Microbiological Quality of Paediatric Oral Liquid Drug Formulations during Consumption

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Abstract- This preliminary study was conducted to evaluate the microbiological quality of paediatric oral liquid drug formulations during consumption. Paracetamol, Salbutamol, Cephalexin, Amoxicillin and Lactulose are few oral liquid medicines prescribed in Sri Lanka. These oral liquid medicines are at a greater risk of microbial contamination during consumption. This can lead to co-infection in paediatric patients. Therefore, it is important to evaluate microbial quality of oral liquid medicines during consumption. In methodology, organoleptic examination, pH determination and assessment of microbiological quality of two bottles from each five commercially available samples were done. Selective media were employed for quantification and isolation of the microbial contaminants. Quantification of viable microbial colonies was expressed as colony forming units per mililitre (cfu/ml). These procedures were conducted in duplicates at each consumption day and cfu/ml was expressed as a mean. All liquid drug formulations had <1000 cfu/ml bacteria during the consumption period except Lactulose, which had 12733 cfu/ml bacteria during the last consumption day. In conclusion, this study has revealed that the good microbial quality of paediatric oral liquid drug formulations should be maintained during consumption period.

Index Terms- paediatric, liquid drug formulations, microbial quality, consumption

I. INTRODUCTION

Paediatrics is the branch of medicine that deals with the medical care of infants and children. The gastrointestinal tract of a normal fetus is sterile. During birth and rapidly thereafter, bacteria from the mother and the surrounding environment colonize the infant's gut. Immediately after the vaginal delivery, the microorganisms of the upper gastrointestinal tract of the babies may have acquired from the mothers' feces (Walker *et al*, 2004). Environmental, oral and cutaneous bacteria are readily transferred from the mother to the infant through suckling, kissing, and caressing. After the introduction of solid food and weaning, the microflora of breast-fed infants becomes similar to that of formula-fed infants.

Orally administered aqueous solutions, suspensions, emulsions, syrups etc. are among the preparations that are at the greatest risk of microbial contamination during consumption. These foods as well as paediatric oral liquid drug formulations may introduce pathogenic microorganisms to infants. Further, these pathogenic organisms may be highly detrimental for immuno-compromised infants. Therefore, microbiological quality of such oral liquid medicines is a very important factor for the above mentioned patients.

According to United States Pharmacopoeial standards, microbial contamination of the raw material should be not more than 10^3 viable micro-organisms per gram or per milliliter; total viable aerobic bacteria count not more than 10^4, fungi not more than 10^2 per gram or per milliliter, enterobacteria and certain other gram-negative bacteria not more than 10^2 per gram or per milliliter. Also these preparations should show absence of *Salmonella* (10 g or 10 ml), *Escherichia coli* (1 g or 1 ml) and *Staphylococcus aureus* (1 g or 1 ml) (USP-NF, 2008).

Many factors can increase microbial contamination during consumption includes improper storage conditions, unhygienic handling of the product, not following aseptic procedures when opening of the bottles and reconstituting. Air, water, reconstituting equipments, reconstituting personnel and the consumer can be taken as the major sources of microbial contamination of oral liquid drug formulations. There have been an increasing number of reports of infections due to above mentioned reasons (Adeshina *et al*., 2009).

Children might well not appreciate a medicine with an unpleasant taste. And it is not just about taste; unusual flavors, texture, mouth feel, color and smell; all add to the complex problem of giving medicines to children. Also with paediatrics there is a need often to provide for variation in dose and for swallowability. Both these are advanced by the use of liquid formulations (Morgan, 2009). Most commonly used oral liquid drug formulations for the paediatrics are aqueous solutions, suspensions, emulsions, syrups etc. They can be easily administered to paediatrics and allow dosage flexibility. Such oral liquid medicines must contain excepients suitable for paediatric use and ensure physical, chemical and microbiological stability (Salgado *et al*., 2005).

Therefore **objectives** of the present preliminary study was designed to evaluate the microbial quality (quantitative and qualitative identification of Staphylococci, Streptococci, *E. coli*) of Paracetamol (oral syrup), Salbutamol (oral syrup), Cephalexin (oral aqueous suspension / dry syrup), Amoxicillin (oral suspension), Lactulose (oral syrup) during consumption and changes of their organoleptic properties (pH/colour/odor) during the same period.

