screw cap used to seal the containers of syrups, thereby preventing its release, is a problem with the storage and use of syrups. This can be avoided by the addition of polyhydric alcohols or by the inclusion of invert syrup, which is a mixture of glucose and fructose.

Syrups as liquids are bulky and therefore inconvenient to transport and store. If the container should break, the whole of the product is immediately and irretrievably lost.

The stability of ingredients in solution is often poorer than if formulated as tablets or capsules.

Syrups often provide suitable media for the growth of microorganisms and therefore require the incorporation of a preservative [Black, 2012].

Microbial quality of pharmaceutical syrups

Non sterile pharmaceuticals are not produced by aseptic processes and therefore are not expected to be totally free from microbial contamination. The degree of contamination in non sterile products is regulated based on the acceptance criteria for microbiological quality established in the pharmacopoeia monographs (Vu et al., 2014).

The term 'bio-burden' is used to describe the population of viable microorganism present on or in a product and/or sterile barrier system. Bio-burden is the sum of microbial contributions from a number of sources including raw materials, assembly processes, manufacturing of components, manufacturing environment, cleaning processes and packaging of the finished product.

The major contaminants of non sterile pharmaceutical products are bacteria, yeast and mould (Vu et al., 2014).

Under current good manufacturing practice, manufacturers are expected to maintain strict adherence to microbial contamination control practices during the production, and to develop microbial specifications for their non sterile products (Vu et al., 2014).

Objectionable microorganisms may be pathogens or opportunistic pathogens with their attendant metabolic activities and their microbial characteristics such as exotoxins, endotoxins, sporulation etc. These microorganisms can grow under suboptimal temperature and nutrients and may affect product quality and safety.

The aim of microbial quality testing is to measure the total number of viable organisms on a medical device or product prior to its use (Denyer et al., 2004). It is important when conducting these tests to ensure that the testing method does not either introduce bacteria into the test sample or kill bacteria in the test sample. To prepare drug products for testing, they must be dissolved in certain substances based on their 'physical characteristics' (Clement et al., 2010). For example a water soluble drug product should be dissolved in 'Buffered sodium chloride-peptone solution pH 7.0, Phosphate buffer solution pH 7.2, or Soybean-casein digest broth'.

The membrane filtration method and the plate count method can be used to determine the number of microbes in a sample. In the membrane filtration method, the sample is passed through a membrane filter with a pore size of 0.45 micrometres or less. The membrane filter is then placed onto the appropriate culture media and incubated. In the plate count method, the sample of drug product to be tested and the appropriate culture media are poured into a petri dish and incubated.

The bio-burden quantification is expressed in colony forming unit (CFU).

Limits of microbial contamination

There are generally established guidelines for the maximum CFU that a drug product can contain. The microbial limit for non-sterile pharmaceuticals must be within an acceptable range that does not pose health hazards to intended patient groups or diminish product stability (Vu et al., 2014).

The table below represents the USP acceptance criteria for microbiological quality of non-sterile dosage forms (Vu et al., 2014).

Table 1: USP acceptable criteria for microbiological quality of non sterile dosage forms CFU- Colony forming unit.

Route of Administration	TAMC CFU/ml	TYMC CFU/ml	Absence of Specified Organisms
Oral (non aqueous)	10^3	10^2	*Escherichia coli*
Oral (aqueous)	10^2	10^1	*Escherichia coli*
Rectal	10^3	10^2	None designated
Oromucosal	10^3	10^2	*Staphylococcus aureus, Pseudomonas aureginosa*
Cutaneous	10^2	10^1	*Staphylococcus aureus, Pseudomonas aureginosa*
Auricular	10^2	10^1	*Staphylococcus aureus, Pseudomonas aureginosa*
Vaginal	10^2	10^1	*Staphylococcus aureus, Pseudomonas aureginosa, Candida albicans*
Nasal	10^2	10^1	*Staphylococcus aureus, Pseudomonas aureginosa*
Gingival	10^2	10^1	*Staphylococcus aureus, Pseudomonas aureginosa*
Transdermal patch (drug matrix, adhesive layer and backing)	10^2	10^1	*Staphylococcus aureus, Pseudomonas aureginosa*
Inhalation	10^2	10^1	*Staphylococcus aureus, Pseudomonas aureginosa* Bile tolerant gram negative bacteria
Pharmaceutical substances	10^3	10^2	None designated

TAMC- Total aerobic microbial count
TYMC- Total combined yeasts and mould

Significance of microbial quality evaluation

Microbial limit tests are intended to determine whether a substance or preparation complies with an established specification for microbial quality and are designed to allow determination of the absence of; or limited occurrence of specified organisms that may be detected under standard conditions. These tests can be applied to pharmaceutical products, both finished and raw materials, and may also be useful in evaluating the presence of organisms on selected materials used in some medical devices or biologics.

