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ASSESSMENT OF MICROBIAL CONTAMINATION IN PEDIATRIC ORAL LIQUID FORMULATIONS MARKETED IN KATSINA STATE, NIGERIA

***Mukhtar, G.L.,[1] Mukhtar. M.D.[2] and Magashi, A. M.[2]**
[1]Department of Microbiology, Umaru Musa Yar'adua University, P.M.B 2218 Katsina
[2]Microbiology Department, Bayero University, P. M. B. 3011, Kano, Nigeria
*Corresponding author: mukhtar.gambo@umyu.edu.ng(08060839389).

ABSTRACT
Pharmaceutical oral liquid dosage forms are basically formulated as non-sterile products, however, must satisfy the appropriate microbiological purity criteria. Such pharmaceutical products are susceptible to contamination by a variety of microorganisms during manufacturing and use. In this study, sixty seven (67) samples of four (4) different pediatric oral liquid drugs (Ibuprofen, Albendazole, Metronidazole and cotrimoxazole suspensions) were evaluated for the presence of microbial contamination using standard procedures described in the official monograph of the British and United Stated pharmacopoeia. Microbial contamination was observed in 44 (65.7%) of the tested samples with 25 (37.3%) and 17 (25.4%) of the examined products having Total Aerobic Microbial Counts (TAMC) and Total Yeasts Moulds Counts (TYMC) greater than the USP and BP acceptable limit of 10^2 cfu/ml and 10^1 cfu/ml respectively. High level of microbial contamination was observed in samples of pediatric oral liquid formulations particularly in samples of Ibuprofen and metronidazole suspensions. Microbial contaminants recovered from the examined products include Bacillus sp., Staphylococcus aureus, Micrococcus sp., Pseudomonas aeruginosa, Enterobacter sp., Escherichia coli, Penicillium sp., Aspergillus sp., and Candida sp. Strict adherence to guidelines of the current good manufacturing practices and good hygienic practices during handling, sales and usage of the products is recommended.
Keywords: Microbial contamination, oral liquids, TAMC, TYMC and Acceptance criteria

INTRODUCTION

Pharmaceutical products of different forms and dosage are susceptible to contamination by a variety of microorganisms during manufacturing, storage, handling or use by the consumer (Itah *et al*., 2004; Oviasogie *et al*., 2015). Oral dosage forms such as aqueous solutions, suspensions, emulsions, syrups and tablets are among the preparations that are at the greatest risk of microbial contamination (Netish *et al.,* 2018). As a result of the lack of good manufacturing practices, liquid dosage forms being non-sterile are likely to contain different types of microbial species. Additionally, pharmaceutical products that contain moderate to high water activity, products containing sweeteners and products in multidose containers are more prone to microbial contamination (Atata *et al.,* 2007). Thus, moisture and a high amount of sugar in the oral liquid drugs in particular can support microbial growth (Khanom *et al.,* 2013). Netish *et al.* (2018) further emphasized that a high percentage level of sucrose contained in liquid dosage forms especially syrups, makes them more susceptible to microbial growth.

Several studies have reported cases of microbial contamination of liquid dosage forms. Moniruzzaman *et al.* (2012) reported microbial contamination in antacids and paracetamol samples from different drug stores of Dhaka, Bangladesh, with 75% of the antacid and 60% of the paracetamol samples showing microbial count exceeding the USP limit. In another study that investigated the microbial contamination of brands of multivitamin syrups marketed in Maiduguri metropolis, Borno State, Tukur *et al.* (2012) reported that one out of the seven different samples contains pathogenic organisms while the other six samples contain non-pathogenic organisms, but were below the limit count set by the microbiological quality of syrups. Similarly, a study by Adeshina *et al.* (2009) has shown that among some pediatric anti-malarial and cough preparations sold in retail outlets in Ilorin, Nigeria, 14 (70%) were heavily contaminated and exceeded the official tolerance limit (Greater than 10^2CFU/mL) of permissible microorganisms specified for syrups and suspensions.