II. METHODS AND MATERIALS

A. Oral liquid solutions selected for the study

Three bottles (triplicate) of the each from the following oral liquid medicines were taken (Paracetamol, Salbutamol,

Cephalexin, Amoxicillin and Lactulose).One bottle from the each was taken as the control and other two were taken as the test samples. Sample bottles of the infant oral liquid medicines were opened aseptically, according to their dose and frequency (Salgado *et al*., 2005). In this study, dose regimen of infants was considered (Table 01) (BNF:Paediatrics, 2005).

Table 01 -The dose frequencies, dose and the consumption period for each oral liquid medicine

Oral liquid medicine	Consumption period	Dose			Dose frequency		
		Infants	Children 1–5 yrs	Children 6–14 yrs	Infants	Children 1–5 yrs	Children 6–14 yrs
Paracetamol	3 days	60-120 mg	250 mg	500 mg	8 hrly	6 hrly	6 hrly
Salbutamol	3 days	100 mcg	1 mg	2 mg	6 hrly	6 hrly	6 hrly
Cephalexin	7 days	125 mg	125 mg	500 mg	bd	tds	Tds
Amoxicillin	5 days	62.5 mg	125 mg	250 mg	8 hrly	8 hrly	8 hrly
Lactulose	3 days	2.5 ml	5 ml	15 ml	bd	bd	Bd

B. Enumeration of microbial contaminants in paediatric solutions

The following selective and non selective culture media were employed for quantification and identification of the microbial contaminants: MacConkey Agar (Oxoid CM0007), Blood Agar (Oxoid CM0055), Sabouraud Dextrose Agar (Oxoid COM0041).

A dilution gradient (10^{-1} to 10^{-5}) of the original paediatric solutions was prepared in normal saline (0.9% NaCl) and 100 µl of each diluents and that of the original sample was cultured using spread plate method on above mentioned media in duplicates. Thereafter, the plates were incubated for 24 – 72 hours at 37 ^{0}C. Colonies were counted and number of viable cells in original sample and each diluent was expressed as colony forming units per mililitre (cfu/ml). Colony forming units per mililitre was calculated by using the following equation.

CFU/mL =CFU/plate x dilution factor x 1/aliquot

C. Identificaion of microbial contaminants in paediatric solutions

Solutions were analysed for the presence and absence of *S. aureus*, *Strepococcus*sp and *E. coli* and they were identified by using their Culture characteristics, morphological features by the Gram stain, catalase test, oxidase test and coagulase test.

D. Examination of organoleptic properties

Label information such as manufactured date, expiry date and batch number of the packaging were checked. The pH of the samples was determined by using a pH meter during consumption period and colour, odour, taste of the samples were tested by using the sense of organs.

III. Results

A. Results in summary

Microbial growth was higher on blood agar medium than on Mackonkey agar medium. Significant microbial growth observed in Lactulose on the third consumption day. No growth observed in Salbutamol during the particular consumption period. Minute microbial growth observed in Paracetamol, Amoxicillin and Cephalexin during the consumption period. The microbial growth of Paracetamol was high in blood agar medium on the third consumption day comparing to Amoxicillin and Cephalexin.

E.coli and *streptococci* species observed in Paracetamol, Amoxicillin, Cephalexin and Lactulose except Salbutamol during the particular consumption period.

The results of microbiological quality assessment of samples are shown in table 02.

Table 02 - Microbial assessment during consumption period.

Drug	Consumption day	Mean Cfu/ mL in BA*	Mean Cfu/ mL in MA
Paracetamol syrup	1	120	0
	2	198	10
	3	925	60
	Control	0	0
Salbutamol syrup	1	0	0
	2	0	0
	3	0	0
	Control	0	0
Amoxicillin suspension	1	0	0
	3	0	0
	5	0	10
	Control	0	0

Cephalexin suspension	1	0	0
	3	0	10
	5	20	55
	Control	0	0
Lactulose syrup	1	850	10
	2	1322	55
	3	12733	55
	Control	145	0

*MA- Mackonkey Agar, BA- Blood Agar

The pH variations observed in Paracetamol, Amoxicillin, Cephalexin and Lactulose except Salbutamol during the particular consumption period. Slight colour change observed only in Lactulose during the second and the third consumption days.

Paracetamol showed a pH change of 0.06 compared to the control. The pH reduction of Amoxicillin suspension was 0.13 and pH reduction of Cephalexin suspension was 0.14 in the particular consumption period. The pH change of lactulose was 0.11 during the particular consumption period.

The results of organoleptic examination and pH determination during the particular consumption period are shown in Table 03.