Microorganism

A microorganism is defined as a living thing that is so small, it must be viewed with a microscope and include bacteria, protozoa, some fungi and algae. Microorganisms live in every part of the biosphere, including soil, water and atmosphere. Microorganisms may be unicellular or multicellular and the study of microorganisms is called microbiology.

Microorganisms may be

a. Prokaryotes which are organisms without a nucleus or membrane bound organelles e.g. bacteria and archaea (Gold, 1992).
b. Eukaryotes which are organisms with complex cells in which the genetic material is organized into a membrane bound nucleus e.g. fungi, algae (Dyall et al., 2004). Some microbiologists classify viruses as microorganisms, but others consider them as non-living (Rybicki, 1990).

METHODOLOGY

Sample Collection

The pharmaceutical syrups of 10 companies having different manufacturing date were collected from various retail pharmacy outlets in Uyo Akwa Ibom for each of the samples, batch number, date of manufacture, date of expiry, dosage form was documented from the label attached to the bottle.

Table 2: Samples.

Product code	Name	Batch number	Mfg date	Expiry date	Manufacturer address	NAFDAC Reg. No
B	Vinaquine syrup	A151752	11-2015	10-2018	May & Baker Nig PLC, Nigeria	04:0444
A	Dr Meyers liquid iron	6316	3-2016	2-2018	Farmex Meyer Ltd, Nigeria	04-1155
C	Benylin expectorant	J0107	9-2015	8-2018	Nigerian-German chemicals PLC, Nigeria	04-0820
D	Zevit	L2E042	1-2016	12-2017	Mecure industries, Nigeria	A4-4945
E	Chazmax	BEL004	4-2016	3-2018	Chazmax Pharmaceutical industries, Nigeria	04-8444
F	Architamol syrup	P81501	12-2015	11-2018	ARCHY Pharmaceutical, Nigeria	04-5621
G	Rophegan	A140450	8-2014	7-2017	May & Baker Nig PLC, Nigeria	04-0290
H	Tussylin	16041	2-2016	2-2019	Afrab chem. Ltd, Nigeria	04-1287
I	Escobic	40032	4-2016	3-2019	ESEHI Pharm Ind. Ltd, Nigeria	04-9597
J	Nosak	SK793001	10-2015	9-2017	Nosak Health Care, Nigeria	A4-3155

Method

A three-tube ten-fold serial dilution of the samples was prepared using sterile pipettes for each step under aseptic conditions. Each dilution was evenly mixed.

Microbiological assay of samples

Microbiological analyses included in this study were total aerobic microbial count (TAMC), total coliform count (TCC) and total yeast and mold count (TYMC). For enumeration of the microorganisms, syrups samples were diluted in 0.9% normal saline. An aliquot from different dilutions were plated onto Nutrient agar (NA) for enumeration of TAMC, MacConkey agar for TCC and Sabouraud dextrose agar (SDA) for TYMC. The NA and MacConkey agar plates were incubated at 37 °C for 24-48 h. The SDA plates were incubated at room temperature for 5 days. The characteristic colonies grown on the Nutrient and MacConkey agars plates were isolated and purified for morphological and biochemical study. On the basis of morphological, cultural, Gram staining and biochemical characteristics, no E. coli detected [Black, 2012].

RESULT OF MICROBIAL QUALITY TEST

The result of the microbial quality tests of the samples in CFU/ml are shown in the table below:

Table 3: Nutrient agar and MCkonkey agar microbial count (CFU/ml).

	TAMC				TCC
Samples	**I**	**II**	**III**	**Mean ± SEM**	**ND**
A	0	1×10^3	1×10^3	666.67 ± 333.33^a	ND
B	0	1×10^3	1×10^3	666.67 ± 333.33^b	ND
C	0	0	0	0^c	ND
D	4×10^3	5×10^3	6×10^3	5000 ± 577.35^d	ND
E	4×10^3	4×10^3	4×10^3	4000 ± 0^e	ND
F	3×10^3	2×10^3	1×10^3	2000 ± 577.35^f	ND
G	0	0	0	0^g	ND
H	1×10^3	2×10^3	1×10^3	1333.33 ± 333.33^h	ND
I	2×10^3	2×10^3	2×10^3	2000 ± 0^i	ND
J	1×10^3	2×10^3	1×10^3	0^j	ND

a - Not significantly greater than USP (P>0.05)
b - Not significantly greater than USP (P>0.05)
c - Not significantly lesser than USP (P>0.05)
d - Not significantly lesser than USP (P>0.05)
e - Not significantly greater than USP (P>0.05)
f - Extremely significantly greater than USP (P<0.001)
g - Not significantly lesser than USP (P>0.05)
h - Moderately significantly greater than USP (P<0.01)
i - Not significantly greater than USP (P>0.05)
j - Not significantly greater than USP (P>0.05)

Table 4: Sabouraud dextrose agar.