Some of the preparations were contaminated with *Bacillus subtilis, Escherichia coli, Staphylococcus aureus,* and *Pseudomonas aeruginosa.*

Microbial contaminants when present in non-sterile preparations can result in several undesirable consequences that ranged from spoilage resulting to physical and chemical changes, risk of infection to the consumer as well as products recalls and business loss (Calistus *et al.*, 2011; Oviasogie *et al.,* 2015). Such changes in the physical and chemical characteristics of contaminated pharmaceuticals include breaking of emulsions, thinning of creams, and fermentation of syrups, appearance of turbidity or deposit. Likewise, alterations in the organoleptic properties of the drugs such as changes odour, colour, and taste, thus rendering them unacceptable to the patients (Moniruzzaman *et al.*, 2012).

Furthermore, microbial contaminants when present in non-sterile preparations can possibly reduce or even inactivate the therapeutic activity of the product (United States Pharmacopoeia, 2009). Use of such kind of products even when the level of contamination is low is also considered to be microbiologically unsafe because it may present potential health hazards to patients (Essam *et al.*, 2012). There have been several published reports describing clinical hazards that are attributable to microbiologically contaminated pharmaceuticals (Mugoyela and Mwambete, 2010).

Pharmaceutical formulations of Syrups and suspensions contain sweetening agents and other excipients that make them susceptible to microbial contamination even when stored at particular conditions (Sudeshika *et al.*, 2014). Increased attention is needed on the microbiological quality of liquid oral formulations because these represent the most common category of drugs that are administered to pediatric patients whose immune system is yet matured. This study therefore aimed at investigating the presence of microbial contamination in oral liquid drugs obtained from hospitals and patent medicine stores in Katsina State, Nigeria.

MATERIALS AND METHODS

Collection and processing of drug samples

A total of sixty seven (67) samples of four (4) different pediatric oral liquid drugs were obtained from the hospital, private pharmacies and patent medicine stores randomly selected within the state. The samples included liquid formulations of Ibuprofen, Albendazole, Metronidazole and cotrimoxazole suspensions.

Prior to opening of the samples, the outside surfaces of sample containers such head cap of the oral suspensions were wiped with 70% v/v ethanol as described by Shaqra *et al.,*(2014). Ten (10 ml) of the products to be examined were diluted in 90ml of Trypticase Soy Broth (TSB) and serially diluted to get ten-fold dilutions of 10^1-10^5.

Assessment of microbial contamination

Microbial Enumeration Tests: One (1ml) of the first three (10^1-10^3) dilutions of each test samples was inoculated onto plates of casein soya bean digest agar (CSBDA) and incubated at 37°C for 3–5 days, and Sabouraud-dextrose agar at 25 °C for 5–7 days. After the incubation period, colonies were counted and the results were expressed as colony forming units per milliliter (cfu/ ml) of the sample (United States Pharmacopeia, 2018).

Isolation and identification of bacterial contaminants

After the incubation period, colonies were sub cultured on the MacConkey agar, *Salmonella Shigella* agar, Mannitol salt agar and Nutrient agar plate to get pure culture for identification. Gram staining and microscopy, an array of biochemical tests which include catalase, coagulase, oxidase, indole, citrate utilization, Voges prauskeur test, methyl red, urease production, motility was carried out as described by Cheesbrough (2006).

Identification of fungal contaminants

Fungal growths from the SDA plate were identified using colonial morphology such as growth pattern, pigmentation and size of colonies. Further identification was achieved by lactophenol blue stain and microscopically (Davise, 2012).

RESULTS

The results of the assessment of the microbial contamination of the analyzed oral drug samples as depicted on Figure 1 revealed the presence of microbial contamination in 44 (65.7%) of the tested samples, of which 12 (27.3%) samples even though were contaminated, complied with the USP and BP acceptance criteria of non-sterile oral preparations while 32 samples were heavily contaminated above the acceptable limit. Microbial loads in the tested samples, as shown in Table 1, showed that 25 (37.3%) of the examined products had total aerobic microbial count greater than 10^2 cfu/ml. Ibuprofen suspension had the highest percentage of samples (45.0%) with total aerobic microbial count greater than 10^2 cfu/ml, followed by samples of metronidazole suspensions of which 41.2% had total aerobic microbial count greater than 10^2 cfu/ml.

On the other hand, the proportions of the tested samples having total yeast and mould count greater than 10^1 cfu/ml was 17 (25.4%). Similarly, samples of ibuprofen suspension had the highest percentage of samples (35.0%) with total yeast and mould count greater than 10^1 cfu/ml, followed by samples of metronidazole suspensions of which 29.4% had total aerobic microbial count greater than 10^1 cfu/ml.

