COMPARATIVE STUDIES ON THE MICROBIOLOGICAL AND PHYSICAL QUALITY OF EXPIRED AND UNEXPIRED PEDIATRIC SYRUP

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ABSTRACT

Background

The recent increase in the number of cases of contaminated drugs had necessitated the need for research involving microbial contamination and subsequent deterioration of drug quality (Tanko and Antai, 2006). The microbiological and physical quality of selected expired and unexpired pediatric syrups were assessed in selected drug outlet in Owerri Imo state Nigeria.

Aim: this study is aimed at assessing and comparing the quality of expired and unexpired drugs at pharmaceutical outlets at Douglas and Wetheral roads in Owerri, Imo State.

Materials and methods: comparative study on the microbiological and physical quality of unexpired and expired pediatric syrup was carried out in the medical microbiology laboratory using different categories of syrup samples. A total of twenty samples where used in this study, ten syrup in each case, with each syrup having the expired and unexpired form. Different culture media were used for microbial cultivation, The microbiological and physical changes in syrups were observed and assessed..The unexpired syrup sample were found to maintain their original taste, color and viscosity, while having the lowest load of contamination ranging from (Log_{10} 1.53cfu/ml – Log_{10} 1.71cfu/ml) for bacteria and (Log_{10} 0.68cfu/ml – Log_{10} 1.70cfu/ml) for fungi. While expired syrup sample showed changes in taste, color and viscosity ,with the highest load of contamination ranging from (Log_{10} 2.25cfu/ml – Log_{10} 1.79cfu/ml) for bacteria and (Log_{10} 0.9cfu/ml – Log_{10} 1.96cfu/ml) for fungi. Microbial analysis on the unexpired syrups revealed the presence of *Aspergillus species* (*3.8%*) *Candida albican*(*1.7%*),*Escherichia coli* (5.4%). While Analysis on the expired syrups revealed the increase in the presence of *Staphylococcus species* (18.0%), *Escherichia coli* (14.0%), while fungal isolates were *Aspergillus* species (8.3%), *Rhizopus* species (0.2%), *Candida albican* (3.6%)

INTRODUCTION

Pediatric unit is a medical specialty in hospital concerned with the health, growth and development of infants and children, as well as opportunity to achieve full adult potential. (Behrman *et al.,*2000).Child development is a gradual process that takes different stages, thus a lot of considerations are taken during drug development, administration and metabolism. Considering the delicate body structures, oral dosage administrating is preferred in children especially the use of syrups, this is because liquids generally have a faster rate of absorption than tablets (strickley *et al*., 2008).

Drug formation has an important role to play especially in infants and young children who cannot swallow tablets or capsules. Also they require doses based on body weight, and fixed doses in tablets or capsules intended for adults cannot be given to children or infants. (Griessmann *et al.,*2007).

In formulating syrups, certain factors are assessed, body weight of the patient to the dose , the ability of the drug to undergo chemical reactions (making it unstable), effect of pH and temperature on prolonged storage of syrups amongst other factors (Nahatta and Hipple;1997).

Microbiological effect on the syrup is an important factor in syrup formulation and production because any effect left unchecked would in one way or the other affect the quality of the syrup and the final consumer health may be affected complications.(Nahatta and Hipple;1997).

Microorganism because of their ubiquity form an integral part of our environment, and also reside on the skin and gastrointestinal tract (GIT) of man and their presence in most products has been reported (Kallings*etal*,1996; Ogbulie*et al*,2000). Pharmaceutical products of various dosage forms are susceptible to various contamination by a variety of microorganisms during manufacture and usage (Kallings *et al*,1996; Ogbulie *et al*,2000). The hazard of using contaminated products are due principally to the effect of microorganisms or their harmful by-products on human health. This can be very deleterious, particularly on critically ill, traumatized or convalescing patient. Spoilt pharmaceutical products constitute wastage and may have very serious economic implication for the manufacturer (Mendie *et al,* 1993). Orally administered drugs often contain non-pathogenic microorganisms. The products (syrups) are contaminated by aerobic spore bearers and fungi. The Gram positive bacteria are *Staphylococcus aureus* and *Micrococcus* species, while *Klesiella* species ,*Escherichia coli* and *Pseudomonals* species constitute the Gram negative rods. Controls of pharmaceutical products are largely a matter of identity of hazards and utilizing good manufacturing practice (GMP). (Russel *et al,*1992).

