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ORIGINAL ARTICLE

Microbiological control of adapted liquid dosage forms in a pediatric hospital from Manaus

Controle microbiológico de formas farmacêuticas líquidas adaptadas em um hospital pediátrico de Manaus

Control microbiológico de formas farmacéuticas líquidas adaptadas en un hospital pediátrico de Manaus

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ABSTRACT

Background and Objectives: Ideal dosage forms for pediatric use are liquid because this population has difficulty swallowing. However, the pharmaceutical market does not have a large arsenal. To solve this situation, it is necessary to adapt drugs intended for the adult public in the form of oral solutions that allow the use of pediatric patients. Such practice changes the physicochemical and microbiological properties of these drugs. Most studies on pharmaceutical adaptations are directed to the physicochemical stability. Therefore, this study aimed to perform microbiological control of liquid pharmaceutical forms adapted in a pediatric hospital. **Methods:** Microbiological analysis was performed according to the specifications of the Brazilian Pharmacopoeia for non-sterile products. The total number of mesophilic microorganisms and the presence of pathogenic microorganisms were counted. **Results:** During the study period, 36 pharmaceutical adaptations were prepared in the hospital and then, after applying exclusion criteria, 16 samples were selected for microbiological analysis. The most common classes were diuretics, antihypertensives and psycholeptics. No preservatives were used in the preparation of the analyzed pharmaceutical adaptations. Half of the adaptations had a total number of mesophilic microorganisms above the allowed limit on the day of manipulation, 43.75% in the middle of the shelf life and 62.5% in the end of the shelf life. **Conclusion:** Only 03 (18.75%) adaptations were within the acceptable microbial limits established throughout the study. Regarding the presence of pathogens, all were free from the pathogens Escherichia coli, Salmonella sp., Pseudomonas aeruginosa and Staphylococcus aureus during the study period**.**

Keywords: *Brazilian Pharmacopeia. Pediatric Hospitals. Pharmaceutical Preparations.*

RESUMO

Justificativa e Objetivos: Formas farmacêuticas líquidas são ideais para pacientes pediátricos, uma vez que esta população apresenta dificuldades de deglutição. Entretanto, o mercado farmacêutico não dispõe de um grande arsenal terapêutico. Para contornar esta situação, é necessária a adaptação de medicamentos voltados ao público adulto para a forma de soluções orais, que permitem o emprego em pacientes pediátricos. Todavia, esta pode alterar as propriedades físico-químicas e microbiológicas destes fármacos. De acordo com a literatura, grande parte dos estudos com adaptações farmacêuticas são direcionados a estabilidade físico-química, e os parâmetros microbiológicos são menos avaliados. Portanto, o objetivo deste trabalho foi realizar o controle microbiológico das formas farmacêuticas líquidas adaptadas em um hospital pediátrico. **Métodos:** A análise microbiológica foi realizada conforme especificações da Farmacopeia Brasileira para produtos não estéreis. Realizou-se os testes de contagem do número total de microrganismos mesófilos e da presença de microrganismos patogênicos. **Resultados:** No período do estudo, 16 amostras foram selecionadas para análise microbiológica. Dentre os medicamentos as classes mais frequentes foram diuréticos, anti-hipertensivos e psicolépticos. Não foram utilizados conservantes no preparo das adaptações farmacêuticas analisadas. Metade das adaptações apresentaram contagem do número total de microrganismos mesófilos acima do limite permitido no dia da manipulação, 43,75% na metade do período de validade e 62,5% no prazo de validade. **Conclusão:** Apenas 03 (18,75%) adaptações estavam dentro dos limites microbianos aceitáveis estabelecidos durante todo o estudo. Com relação a presença de patógenos, todas foram isentas dos patógenos *Escherichia coli*, *Salmonella sp.*, *Pseudomonas aeruginosa* e *Staphylococcus aureus* no período do estudo.

Descritores: *Farmacopeia Brasileira. Hospitais Pediátricos. Preparações Farmacêuticas*.

