

Microbial analysis of brands of multivitamin syrups marketed in Maiduguri, Northeast Nigeria

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ABSTRACT

This study investigated the microbial contamination of seven (7) brands of multivitamin syrups marketed in Maiduguri metropolis, Borno state. The study is aimed at evaluating the microbial quality of different brands of multivitamin preparations from various manufacturers marketed in Maiduguri. Pour plate method was used. Preliminary investigation revealed that one out of the seven different samples contains pathogenic organisms which became pertinent for further investigation to reveal the identity of the organism. Escherichia coli, Staphylococcus and Salmonella were investigated but only salmonella was found to be present. The other six samples also contain non pathogenic organisms, but were below the limit count set by microbiological quality of syrups.

This finding showed that most multivitamin syrups marketed in Maiduguri followed Pharmacopoeia specifications on microbial quality.

Key words: contamination, pathogenic, multivitamin syrup, brands.

INTRODUCTION

Micro-organisms cannot be seen by the naked eyes but only by the use of microscopes. They are microscopic forms of life which are ubiquitous and are present everywhere in the environment including the human body. They were first detected in 1675 by a Dutch draper Anthony Van Leeuwenhoek, who noticed tiny 'animalcules' in droplets of rain water under his microscope. He went on to discover that they were present in dental plaque, faeces and many other substances [1]. Most micro-organisms are harmless or even beneficial to man. Only minority cause disease in healthy humans (although many more may do so in patients with damaged immune system). After about another 12 years of Van Leeuwenhoek's discovery, it was shown that these minute creatures were also under certain circumstances the agents of disease. This was first demonstrated by Agostino Bassi in 1835 for a bacterial infection of silkworms [1]. The German Robert Koch was the first to prove that a bacterium could cause a human disease, namely anthrax in 1876. Naturally the discovery aroused huge scientific and public interest, although they were those in both the lay and the scientific communities who were opposed to the new theory of infection.

The study of micro-organisms is known as microbiology and not surprisingly the much of the study is directed at those organisms which do cause human disease. It is now realized that not only are micro-organisms responsible for what are conventionally considered infectious diseases but they may also contribute to such diverse illnesses as peptic ulcer, angina pectoris and cervical cancer. Bacteria have the genetic ability to transmit and acquire resistance to drugs used as therapeutic agents [2]. There is no doubt that in the future, micro-organisms will be found to be involved in many more non-infectious diseases. However they are also essential to human life. Every square inch of our body surface is colonized by many thousands of organisms which help to protect the body from invasion by other potentially harmful organisms. If this normal 'flora' is damaged for instance by antibiotics, it leaves the way open for the harmful organisms to get a foothold and establish themselves instead.

Syrups are non-sterile liquid dosage form that contain active medicaments and constitute the most convenient dosage form for babies, children and the elderly [3].

Syrups are mostly prepared for oral administration in children since tablets and capsules cannot be easily or conveniently administered to them [4].

Syrups are characterized by sweet taste and a viscous consistency. Concentrated aqueous solution of sucrose or other sugars serve as the general vehicle for all syrups

Flavoured and medicated syrups are the preferred dosage forms of choice for both children and adults, because they are palatable and easily absorbed by the body. Patients frequently use multidose syrups and most of them have been found to be potent. For this reason, manufacturers should use preservatives to prevent accidental contaminations to opened bottles of syrup. Preservatives are widely employed in the cosmetics and pharmaceutical industries as well as in a variety of other manufacturing industries [5].

The preservatives include both ingredients with known antimicrobials activity and ingredients that may contribute directly or indirectly to antimicrobial activity [6]. Antimicrobial preservatives work by reducing the number of organisms and inhibiting the growth of micro-organisms that may be introduced during repeated use accidentally [7]. The amount of a preservative varies with the proportion of water available for growth, the nature and inherent preservative activity of some formulative materials and the capability of the preservative itself. All handling and storage methods are therefore primarily concerned with minimising microbial contamination and retarding microbial growth and activity [8].

In an attempt to examine the general information on the microbial content of 93 samples of non-sterile pharmaceutical preparations from 34 different manufacturers comprising of 31 cough syrups and 18 multivitamin syrups. Results revealed that three samples out of the 31 cough syrups were contaminated with gram positive bacilli, but the total aerobic count was not more than 1×10^3 cfu/ml while 1 out of the 18 multivitamin was contaminated but all samples were found to be free from *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella* [9].