Antimicrobial preservatives are usually included in pharmaceutical products to discourage microbial contaminants, though, excessive use of preservatives like sorbitol causes diarrhea, while propylene glycol cause hyper-osmolarity (Glasgow *et al,*1983). Over the years, microbiological quality control has assumed a significance in the formulation of pharmaceutical products. The nature and extent of contamination in pharmaceutical products was first fully appreciated by (Kalling*et al* 1966).and has since then been reported to cause more problem for patients especially in the developing economy where dispensing drugs are done under a contamination prone environment. Several research works has been carried out on possible microbial contamination of syrups which can be as a result of increase in number of pharmaceutical products, poor storage and dispensing system and packaging as well as microbiological impurity of starting materials. Apart from all these, syrups themselves are highly nutritive and will readily become contaminated with adventitious microorganisms.

This study was therefore designed to investigate some pediatric syrups with a view to ascertaining the difference between the physical and microbiological qualities of unexpired and expired syrups.

MATERIALS AND METHODS

Samples for this study were purchased from Ekeonuwa market and retail pharmaceutical outlets at Douglas and Wetheral roads in Owerri, Imo State. Twenty samples of syrup were used, which consist of different brands including, Kp multivitamin, Emzorparacetamol, Petaxparacetamol, Hb2 Heamoglobin syrup, Tutolincought, Chloroquine syrup, Septrineclotromazole , Flagyl Metromazole syrup, Chloramphenicol syrup, and Emzor ampicillin syrup.

Reagents:

Lacto-phenol cotton blue,Gram's alcohol, crystal violet, carbolfuschin, in-dole reagent, kovac's reagent, fresh human serum plasma.1% peptone water.

METHODS

Preparation and sterilization of materials

Media were prepared according to the manufacturer's instruction. Glass wares (petri dishes, conical flask, test tubes, beakers e.t.c)

were sterilized in hot air oven under pressure, in an autoclave at 121^{o}C for 15 minutes (Ogbulie *et al.,*2001) while tops of benches and test tubes racks were disinfected with 98% ethanol.

Sample Processing

A ten- fold dilution of the syrup was prepared by transferring 1ml of it to a test tube containing 9ml of normal saline with the help of sterile pipette.0.1ml of the appropriate dilutions were inoculated into corresponding petri dishes containing the already solidified and aseptically prepared media.

Cultural methods

Using a sterile L shaped glass rod the inoculums was spread uniformly over the surface of each agar medium. These were then incubated at 37^{o}C for 18-24 hours for Nutrient Agar, MacConkey Agar, and at ambient temperature for 2-3 days for Sabouraud Dextrose Agar. At the end of incubation period typical colonies of microorganisms where observed

Isolation and identification of isolates

Isolation

This was based on colonial appearance or morphology. They were purified and subculture into a slant for identification.

Identification of Bacteria

All isolates on Nutrient Agar (NA) were sub-cultured onto fresh NA to obtain pure culture which were later inoculated on Nutrient Agar slants on McCartney bottles to prepare stock culture which were preserved under refrigerator temperature of about 4^{o}C for Gramm's staining , and Biochemical tests. All isolates where Gram stained and appropriate biochemical done for proper identification

Identification of fungi

All isolates from sabouraud Dextrose Agar were Gram stained and their hyphae were teased on a sterile glass slide with a few drops of lacto phenol cotton blue, a cover slip was applied, without entrapping air bubbles and then examined under a compound microscope with the x10 and x40 objectives.