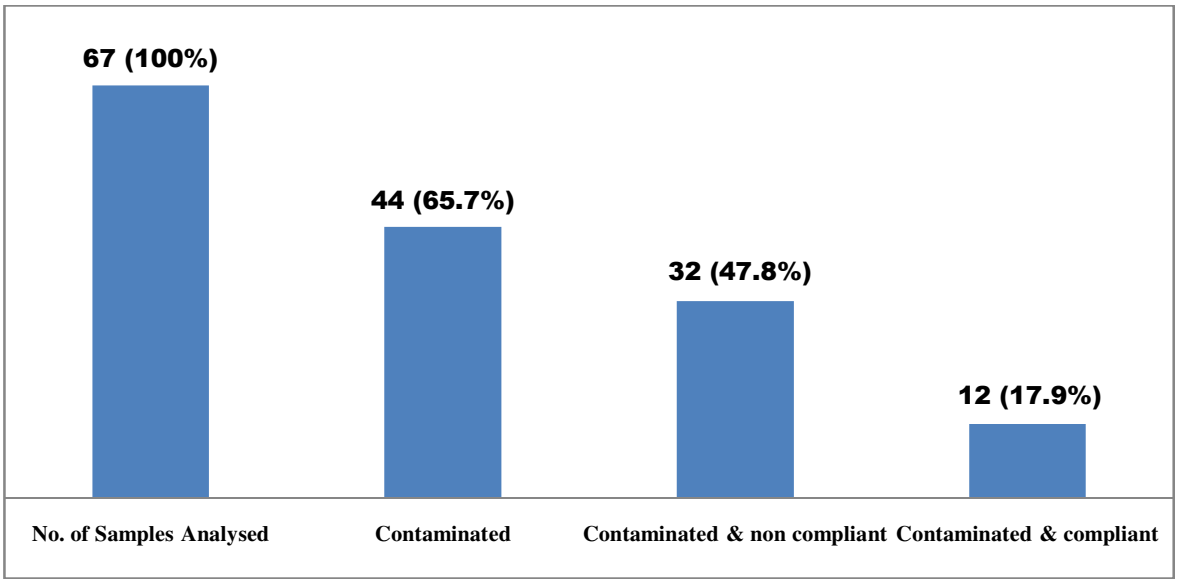

Figure 1: Percentage contamination of some pediatric oral liquid formulations sold in Katsina State, Nigeria.

Table 1: Microbial Load (cfu/mL) in the Tested Samples

Drug type	No. of Samples Tested	Total Aerobic Microbial Count > 10^2 cfu/ml		Total Yeast and Mould Count > 10^1 cfu/ml	
		N	%	N	%
Albendazole suspension	13	03	23.1	02	15.4
Ibuprofen suspension	20	09	45.0	07	35.0
Cotrimoxazole suspension	17	06	35.3	03	17.6
Metronidazole suspension	17	07	41.2	05	29.4
Total	**67**	**25**	**37.3**	**17**	**25.4**

N-number

Distribution of microbial contaminants from the tested products

The different oral liquid preparations were found to be contaminated with various microbial species of bacteria and fungi. In general, 63 microbial contaminants were successfully identified, of which 42 (66.7%) were bacteria and the remaining, 21 (33.3%) were fungi. Table 2 presents the distribution of the identified bacterial contaminants recovered from the tested preparations. The most frequently found bacterial contaminants were *Bacillus species*, 12 (28.6%) followed by *Staphylococcus* sp. Other bacterial contaminants recovered include *Micrococcus* sp., *Pseudomonas aeruginosa*, *Enterobacter* sp., *Escherichia coli* and *Klebsiella sp.* While table 3 present the percentage occurrence of the fungal isolates, *Candida* sp., were the predominant fungal contaminants, 09 (42.9%) followed by *Penicillium* sp. and *Aspergillus flavus.*

Table 2: Distribution of the bacterial contaminants recovered for the tested preparations

Bacterial isolate	Number	Percentage (%)
Bacillus sp.	12	28.6.0
Staphylococcus aureus	05	11.9
Other Staphylococcus sp.	07	16.7
Micrococcus sp.	03	7.1
Pseudomonas aeruginosa	04	9.5
Escherichia coli	02	4.8
Enterobacter sp.	04	9.5
Klebsiella sp.	03	7.1
Citrobacter sp.	02	4.8
Total	**42**	**100**