RESUMEN

Justificación y Objetivos: Las formas farmacéuticas líquidas son ideales para pacientes pediátricos, ya que esta población presenta dificultades para deglutir. Sin embargo, el mercado farmacéutico no dispone de un amplío arsenal terapéutico. Para superar esta situación, es necesaria la adaptación de medicamentos destinados al público adulto a la forma de soluciones orales, que permitan su uso en pacientes pediátricos. No obstante, esto puede alterar las propiedades físico-químicas y microbiológicas de estos fármacos. De acuerdo con la literatura, gran parte de los estudios sobre adaptaciones farmacéuticas están dirigidos a la estabilidad físico-química, y los parámetros microbiológicos son menos evaluados. Por lo tanto, el objetivo de este estudio fue realizar el control microbiológico de las formas farmacéuticas líquidas adaptadas en un hospital pediátrico. **Métodos:** El análisis microbiológico se realizó conforme a las especificaciones de la Farmacopea Brasileña para productos no estériles. Se realizaron pruebas de recuentos del número total de microorganismos mesófilos y de la presencia de microorganismos patógenos. **Resultados:** Durante el período del estudio, se seleccionaron 16 muestras para el análisis microbiológico. Entre los medicamentos, las clases más frecuentes fueron: diuréticos, antihipertensivos y psicolépticos. No se utilizaron conservantes en la preparación de las adaptaciones farmacéuticas analizadas. La mitad de las adaptaciones presentaron un recuento del número total de microorganismos mesófilos por encima del límite permitido el día de la manipulación, el 43,75% en la mitad del período de validez y el 62,5% en el período de validez. **Conclusión:** Solo 03 (18,75%) adaptaciones estaban dentro de los límites microbianos aceptables establecidos a lo largo del estudio. En cuanto a la presencia de patógenos, todos extensas de los patógenos *Escherichia coli, Salmonella sp., Pseudomonas aeruginosa* y *Staphylococcus aureus* durante el período de estudio.

Palabras Clave: *Farmacopea Brasileña. Hospitales Pediátricos. Preparaciones Farmacéuticas.*

INTRODUCTION

In the case of active substances, liquid pharmaceutical forms are ideal for use in the pediatric population, as they are easily swallowed and allow greater control over the dose administered. Such adjustments are essential, since pediatric patients have pharmacokinetic and pharmacodynamic differences that vary rapidly throughout childhood, and are more exposed to drug-related adverse effects[1-4].

Although ideal, there is a notable absence of drugs in liquid pharmaceutical form in pediatric clinical practice, due to legal, ethical and economic issues, where this section of the population is generally not included in clinical trials for the development of new drugs, or due to the fact that some drugs have poor bioavailability or effectiveness when prepared in aqueous solution[5,6].

In this context, a common practice in many children's hospitals is the adaptation of pharmaceutical forms for administration to children, particularly inpatients. These adapted formulations can be obtained by different methods, from crushing a tablet or opening a capsule to use its contents. The resulting powder can be dissolved or suspended in various excipients to produce a liquid consistency medicine for oral use, or put back into smaller capsules or sachets[3,7].

The act of reformulating medicines into an oral extemporaneous preparation for prescription outside the approved indications (age range, dosage, presentation) or for use by a route of administration other than that originally developed is defined in science as unlicensed or *off-label* use[8].

Although the overall burden of inefficient and unsafe pharmacotherapy in children has never been established, the high use of *off-label* medicines is worrying[6]. The prevalence of prescriptions with adapted pediatric medicines is estimated at 3.2% to 95%; where, 26% to 95% in neonates, 2.7% to 51.2% in outpatients and 9.0% to 79.0% in inpatients. Developing countries are the most severely affected, as people aged between 0 and 18 make up a large proportion of the population and are the most vulnerable to disease[3].

It can be seen that adapted medicines are a solution not only for personalizing therapy in paediatric patients, but also for specific therapies when there are no commercial alternatives available. However, splitting the tablet, crushing and/or dissolving it to obtain a liquid pharmaceutical form offers different risks to the patient since there is not enough safety

information on drug interactions, stability or efficacy caused by potential changes in bioavailability[3,9].

Environmental factors can affect the physicochemical and microbiological stability of these drugs, such as temperature, light, humidity, radiation, air, particle size, solvents, pH and the presence of contamination or the intentional mixing of different products, as well as the hygiene and sterility of the place where they will be handled[10].