Gram staining procedure

Pure stoke cultures were sub-cultured on Nutrient Agar plates, to obtain 24 hour old culture for Gram's staining, and Biochemical tests.tin smears were made, allowed to dry and heat fixed by passing the slides over a Bunsen burner 2-3 times. The smear were flooded with crystal violet (a primary dye) for one minute. The stain was washed off quickly with clean distilled water, the water allowed to drain off, and the smear flooded with Gram's iodine and allowed to stand for one minute. The iodine was washed off with distilled water. The primary dye was decolorized with 95% ethyl alcohol for 30 seconds. This was quickly washed off with distilled water. The smear was covered with safranine for 30 seconds this was washed and examined under the microscope.

Biochemical test

Catalase Tests

This test was used to differentiate those organisms that produce the enzyme catalase from non-catalase producing ones. It is based on the principle of catalase, which acts as a catalyst in the breakdown of hydrogen peroxide to oxygen and water. In the procedure, 1 drop of 3% hydrogen peroxide was dropped on a slide. Using a glass rod, a growth of the test organism was mixed with the solution. It was observed for active bubbling which indicated a positive test or no release of bubbles that indicated a negative test.

Coagulase Test

This is used to identify organisms that produce the enzyme coagulase from those that do not. In the procedure, a drop of physiological saline was placed on a clean grease free slide. A colony of the isolate was taken using a clean glass rod and emulsified with the drop to make a thick suspension. A drop of plasma was added to the suspension and mixed gently .It was observed for clumping with 10 seconds for positive test or no clumping for negative test.

Indole Production

This is based on the ability of microorganisms to break down amino acid tryptophan releasing in-dole. In the procedure, 1% peptone water was inoculated with the unknown isolate. It was incubated at 37^{o}C for 24hour, 0.5ml of kovac's reagent was added. It was shaken and observed for appearance of red color which showed the presence of in-dole (positive test) or no change in change (negative test).

Physical test

Physical changes were observed in the samples and were based on changes in color, and viscosity, findings were noted.

RESULTS

Twenty brands of pediatric syrups were analysed in the laboratory for their microbiological and physical quality changes. The sample include 10 expired and 10 unexpired syrups, which are Hb2 Heamoglobin syrup (expired and unxpired), Tutolincought syrup (expired and unxpired), Chloroquine syrup (expired and unxpired), Kpmuitivatamin syrup (expired and unxpired), Septrineclotromazole syrup (expired and unexpired), FlagylMetromazole syrup(expired and unexpired), Emzorparacetamol (expired and unexpired), Petexparacetamol syrup(expired and unexpired), Chloramphenicol syrup(expired and unexpired), and Emzor ampicillin syrup(expired and unexpired).The microbiological identification and physical changes of the drugs are as shown below.

Table 1 Morphological and biochemical characteristics of bacterial isolates

Colonial morphology	**Creamy to yellow, opaque, round cocciin clusters and singles, gram positive rods.**
Gram stain	+
Indole test	-
Catalase test	+
Coagulase test	+
Organism	***Staphylococcus aureus***
Colonial morphology	**Creamy, mucoid, raised smooth colonies gram negative rods**
Gram stain	-
Indole test	+
Catalase test	-
Coagulase test	-
Organism	***Escherichia coli***
Colony morphology	**Large grey white colonies, gram negative rods**
Gram stain	-
Indole test	-
Catalase test	-
Coagulase test	-
Organism	***Klebsiella spp***.