Samples	**I**	**II**	**III**	**Mean ± SEM**
A	0	1×10^3	1×10^3	666.67 ± 333.33^a
B	1×10^3	1×10^3	1×10^3	1000 ± 0^b
C	0	0	0	0^c
D	0	0	0	0^d
E	1×10^3	1×10^3	1×10^3	1000 ± 0^e
F	2×10^3	2×10^3	2×10^3	2000 ± 0^f
G	0	0	0	0^g
H	1×10^3	2×10^3	1×10^3	1333.33 ± 333.33^h
I	1×10^3	0	1×10^3	666.67 ± 333.33^i
J	0	1×10^3	1×10^3	$666.67^j \pm 333.33^a$

a - Not significantly greater than USP (P>0.05)
b - Not significantly greater than USP (P>0.05)
c - Not significantly lesser than USP (P>0.05)
d - Not significantly lesser than USP (P>0.05)
e - Not significantly greater than USP (P>0.05)
f - Extremely significantly greater than USP (P<0.001)
g - Not significantly lesser than USP (P>0.05)

h - Moderately significantly greater than USP (P<0.01)
i - Not significantly greater than USP (P>0.05)
j - Not significantly greater than USP (P>0.05)

The total aerobic microbial count (TAMC) varied between 1 × 10^3 CFU/ml and 6.0×10^3 CFU/ml. Whereas, total yeast and mold count (TYMC) ranged between 1.0×10^3 CFU/ml and 2 ×10^3 CFU/ml. Overall, 6 (60%) out of 10 samples exceeded the limit (TAMC ≤1x10^2 CFU/ml; TYMC ≤1x0^1 CFU/ml; and no *E. coli*. detected.

RESULTS OF GRAM STAINING

Table 5: The results of gram staining of the contaminating organisms.

Samples	Gram reaction
A	Gram positive organisms
B	Gram positive organisms
D	Gram positive organisms
E	Gram positive organisms
F	Gram positive organisms
H	Gram positive organisms
I	Gram positive organisms
J	Gram positive organisms

Table 6: The results of Biochemical Test on the contaminating Microorganisms.

Sample	Nitrate reduction	Glucose fermentation	Mannitol fermentation	Citrate utilization	Starch	Inference
A	+	A	-	-	-	*Staphaylococcus aureus*
B	+	A	A	+	+	*Bacillus subtilis*
C	+	+	+	+	-	*Staphylococcus aureus*
D	+	A	-	+	-	*Staphylococcus aureus*
E	+	AG	AG	-	-	*Bacillus cereus*
F	+	A	-	-	-	*Bacillus subtilis*
G	+	A	A	+	-	*Bacillus subtilis*
H	+	A	-	+	+	*Bacillus cereus*
I	+	A	A	+	-	*Staphaylococcus aureus*
J	+	A	-	+	+	*Bacillus subtilis*

Key: A = Acid produced from either mannitol or glucose
AG = Acid and gas produced
+ = Positive - = Negative

DISCUSSION

The findings of the microbial quality testing are depicted on tables 3 and 4, while the results of the gram staining are shown on table 5.

From table 3, the total aerobic microbial count (TAMC) using nutrient agar showed that samples D, E, F and I had similar microbial load which was significantly greater (p<0.05) than the mean. As such they do not conform to the limits of 10^2 CFU/ml. This may possibly due to lack of proper packaging and bad storage condition.

Samples C and G showed similar microbial load. However, it was non-significantly lesser than the mean (p>0.05).

Samples A, B, H and J showed similar microbial load which was non-significantly greater than the mean (p>0.05).

From the total combined yeast and mould count using saboraud dextrose agar, shown on table 4, samples A, B, E, I, and J showed similar microbial load which was non-significantly greater than the mean.

Samples C, D, and G showed similar microbial load which was non-significantly greater than the mean (p>0.05). Sample F showed extremely significantly greater microbial load than control (p<-0.05). This implies that the product was highly contaminated in the course of production, packaging or storage the sources of contamination not ascertained. Sample H had a greater microbial load than control which is moderately significant meaning the sample was contaminated.

From the results of gram staining, the contaminants were gram positive organisms.

The Biochemical test results confirmed the presence of Gram positive organisms of *Staphylococcus aurous, Bacillus subtilis and Bacillus cereus*.