In the literature, most of the quality control work on pharmaceutical adaptations in hospitals focuses on analyzing the physicochemical stability of the formulations. However, it is important to emphasize the need to assess the load of 'non-compliant microorganisms' in these products, which can cause organoleptic changes and interfere in the drug's degradation process, leading to a reduction in its efficacy and safety. In addition, the high microbial load can cause serious infections in these populations[12].

Pharmaceutical professionals must consider microbial contamination and materials that pose a danger to patients, especially vulnerable groups such as pediatric patients. The addition of preservatives and good handling practice measures are crucial in controlling mold, inhibiting yeast growth and protecting against bacterial spread[13,14].

In view of this, the research justifies its raison d'être in the need to carry out microbiological control of these extemporaneous formulations in a hospital in order to contribute to improving the handling process, reducing the risk of contamination of patients or even accelerated degradation of the medicines produced and, finally, to generate sufficient data available on the microbiological control of these adaptations[11,15], since no studies on the subject were found in the city, although the use of adapted products has already been investigated in the city of Manaus[16,17].

Therefore, the aim of this study was to carry out microbiological control of adapted liquid pharmaceutical forms in a pediatric hospital, following the specifications of the Brazilian Pharmacopoeia for non-sterile products, using the most probable number for mesophilic microorganisms and testing for the pathogenic bacteria Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus and Salmonella sp.

METHODS

Study design - A prospective study was carried out on the microbiological control of liquid pharmaceutical forms adapted in the pediatric hospital pharmacy, with collections from September 2017 to May 2018.

Survey and selection of adapted pharmaceutical forms – Initially, a survey was carried out of all the formulations adapted in the hospital pharmacy during the study period, and the total

number of formulations produced was obtained. These formulations were then evaluated in terms of their composition and classified according to their pharmacological classes, determined using the ATC (Anatomical Therapeutic Chemical) methodology.[18] After this process, exclusion criteria were applied to select which formulations would be evaluated microbiologically, including: being an antimicrobial agent, not being for oral use, a shelf life of less than 7 days and production in insufficient quantities for the study's analysis.

Together with the formulations selected for microbiological analysis, an aliquot of the excipients simple syrups and carboxymethylcellulose used in the preparation of the pharmaceutical adaptations were inserted, both of which have a shelf life of 180 days and are prepared in the hospital pharmacy itself.

After selecting the medicines and excipients used in the liquid formulations, the samples obtained for analysis were grouped according to the shelf life established by the site's standard operating procedure: Group 1 (7 to 20 days), Group 2 (30 days), Group 3 (35-48 days), Group 4 (60 days), Group 5 (90 days) and Group 6 (180 days).

For the microbiological analysis, 03 units were requested from the same batch of each selected sample, which were collected at "time zero", i.e. on the day of handling. These were then transported in a refrigerated box to the laboratory and kept under refrigeration (4 °C) until the moment of analysis.

Microbiological analysis – Microbiological analyses were carried out immediately after preparing the formulations (time zero), halfway through the shelf life and when the shelf life was reached, using previously prepared culture media incubated in an oven at 32 ºC ± 2.5 ºC for 24 hours to assess sterility after preparation and sterilization in an autoclave. Tests were carried out for the total count of mesophilic microorganisms and for pathogens, in accordance with the criteria for non-sterile pharmaceutical products as specified in the Brazilian Pharmacopoeia 5th edition.[19]

Counting the total number of mesophilic microorganisms by most probable number

- Mesophiles were analyzed using the most probable number method, which consists of evaluating the growth of viable microorganisms within 5 days, in Casein-Soy broth (KASVI, São José dos Pinhais-PR) incubated in an oven at 32 ºC ± 2.5 ºC. The analysis was carried out by preparing dilutions, in triplicate, at a ratio of 1:10; 1:100 and 1:1000 of the formulations analyzed. Positive and negative tubes were recorded and compared according to the specifications in the Brazilian Pharmacopoeia, where the limit for total aerobic bacteria count

(UFC/g or mL) is 200 UFC/g or mL of product. The result obtained is expressed as Most Probable Number per gram or milliliter of product, MPN per g or MPN per mL of product.