Table 2Fungal identification

Isolate no	Macroscopic features	Microscopic features	Isolates identity
1	Light brownish colony and fluty or dusty with brown on reverse side	Presence of conidiospores and septate hyphae	*Rhizopus species*
2	Yellow surface at first andwooly-like which will turns to brownish black with radia line.	Presence of condiospore with branching non-septate hyphae	*Aspergillus species*
3	White spotted tiny roundcolonies all over the surface with white reverse side.	Presence of budding andpseudohyphae which is septate. presence of germ tube	*Yeast cell*
4	Wooly-like at first and fluty Or dusty which will turn to Greenish surface	Septate hyphae	*Fusiform*

Table 3: Bacterial isolates identified in unexpired drugs

Syrup	Bacteria
Hb12 Heamoglobin syrup	*Staphylocuccus and E.coli*
Tutolin cough syrup	*Staphylococcus and E.coli*
Kp multivitamin syrup	*Escherichia coli*
Septrineclotromazole syrup	*Staphylococcus and E.coli*
Flagymetrommdazole	*Staphylococcus and E.coli*
Emzorparacetamol syrup	*Staphylococcus species*
Petaxparacetammol syrup	*Staphylococcus and E.coli*
Chloramphenicol syrup	*Staphylococcus and E.coli*
Emzor ampicillin syrup	*Staphylococcus species*
Chloroquine syrup	*Staphylococcus and E.coli*

Table 3b: Fungal isolates identified in unexpired syrup

syrup	Fungal isolates
Hb12 Heamoglobin syrup	*Aspergillus* and *Candida albican*
Tutolin cough syrup	*Candida albican*
Kp multivitamin syrup	*Aspergillus, Candida albican,Fusiform*
Septrineclotromazole syrup	*Aspergillus, Candida albican,Fusiform*
Emzorparacetamol syrup	*Aspergillus, Candida albican*
Petaxparacetammol syrup	*Aspergillus, Candida albican*
Chloramphenicol syrup	*Candida albican*
Emzor ampicillin syrup	*Aspergillus, Candida albican*
Chloroquine syrup	*Candida albican*
Flagymetrommdazol syrup	*Candida albican, Fusiform*

Table 4a:Bacterial isolates identified in expired drugs

syrup	Bacteria isolate
Hb12 Heamoglobin syrup	*Staphylocuccus and E.coli*
Tutolin cough syrup	*Staphylocuccus and E.coli*
Kp multivitamin syrup	*Staphylococcus species*
Septrineclotromazole syrup	*Staphylocuccus and E.coli*
Flagymetrommdazor	*Staphylocuccus and E.coli*
Emzorparacetamol syrup	*Staphylococcus* species
Petaxparacetammol syrup	*Staphylocuccus and E.coli*
Chloramphenicol syrup	*Staphylococcus* species
Emzor ampicillin syrup	*Staphylocuccus and E.coli*
Chloroquine syrup	*Staphylococcus,E.coli*
	Klebsiella species

Table 4b: Fungal isolates identified in expired syrup

syrup	Isolates
Hb12 Heamoglobin syrup	*Aspergillus and Candida albican*
Tutolin cough syrup	*Aspergillus species*
Kp multivitamin syrup	*Aspergillus species*
Septrineclotromazole syrup	*Aspergillus and Candida albican*
Flagymetrommdazol syrup	*Aspergillus and Candida albican*
Emzorparacetamol syrup	*Candida albican*
Petaxparacetammol syrup	*Aspergillus species*
Chloramphenicol syrup	*Candida albican*
Emzor ampicillin syrup	*Aspergillus,Candida albican*
Chloroquine syrup	*Aspergillus, Fusiform, Candida albican*

Table 5:Prevalence of organism in unexpired drugs

organism	Percentage occurrence
Staphylococcus species	0.3%
Escherichia coli	3.7%
Aspergillus species	1.3%
Candidiaalbican	14.6%

Table 6:Prevalence of organism in expired drugs

organism	Percentage occurrence
Staphylococcus species	4.6%
Escherichia coli	2.9%
Klebsiella species	0.4%
Aspergillus species	8.3%
Mucor species	0.2%
Candida albican	*3.6%*