[10] Studied the microbiological quality of some commercially available syrups and suspensions in Nigeria, and found that 40% of the syrups did not comply with the official requirement for microbiological quality of syrups. The bacterial load of all the syrups except one was below 10^3 cfu/ml which is the highest permissible limit of bacteria in non sterile pharmaceuticals. Bacillus which was reported in this study to be the most frequent contaminant of syrup had also been found to be number one contaminant of pharmaceuticals [11].

Microbiological quality of 20 paediatric anti malarial and cough preparations revealed that 5 out of the cough syrup had bacterial load of less than 10×3 cfu/ml which implies that they complied with the official requirement for the microbiological quality of syrups while the other 5 were heavily contaminated and thus did not meet the official limit [4]. The United States Food and Drug Administration (FDA) require that drug product be tested for its purity, identity, strength, quality and stability before it can be released for use. Hence pharmaceutical validation and process control are important [12].

The study is aimed at evaluating the microbial quality of different brands of multivitamin preparation from various manufacturers marketed in Maiduguri.

MATERIALS AND METHOD

Materials

- Drug samples (multivitamin syrup)
- Autoclave (Dixons CE model type ST 195, made in U.K)
- Incubator (Genlab, made in Cheshire)
- Foil paper
- Syringes
- Conical flask
- Beakers
- Cotton wool
- Disposable Petri dishes
- Mac Cartney bottles
- Hand gloves

- Spatula
- Air condition
- Weighing machine (Mettler pj 3600, made in Switzerland)
- Test tubes
- Measuring cylinder (Pyrex)
- Colony counter (Stuart sc6, made in U.K)
- Air filter (Hunter, made in China)
- Ultra violent lamp
- Laminar flow (Former scientific,inc made in U.S.A)

Media Used

- MacConkey agar (Darmstadt, Germany)
- MacConkey broth (Darmstadt, Germany)
- Nutrient agar (Darmstadt, Germany)
- Nutrient broth (Darmstadt, Germany)
- Sabourauds dextrose agar (Darmstadt, Germany)
- Sabourauds dextrose broth (Darmstadt, Germany)
- Baird parker agar (Darmstadt, Germany)
- Xylose lysine deoxycholate agar (Darmstadt, Germany)

others

- Distilled water
- Peptone water
- Methylated spirit.

Sample collection

Seven different brands of multivitamin syrups marketed in Maiduguri, Nigeria were purchased from three different pharmacies and corded with numbers 1-7. Each had NAFDAC registration status, shelf life, manufacturing and expiry date.

Analysis of samples

Pour plate method was used for the estimation in accordance with [13]. The culture media (agar and broth) were prepared according to the manufacturers instructions. MacConkey, nutrient and Sabourauds dextrose were used for this analysis. One millilitre was withdrawn aseptically from each sample into corresponding labelled bottle PW1-7, thus comprising of peptone Water and drug sample. The screw caps were tightly covered and shaken well to ensure complete dissolution of the drug sample, 1ml from each bottle was transferred aseptically into duplicate medium plate (agar) and bottles (broth), kept in an incubator set at 37°C. Bacterial colonies were counted and the number of colony forming units per ml of each plate was calculated [14].

Identification of isolated microorganisms

The sample of the syrups were plated on various selective media such as MacConkey for (*Escherichia coli*), Sabourauds dextrose agar for (*moulds and yeasts*), Baird parker agar (for *Staphylococcus*) and Xylose lysin Deoxycholate for (*Salmonella*) and then incubated.

TABLE 1 Labels present on the various drug samples investigated.