Table 03 - Organoleptic examination and pH determination

Drug	Consumption period (Day)	Color	Odour	Taste	pH(mean)
Paracetamol syrup	1	Red	Strawberry	Sweet	4.35
	2	Red	Strawberry	Sweet	4.33
	3	Red	Strawberry	Sweet	4.30
	Control	Red	Strawberry	Sweet	4.36
Salbutamol syrup	1	Colorless	Soda smell	Pungent	3.18
	2	Colorless	Soda smell	Pungent	3.18
	3	Colorless	Soda smell	Pungent	3.18
	Control	Colorless	Soda smell	Pungent	3.18
Amoxicillin suspension	1	Yellowish green	Fruity smell	Sweet	4.60
	3	Yellowish green	Fruity smell	Sweet	4.55
	5	Yellowish green	Fruity smell	Sweet	4.49
	Control	Yellowish green	Fruity smell	Sweet	4.62
Cephalexin suspension	1	Orange	Fruity smell	Sweet	5.25
	3	Orange	Fruity smell	Sweet	5.20
	5	Orange	Fruity smell	Sweet	5.17
	Control	Orange	Fruity smell	Sweet	5.27
Lactulose syrup	1	Yellow	No	Sweet	3.68
	2	Yellow	No	Sweet	3.67
	3	Dark yellow	No	Sweet	3.57
	Control	Light yellow	No	Sweet	3.70

IV. Discussion

The present preliminary study to evaluate the microbiological quality of paediatric oral liquid drug formulations during consumption is an indicator to identify the potential co-infections of paediatric patients. Syrups, suspensions, emulsions and solutions can be taken as the most common oral drug formulations that are used by paediatric patients. Children, neonates and infants differ from adults in their response to drugs. Therefore special care is needed in the consumption period of a paediatric drug to reduce secondary infections because the immune system of paediatric patients is immature. For this study three commercially available paediatric syrups and two commercially available suspensions were used.

This preliminary study has identified drug formulations which had high sweet taste are at a greater risk of microbial contamination during consumption due to consecutive bottle opening for drug administration. In microbial assessment, different culture media has been employed to identify the growth of micro-organisms. MacConkey Agar is used to isolate and differentiate members of the *Enterobacteriaceae* based on the ability to ferment lactose. This is a selective and differential medium containing lactose, bile salt, neutral red, and crystal violet. Bile salt and crystal violet inhibit the growth of gram positive bacteria.

Blood Agar is a bacterial growth medium that can be used to distinguish normal from pathogenic bacteria based on the effect of bacterial hemolytic enzymes on red blood cells. Blood Agar is a culture medium consisting of blood (usually sheep's blood) and

nutrient agar, used in bacteriology to cultivate certain microorganisms, including *Staphylococcus epidermidis, Streptococcus pneumoniae,* and *Clostridium perfringens.* This medium is used for the isolation and cultivation of fastidious and slow growing, obligatory anaerobic bacteria from a variety of clinical and nonclinical materials. It also supports good growth of most aerobic, facultatively anaerobic and micro-aerophilic bacteria if incubated appropriately. Sabauraud's dextrose agar is a culture medium for fungi, containing peptone agar and glucose that has the pH adjusted to 5.6.

Considering the results of microbial assessment in Table-02 microbial contamination was observed in all other commercially available paediatric liquid medicines except Salbutamol. The growth of lactose fermenters and fastidious organisms were identified in Paracetamol syrup during the consumption period. This may be due to the sweetening agents included in the preparation, but the number of colony forming units per mililitre of bacteria is not significant according to the standards (viable bacteria $< 10^3$ and yeasts $< 10^2$). No microbial growth was identified in Salbutamol syrup. This is because of adequately added antimicrobial preservatives in the drug formulation.

For microbial assessment of suspensions, Amoxicillin and Cephalexin were selected. They are antibiotic suspensions catergorized under peniciilins and cephalasporins respectively. Amoxicillin is active against gram positive aerobes, gram positive anaerobes and gram negative aerobes. But Amoxicillin is susceptible for degradation by beta lactamases, therefore the spectrum of activity of this preparation does not include organisms which produce these enzymes, including resistant *staphylococci* and all strains of *Pseudomonas*, *Klebsiella*, and *Enterobacter*. Very minute numbers of microorganism colonies were identified in this reconstituted preparation because of its extended microbial spectrum. The less number of cfu/ml of bacteria may be due to reconstitutional contaminations. Lactose fermenters which were identified during the consumption may be resistant to Amoxicillin, thereby resistant organisms can go into body and lead to cause drug resistance in the future administration. This can be a health hazard to paediatric patients. Cephalexin belongs to the first generation of cephalasporins active against gram positive organisms and has a limited activity against gram negative organisms. Some strains of *Escherichia coli, Klebsiella species, Proteus mirabilis* are susceptible for this drug. Therefore the growth of microbes in the preparation can be resistant organisms. Consuming such preparation may lead to drug resistance and serious health hazards in the future. But the number of cfu/ml of bacteria was not significant according to the standards mentioned in USP.