Table 3: Distribution of fungal contaminants recovered for the tested preparations

Fungal isolate	Number	Percentage (%)
Aspergillus flavus	04	19.0%
Aspergillus niger	03	14.3%
Penicillium sp.	05	23.8%
Candida sp.	09	42.9%
Total	**21**	**100%**

DISCUSSION

The occurrence of microbial contamination of 65.7% in the tested non-sterile pharmaceutical oral drug formulations in this study is comparable to the results of other studies: Calistus *et al*, (2011) reported microbial contamination in 62.5% and 55.5% of metronidazole and co-trimoxazole suspensions sourced from different locations in South-Eastern Nigeria.

The prevalence of microbial contamination observed in our study is much lower than the report of other studies. In related work, Khanom *et al.* (2013) evaluated the microbial contaminations of liquid oral drugs available in Bangladesh, comprising 26 syrups and 14 suspensions. It was reported that all the samples, except one syrup were found to be contaminated with bacteria and fungi. Similarly, in another study by Fatema, (2014) on the assessment of microbiological quality of the pediatric oral liquid drugs in Dhaka metropolis in Bangladesh reported that all the 20 (100%) samples were found to be highly contaminated with bacteria. Moreover, Daniyan and Sangodere (2011) reported that 72.2% of pediatric syrup preparations sold in patent medicine stores in Minna metropolis, Nigeria, were heavily contaminated with bacteria and fungi. On the other hand, lower incidence of microbial contamination has been reported, Tamalli *et al*. (2013) reported microbial contamination in 21.6% of the tested syrups and 26.7% in tablets commonly used at Alkhoms Market, Libya.

In this study, *Bacillus* species accounted for the majority of microbial contaminant in the tested samples. *Bacillus* species have been reported as the predominant contaminant of non-sterile pharmaceutical products by other studies conducted in different areas of the world such as; Adeshina *et al*. (2009) reported *Bacillus subtilis* as the most predominant contaminants in different brands of paediatric anti-malaria and cough preparations sold in Ilorin, Nigeria. Similarly, Mugoyela and Mwambete, (2010) in Tanzania reported that the majority of microbial contaminants isolated from non-sterile pharmaceuticals were *Bacillus* spp. In a related work from Benin, Nigeria, Akerele and Godwin (2002) reported the isolation of *Bacillus* species as the major contaminant of pharmaceutical products. their presence in a product suggests poor environmental hygiene during manufacturing, distribution and storage (Willey *et al*., 2008) or as heavily contaminated or adulterated raw materials (Mugoyela and Mwambete, 2010).

The implication of microbial contamination of non-sterile pharmaceutical products with *Bacillus* species is that even though most species are non-pathogenic, several studies have shown that their presence in non-sterile pharmaceutical products is undesirable because of their spoilage potential, it can reduce the potency and therapeutic activity of the products with their potential adverse effects to the patients taking such medicaments (Mugoyela and Mwambete, 2010; Adeola *et al*., 2012).

In this study, *Staphylococcus aureus,* other coagulase negative *Staphylococcal* sp. and *micrococcus* sp. accounted for about 28.0% of the bacterial species recovered from the tested dosage forms. Agbulu *et al,* (2016) reported the isolation of *Staphylococcus aureus* in addition to *Pseudomonas* sp. in cough syrups produced by different pharmaceutical companies sourced

from pharmacy shop in Benue State, Nigeria. In a related study in Bangladesh, Fatema (2014), reported *Staphylococcus* species as the most predominant microbial contaminant in paediatric oral liquid drugs. The presence of *Staphylococcus* species in non-sterile oral pharmaceutical products could be attributed to unhygienic handling of the products, or possibly contamination from the equipment and/or raw materials (Gad *et al.* 2011).

Pseudomonas aeruginosa was recovered from three samples of syrups and suspensions. These involved two samples Ibuprofen and one samples of Cotrimoxazole suspensions. Contamination of non-sterile pharmaceutical products with *Pseudomonas* species has been reported worldwide. Jimenez (2007) had reported *Pseudomonas aeruginosa* as the most frequently found contaminant in pharmaceutical products from all over the world. The presence of *Pseudomonas aeruginosa* in non-sterile pharmaceutical products possibly reflects contamination of the raw materials used as well as the conditions of the manufacturing environment in which the products are manufactured and packaged (Rania *et al.*, 2013).