SAMPLES	MANUFACTURING DATE	EXPIRY DATE	BATCH NUMBER	NAFDAC NUMBER
1	10/2010	09/2013	+	+
2	07/2010	07/2012	+	+
3	10/2010	10/2012	+	+
4	06/2009	06/2011	+	+
5	02/2011	02/2014	+	+
6	06/2010	05/2012	+	+
7	06/2009	05/2011	+	+

+ = INDICATED

RESULTS AND DISCUSSION

Table 1 shows the different drug samples used indicating their manufacturing and expiry dates, batch numbers and NAFDAC registration numbers. These labels confirmed their authenticities and their free circulation in market are

fully accepted and recognised. It also reveals that their respective active ingredients are still valid as they were within their shelf-lives

Majority (table 2) of the contamination came from the nutrient agar. The least number of contaminations came from MacConkey agar which had two pathogenic organisms in contrast to MacConkey broth which had no contaminant. Only samples 1, 2 and 5 indicated the absence of growth in nutrient broth while samples 3, 4, 6 and 7 had growth in both nutrient agar and nutrient broth respectively.

TABLE 2: number of organisms recorded per millilitre

SAMPLES	NA	MCA	SDA	NB	MCB	SDB
1	80	-	4	-	-	-
2	60	-	2	-	-	-
3	60	2	1	+	-	-
4	50	-	2	+	-	-
5	40	-	2	-	-	+
6	60	-	-	+	-	-
7	40	-	-	+	-	-
CONTROL	-	-	-	-	-	-

NA = NUTRIENT AGAR
 MCA = MacConkey AGAR
 SDA = SABOURAUDS DEXTROSE AGAR
 NB = NUTRIENT BROTH
 MCB = MacConkey BROTH
 SDB = SABOURAUDS DEXTROSE BROTH
 + = PRESENT
 - = ABSENT

TABLE 3: shows the distribution pattern of isolated contaminants suspected in sample 3 under the influence of MacConkey agar.

MEDIA	ORGANISM	QUANTITY
MCA	E.COLI	NIL
BPA	STAPH	NIL
XLD	SALMONELLA	2

MCA = MacConkey agar
 BPA = Baird parker agar
 XLD = xylose – lysine deoxycholate agar

In addition, samples 1- 4 revealed the presence of growth in Sabouraud dextrose agar only as not evidenced in the broth, while sample 5 proved the presence of growth in both Sabouraud dextrose agar and the broth, lastly sample 6 and 7 revealed no growth in both Sabouraud dextrose agar and broth respectively. Sample number three was found to be contaminated with pathogenic micro-organism higher than the official specification limit. This prompted a further study to investigate for the presence of *Escherichia coli*, *Staphylococcus* and *Salmonella*. Both *Escherichia coli* and *Staphylococcus* were negative (absent) while *Salmonella* was present (table 3). This contamination suggests the route of contamination is possibly water.

The lower count recorded in the other syrups may be attributed to the sugar content of the syrups which provide high osmotic pressure that is inhibitory to many micro-organisms[15]. Moreover syrups are usually filtered prior to bottling.

Contamination by *Salmonella* could also be as a result of serious microbial pollution of the factory equipment or from an infected worker working under unhygienic practices [16]. *Escherichia coli* are not always confined to the intestine and their ability to survive for brief periods outside the body makes them an ideal indicator organism to test environmental samples for faecal contamination [17].

Water is one of the fundamental requirements of life and any undesired addition of chemical substances leads to its contamination and makes it unfit for human utility [18].The major sources of contamination of pharmaceuticals have always been water, the production environment, personnel and packaging material [19]. Therefore proper attention should be given to the prior treatment of these factors to ensure reduction in the level of microbial contamination.

It can also be observed that from the results obtained, the extend of microbial contamination in the different brands of multivitamin syrup samples used was very low as only one sample was found to contain pathogenic organism above the accepted purity limit. Other non-pathogenic micro-organism including mould and yeast were within the accepted level implying that they comply with the official requirement for the microbiological quality of syrups according to FIP working committee 1975.

The result of this study while complementing those of other studies have shown that non-sterile pharmaceutical mixtures such as paediatric preparations (syrups and suspensions) showed compliance with the official requirement for microbiological quality of syrups as only one (1) out of the seven (7) samples tested showed contamination greater than the accepted level. However these can serve as silent and unsuspected sources of infection to infants.

CONCLUSION

It can be concluded that one of the multivitamin syrup was contaminated while the other six have passed the official requirement for microbiological quality of syrups. *Salmonella* was found to be the contaminant present in one of the multivitamin samples and the source of contamination could be from factory equipment, water or an infected personnel.

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