Microbial contamination is very high in Lactulose syrup during the consumption period. Lactulose syrup contains lactulose with other sugars such as galactose and lactose is used in the treatment of constipation and hepatic encephalopathy. Lactulose is a disaccharide containing two sugar molecules bounded together, in here Fructose and Galactose. This is a solid substance, which is very soluble in water and has a sweet taste. It is very sticky and viscous. Sugar bases are favorable for microbial growth. One of the mechanisms of action of Lactulose is metabolizing lactulose by bacteria in colon. It can stimulate peristalsis and decrease stool transit time. There is no enzyme capable of hydrolyzing this disaccharide present in human gastro intestinal tissue. Following administration by mouth, lactulose pass essentially unchanged in to large intestine. If it has metabolized earlier due to microbial contamination during consumption period, the patient may not get the desired therapeutic effect from the drug.

Microbial growth in liquid preparation may cause foul odour, turbidity and appearance. In this study, no colour, odour and taste differences were detected during the particular consumption period of Paracetamol, Amoxicillin, Salbutamol, Cephalexin except Lactulose. Considering the results that were obtained during the particular consumption period, (Table-03) slight differences in colour and viscosity was detected in Lactulose. This may be due to microbial byproducts of microbial contaminants during the consumption period.

High titers of microbial contamination of oral liquid drug preparation may cause spoilage of final product and health hazards to paediatric patients. Byproducts of microbial contamination may cause a change in the pH of liquid drug preparations and reduce the chemical stability and solubility of the drug. According to USP standards pH of Paracetamol ranges in between 3.8 to 6.1. When considering the results of pH determination of Paracetamol syrup during consumption period, it shows pH change of 0.06 compared to the control. But that pH reduction does not affect the standards and the pH of Paracetamol syrup lies within the standard range in the particular consumption period. No pH reduction observed in Salbutamol with comparing to the control during the consumption period. This may be due to the added preservatives and flavors in the preparation.

Amoxicillin and Cephalexin are two beta lactam antibiotic suspensions which have to be reconstituted before use. The pH reduction of Amoxicillin suspension is 0.13 and pH reduction of Cephalexin suspension is 0.14 in the particular consumption period, but according to the USP standards pH of Amoxicillin ranges in between 3.5 to 6 and pH of Cephalexin ranges in between 3 to 6. With reference to that, pH change of Amoxicillin and Cephalexin during the consumption period is not significant. This pH reduction may be due to chemical degradation of the preparation. When these medicines are once reconstituted Amoxicillin and Cephalexin can be used only for 14 and 7 days respectively.

Lactulose is sticky, viscous syrup. The pH of Lactulose ranges between 2.5 to 6.5 according to the USP standards. The pH change is 0.11 during the particular consumption period. This can be due to microbial byproducts, but these pH values stand within the range of the standards and therefore pH change is negligible.

Liquid preparations are less portable and less stable. Therefore expiration dates tend to be shorter. Careful attention is required to assure that the pharmaceutical product will not allow a heavy microbial burden to develop on standing or under normal conditions of use once opened. Microbial contamination may be easily prevented by adding anti-microbial preservatives to the preparations. But high amount of preservatives can make toxicities in paediatric patients. Suitable preservatives should be added according to the standards. Also special care should be given when bottle opening in consumption and in drug reconstitution. Minimal opening time should minimize the microbial contamination during the consumption period. Finally

microbiological quality should be taken into account by caregivers when administering paediatric oral liquid drug formulations to their children.

V. Conclusion

This study has revealed that the microbial contamination is very high in Lactulose solution during the consumption period. *E.coli* and *streptococci* species were found in Paracetamol, Amoxicillin, Cephalexin and Lactulose except Salbutamol.

Addition to the above, this study has identified that good microbial quality of paediatric oral liquid drug formulations can be maintained by careful opening of the bottles during consumption period. However, the presence of sweetening agents makes the formulations susceptible for microbial contamination even when stored at particular conditions. Consecutive bottle opening, air, water, reconstituting equipments, reconstituting personnel and the consumers are the factors leading to microbial contamination. Therefore microbiological quality of oral liquid drug formulations is a crucial issue to be considered when paediatric patients consume them.

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