Table 7: Physical quality changes in unexpired syrups

Sample	Color	Taste	Viscosity
Hb 12 haemoglobin	Black brown	Sweet	Low
Tutolin cough syrup	Orange	Sweet	Sticky and Low
Kpmuitivitamin	Light yellow	Sweet	High
Septrineclotrommdazole	Pink	Sweet	Low
Flagymetrodazole	Light yellow	Sweet	High
Emzorparacetamol	Light pink	Sweet	Low
Petaxparacetammol	Light pink	Sweet	Low
Chloramphenicol	White	Bland	Sticky and low
Emzor ampicillin	White	Bland	Low
Chloroquine syrup	Light pink	Bitter	High

Table 8: physical quality changes in expired syrup

Sample	color	Taste	Viscosity
Hb12 Haemoglobin syrup	Black -brown	Bitter sweet	High
Tutolin cough syrup	Dark-orange	Bitter	Low
Kp multivitamin	Brownish	Bitter	Low and sticky
Septrineclotrommdazole	Pink	Bitter	Low
Flagymetrodazole	Brownish	Bitter	High
Emzorparacetamol	Light yellow	Bitter	Low
Petaxparacetammol	Brownish	Bitter	Low
Chloramphenicol	White	Bland	Sticky and low
Emzor ampicillin	White	Bland	Low