The presence of *Escherichia coli*, *Enterobacter* species and other members of the enterobacteriaceae in some of the tested preparations is a good indicator of faecal contamination which may be principally from production personnel or from water supply (Adeshina *et al.,* 2009). In this study, one sample each of Cotrimoxazole and Metronidazole suspensions showed the presence of *Escherichia coli* and therefore failed the qualitative test for the absence of objectionable microorganism in non-sterile oral dosage forms. The presence of *Escherichia coli* is objectionable in oral dosage forms as clearly stated by the pharmacopoeias worldwide (BP, USP, European pharmacopoeia and the International pharmacopoeia) because its presence is an indication of pathogenic contamination of the products.

In this study, the identified fungal contaminants were mainly moulds which comprised of *Penicillium* sp.*, Aspergillus flavus* and *Aspergillus niger while Candida* sp. was the only identified yeast. Fungal contamination of pharmaceutical products is undesirable because they can produce metabolites/toxins that may be harmful to the consumers and additionally can cause deterioration of the products due to the biodegradation of the various components that constitute the formulation, and thus interfere with the therapeutic function of the product (Gad *et al.,* 2011).

CONCLUSION AND RECOMMENDATION

High level of microbial contamination was observed in samples of pediatric oral liquid formulations particularly in samples of Ibuprofen and metronidazole suspensions. Moreover, bacterial contamination was the most common among the tested samples.

Thus stringent quality control processes and compliance with the guidelines of the current good manufacturing practices during the manufacture, storage, distribution of these products is recommended. Also there should be good hygienic practices during handling, sales and usage of the products.

REFERENCES

Adeola, A.R., Opara M.I., and Adeleye, I.A. (2012). Microbial Quality of Some Non-sterile Pharmaceutical Products Sourced from Some Retail Pharmacies in Lagos, Nigeria. *African Journal of Microbiology Research,* Vol. 6(23), pp. 4903-4907. http://www.academicjournals.org/AJMR DOI: 10.5897/AJMR11.1158.

Adeshina, G. O., Ajayi, S., Onaolapo, J. A. (2009). Microbiological quality of some commercially available paediatric anti-malarial and cough preparations in Ilorin, Nigeria. *Nigerian Journal of Pharmaceutical Sciences,* Vol. 8, P. 109 – 117

Agbulu, C.O., Ameh, E.A and Ocharifu, S.A. (2016). Microbiological Quality of Cough Syrups and Herbal Solutions 'Agbo' Sold in Makurdi Metropolis of Benue State, Nigeria. *Journal of Microbiology and Microbial Technology*; 1 (1).

Akerele, J.O and Godwin, C.U. (2002). Aspects of microbial contamination of tablets dispensed in hospitals and community pharmacies in Benin City, Nigeria. *Tropical Journal of Pharmaceutical Research.* 1 (1): 23-28.

Atata, R.F, and Biyaosi, S.Y. (2016). Microbiological Quality of Some Selected Brands of Tablets and Syrups Produced in Nigeria. *The Pharmaceutical and Chemical Journal,* 3(3):191-196.

Calistus, D.N., Uduma, E.O., Ogechukwu, C.O., Christian, C.O.,and Christopher, E.N. (2011). Microbial and Physicochemical qualities of selected Co-trimoxazole and Metronidazole formulations in South Eastern. *Nigerian. Journal of Advanced Pharmacy Education & Research*, 2: 81-89. ISSN 2249-3379

Cheesbrough, M. (2006). District Laboratory Practice in Tropical Countries. Part 2; Second Edition. Cambridge University Press, New York, USA.

Daniyan S.Y and Sangodere T.A. (2011). Microbial Assessment of Some Syrups Sold in Patent Medicine Stores in Minna Metropolis, Nigeria. *International Research Journal of Pharmacy*, 2(8); 58-61.

Davise, H. Larone. (2002). Medically Important Fungi- A Guide to Identification. ASM Press, American Society for Microbiology, Washington DC. 4th Edition, pg 351-378.