The USP official monograph specifies the absence of Escherichia coli which is a gram negative organism as acceptance criteria for the consumption of the syrups.

This implies that the samples were not contaminated with offending organisms that are dangerous for human consumption.

CONCLUSION

These findings suggest that samples C and G are safe for consumption due to their low microbial load. Samples A, B, and J are also relatively safe while samples D, E, F, I, and H were heavily contaminated though not with offending microorganisms, patients should be cautioned in taking such syrups or the syrups be withdrawn from counter for safety reasons. The results have shown that the prime contaminants were gram positive organisms. This can be attributed to poor handling of pharmaceuticals and their improper storage. Proper hygiene should also be maintained by the personnel involved in the manufacturing of pharmaceuticals syrups. Determination of the microbial quality of finished pharmaceutical products is an important aspect of quality control to ensure that pharmaceutical products conform to the specified standards. Numerous actions are necessary to decrease microbial contamination of non-sterile pharmaceutical products that may include equipment mechanisation, monitoring and post marketing surveillance.

There is no presence of any objectionable bacteria E. coli showing that the syrups are fit for consumption.

Conflicts of Interest

No conflicts of interest in this research work. The results has little or nothing to do with the company's manufacturing practice but more on storage facilities and conditions of the Pharmacy stores in Uyo LGA, Akwa Ibom State.

REFERNCES

1. Black, Jacquelyn. Microbiology: Principles and Exploration (8th edition). John Willey and sons, 2012; 68.
2. Jackson, C., Emmanuel I., Agboke A., Mfon, U., Hillary, O., "Research in Pharmaceutical Biotechnology". 2010; 51-53.
3. Denyer. S.P., Hodges, N.A., Gorman, S.P., Hugo and Russel's Pharmaceutical Microbiology, Blackwell Science, 2004.
4. Dyall, S., Brown, M., Johnson, P., "Ancient Invasion: From Endosymbionts to Organelles". Science, 2004; 304.
5. Eckburg, P., Lepp, P., Relman, D., Archea and Their Potential Role In Human Disease, European Pharmacopoeia, 2003; 4451-4452
6. Feng, P., Weagant, S., Grant, M., "Enumeration of *Escherichia coli* and the Coliform Bacteria". Bacteriological Analytical Manual (8th ed.), 2002.
7. Gillen, Allan L., The Genesis of Germs: The Origin of Diseases and the Coming Plagues. New Leaf Publishing Group, 2007; 10.
8. Goff, H.D., Dairy Science and Tech Education Series, 2006.
9. Gold, T. "The Deep, Hot Biosphere" Proc. National Acad Sci U.S.A, 1992; 89(13): 6045-9.
10. Goldman, Emmanuel., Green, LorenceH (24 August, 2008). Practical Handbook of Microbiology, Second edition (2nd ed.). USA: CRC Press, Taylor and Francis Group p.864.
11. Hogg, S., Essential Microbiology (1st ed.). Wiley, 2005; 99-100.
12. Jones, D., Pharmaceutics-Dosage Form and Design. Pharmaceutical press, 2008.
13. Kadner, R., Encyclopaedia Britanica: Importance of Bacteria to Man, 2014.
14. Madappa, T., Cunha, B.A., "*Escherichia coli* infections". Medscape e-Medicine Health, 2014.
15. Madigan, M., Martinko, J., Brock biology of microorganisms (13th edition). Pearson education, 2006; 1096.
16. Nwakile, D.C., Osonwa, E.U., Okechi, C.O., Opurum, C.C., Nwanyanwu, E.C, Journal of Advanced Pharmacy Education and Research, 2011; 1-89.
17. O'Hara A., Shanahan, F., "The gut flora as a forgotten organ". EMBO Rep., (7): 6882-93.
18. Razvi, N., Awan, R., Naqvi, S.B., Anjun, F., Hussain, Z., Farooqi, S (2014): World Journal of Pharmacy and Pharmaceutical Sciences, 2006; 1771-1777.
19. Ryan, K.J., Ray, C.J., Sherris Medical Microbiology (4th ed.). McGraw Hill, pp. 261-271, 273-296.
20. Rybicky E.P. "Declassification of organism at the edge of life". Problems with virus systematic, 1990.
21. Singleton, P., Bacteria in Biology, Biotechnology and Medicine (5th ed.), Wiley, 1999; 444-454.
22. Todar, K., Todar's Online Textbook of Bacteriology, 2008; 3-8.
23. Vireze, Jopde. "The littlest farmhands". Science, 2015- 08-14; 349(6249): 680-683.
24. Vu, N., lou, J.R., Kupiec, C.T., "International Journal of Pharmaceutical Compounding", 2014; 213-221.