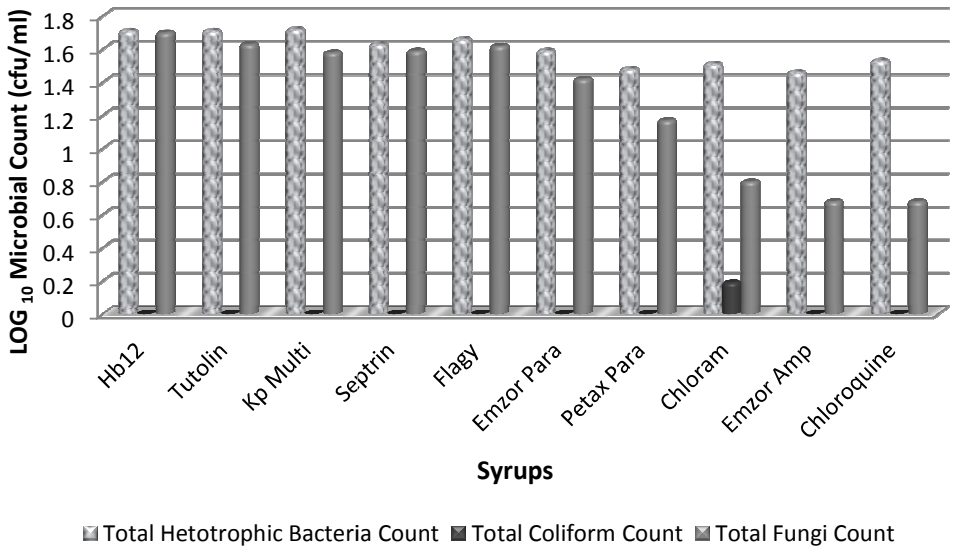

Figure 1 : GRAPHICAL REPRESENTATION ON MICROBIAL LOAD OF UNEXPIRED SYRUPS

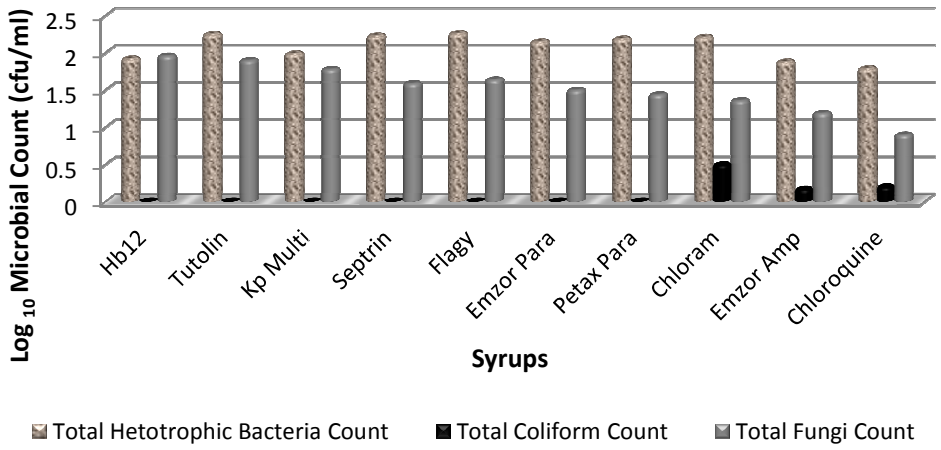

Figure 2 : Graphical Representation On Microbial Load Of Expired Syrups

DISCUSSION

Microorganisms are ubiquitous; and are found in food, beverages and pharmaceuticals (Ogbulie *et al.,* 1998; Nahata, 1999; Prescot *et al.,* 2008) pharmaceutical products become less effective when contaminated with pathogenic microorganism and hinder treatment of disease (Eka *et al.,* 1997; Okeke and Lamikanra 2001).

Organisms found in some of these syrup are principally due to poor aseptic methods in the production processes as well as post production contamination thus, the effect of these microorganisms or their harmful by product on human may be dangerous his health (Mendie *et al.,*1993). This may also have some serious economic implication for the manufacturer as it indicates poor manufacturing practice, unsanitary filling corking method and post production contamination (Mendie*et al*.,1993).

This research study carried out on the expired drugs (syrups) revealed the presence of both bacterial and fungal isolates which include, *Echerichia coli, Staphylococcus specie, Aspergillus species, Klebsiella species and Candidia spcies.* While unexpired revealed *Staphylococcus species, Escherichia coli, Fusiform species, Aspergillus species and Candidia species*. This is supported by the work of the work of (Hugo and Russel, 1997; Ogbulie *et al.* (2009),which he reported the presence of fungi and bacteria contaminants which include *Penicillium, Rhizopus, Aspergillus, Mucor, Fusiform species, Staphylococcus auerus, Micrococcus species, Klebsiella species, Escherichia coli, Pseudomonas aeroginosa*

The unexpired drugs showed the present of bacterial contamination, which reveals poor manufacturing practice.(Russel*et al.,*1992) while the presence of fungal isolate may be due to the excessive use of preservative (Glasgow *et al.,1983*). A similar result was also obtained by (Takon and Eyong, 2018) which reported the presence of fungi and bacteria isolates as contaminants of unexpired drug samples including *Pseudomonas aeroginosa Echerichia coli, Staphylococcus specie, clostridium spp and Candidia species*.

Conclusion and Recommendation

Expired and unexpired pediatric syrups showed varying level of microbial contamination. While contamination of unexpired drugs were associated with poor drug production process that of expired pediatric syrup was due to life span of the drug itself, hence the high level of contamination. Consumers should be enlightened on the danger of consuming expired syrups.

Proper handling of unexpired syrups and storage should be put into considerations and repackaging of the syrups purchased in bulk into smaller container which are not sterilized should be avoided. Pharmaceutical industry should improve on the quality of syrups they produce and good drug quality should be met before being inspected and released into the market for consumption. They should really check most of the manufacturing processes of syrups because they are used for children and should attain the hazard analysis critical control point (HACCP) of a good manufacturing industry. Finally producers should be subjected to routine supervision by agencies such as Nigerian Drug Law Enforcement Agency of Food and Drug Administration and Control (NAFDAC), so as to maintain easy good drug standard. Poor manufacturing practice, unsanitary filling and corking method are possible routes of contamination. These contaminants may cause serious gastrointestinal tract disorders in patients. Therefore, consumers should be enlightened on the danger of consuming expired syrups, while manufactures should improve on their quality control methods to meet the expected drug quality standards

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