Essam, A. Makky., Mohamed M. Ibrahim., and Mamdoh S. El-Gamal (2012). Survey of Hospital Drains Antibiotic ResistantBacteria for Hygiene and Health Care. International Conference on Chemical, Biological and Medical Sciences (ICCBMS'2012) August 25-26, 2012 Kuala Lumpur (Malaysia)

Fatema, K., Sowmitra, R.C, Tohora, S., Md. Mokhlesur, R., Niaz, M.K, Kamal, K.D, and Rashed, N. (2014). Assessment of microbiological quality of the pediatric oral liquid drugs. *Journal of Pharmacognosy and Phytochemistry.* 3(1): 165-171.

Gad, Gamal Fadl M, RehamAIbrahem Aly and Mohamed S El-din Ashour. (2011). Microbial Evaluation of Some Non-sterile Pharmaceutical Preparations Commonly Used in the Egyptian Market. *Tropical Journal of Pharmaceutical Research*; 10 (4): 437-445.

Itah A.Y., Udokpoh A.E., and Ofum M.U., (2004) Bacteriological quality of some pharmaceutical products marketed by drug vendors in Uyo, Nigeria. *African Journal of Health Sciences,* 11(3-4): 128-133.

Jimenez L., (2007). Microbial diversity in pharmaceutical product recalls and environments. *PDA Journal of Pharmaceutical Science and Technology,* 61 (5): 383-399.

Khanom S, Das KK, Banik S, Noor R (2013). Microbiological analysis of liquid oral drugs available in Bangladesh. *International Journal of Pharmacy and Pharmaceutical Science,* 5(4):479- 482.

Moniruzzaman, Mohammad, MD. Farukh Faisal Ashrafi and Zakaria MIA (2012). Qualitative and Quantitative Microbiological Studies of Antacid and Paracetamol Suspensions from Different Drugstores of Dhaka. *Dhaka University Journal of Biological Sciences,*21(1): 105- 107.

Mugoyela, V. and Mwambete, K.D (2010). Microbial contamination of non-sterile pharmaceuticals in public hospital settings. *Therapeutics and Clinical Risk Management,* 6 443–448, DOI: 10.2147/TCRM.S12253

Netish, K.K., Chand Sultana, Mohammad Sayful Islam, Md. Ashraf Ali, Marina Khatun, A. H. M. Mazbah Uddin, Pijush Kanti Roy, Rana Ahmed, Md. Mahabubur Rahaman, Md. Arafat Rahman (2018). Microbial and Physicochemical Evaluations of Paracetamol in Different Brands of Analgesic Syrups Sold in Bangladesh. *Journal of Innovations in Pharmaceutical and Biological Sciences*. 5(1), 81-85: 2349-2759

Oviasogie, F.E., Igbinosa, E.O., and Evbuomwan, R.O. (2015). Microbial assessment of some pharmaceutical products sold in Benin City: Public health implication. *African Journal of Pharmaceutical Research & Development,* 7(1); 45-49.

Shaqra, Qasem M Abu Shaqra, Maissa T Shawaqfahand Walid Al Momani (2014). Microbiological Quality of Blister Pack Tablets in Community Pharmacies in Jordan. *Tropical Journal of Pharmaceutical Research,* 13(2): 261-266.

Tamalli M., M.A.B. Gamal, Bioprabhu Sangar and Alghazal M.A. (2013). Microbial Evaluation of Some Non-Sterile Pharmaceutical Preparations Commonly Used at Alkhoms Market, Libya. *African Journal of Microbiology*, 1(4), 052-056.A

Tukur, M.A., Muazu, J., and Mohammed, G.T. (2012). Microbial analysis of brands of multivitamin syrups marketed in Maiduguri, Northeast Nigeria. *Advances in Applied Science Research,* 3(5):3124-3128.

United States Pharmacopoeia 32 National Formulary 27 (2009). United States Pharmacopoeial Convention Inc., Rockville, MD, pp 605.

United States Pharmacopoeia 41 National Formulary 36 (2018). United States Pharmacopoeial Convention Inc., Rockville, MD, pp 605.

Willey, J.M., Sherwood, L.M, Woolverton, C.J. (2008). *Prescott, Harley and Klein's Microbiology.* 7th ed. McGrawHill Company, New York, America. pp 578.

Sudeshika, S.H.T., Panagoda, G.J., and Tennakoon, I.U.K. (2014). Microbiological Quality of Paediatric Oral Liquid Drug Formulations during Consumption. *International Journal of Scientific and Research Publications,* Volume 4, Issue 9, ISSN 2250-3153