Testing for *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* - The tests for *E. coli, P. aeruginosa and S. aureus* were initially carried out together, as the sample preparation and pre-incubation stages followed the same procedure. To prepare the sample, 1 mL of the formulation was inoculated into a tube containing 9 mL of Casein-Soy broth. The contents of this tube were transferred to a flask containing 90 mL of Casein-Soy broth (pre-incubation stage) and incubated for 18-24 hours at 32 ºC ± 2.5 ºC. After this period, the plates were sown on Cetrimide agar (HiMEDIA, Mumbai, Maharashtra, India) to test for Pseudomonas sp. and sown on Salted Mannitol agar (HiMEDIA, Mumbai, Maharashtra, India) to test for Staphylococcus sp. The plates were incubated in an oven for 18-24 hours at 32 ºC ± 2.5 ºC and colony growth was observed. For Escherichia coli, an aliquot of 1 mL of the pre-incubation medium was transferred to an erlenmeyer flask containing 100 mL of MacConkey broth (enrichment) (KASVI, São José dos Pinhais-PR), which was incubated for 18-24 hours at 43 ºC ± 1 ºC and then sown on a plate (subculture) containing MacConkey agar (HiMEDIA, Mumbai, Maharashtra, India), and incubated for 18-72 hours at 32 ºC ± 2.5 ºC.

The results obtained, following the limits determined by the Brazilian Pharmacopoeia, after the tests, should be negative, i.e. the absence of these pathogens, in the samples of the products analyzed.

Search for *Salmonella sp.* - For the sample preparation and pre-incubation period, 10 mL of sample was added to a flask containing 90 mL of Casein-Soy broth, which was then incubated for 18-24 hours at 32 ºC ± 2.5 ºC. After incubation, a 0.1 mL aliquot of the solution containing the sample was inoculated into a tube containing 10 mL of Selenite-Cystine broth (HiMEDIA, Mumbai, Maharashtra, India). It was incubated for 18-24 hours at 32 ºC ± 2.5 ºC. A loop of the Selenite-Cystine broth was removed using a bacteriological loop and sown on a plate containing Xylose Lysine Deoxycholate - XLD agar (Biokar Diagnost, Beauvais Cedex France), which was incubated for 18-72 hours at 32 ºC ± 2.5 ºC.

The results obtained, following the limits determined by the Brazilian Pharmacopoeia, after the tests, is the absence of this pathogen in the samples of the products analyzed.

Ethical aspects – This project was carried out without the need for approval by the Research Ethics Committee. However, a consent form was previously issued by the Health Unit's Board of Directors for the use of the formulations employed in the study.

RESULTS

Survey and selection of pharmaceutical adaptations– Data was collected from the register of formulations produced in 2017. During this period, 36 pharmaceutical adaptations were prepared. Half of them (50%) were antihypertensives, antibacterials, psycholeptics and diuretics, in the proportion of 11.1% each respectively, and antiepileptics (5.6%), according to the ATC (Anatomical Therapeutic Chemical) methodology[18]. The other half was distributed among other ATC classes. In view of this data, the exclusion criteria were applied, as shown in Table 1.

Table 1. Number of pharmaceutical adaptations excluded from the study after applying the exclusion criteria (N=22).

Exclusion criteria	Number of adaptations included (%)
Contains antimicrobial agent in its formulation	6 (16,6%)
Not for oral use	1 (3%)
Expiration date less than 7 days	0 (0%)
Insufficient quantity for study	15 (41,6%)

Among the exclusion criteria applied, 14 (41.6%) of the excluded adaptations did not meet the criterion of sufficient quantity for the study. As an example, we can mention the antiepileptic drug topiramate, which had its adaptation suspended because the tablets were out of date and would be taken for disposal. This was followed by the exclusion of 6 (16.6%) adaptations containing antimicrobial agents and 1 (3%) formulation because it was not administered orally. Adapted formulations with a shelf life of less than 7 days were not excluded.

As a result, 14 pharmaceutical adaptations were selected for the study, as shown in Table 2.

Table 2. Pharmaceutical adaptations selected for the study (N=14).

Group	Adapted drug	ATC classification	Expiration date
01	Clobazam	psycholeptic	15 days
01	Sildenafil	urological	20 days
02	Spiranolactone	diuretic	30 days
02	Losartan	antihypertensive renin angiotensin antagonist	30 days
02	Methadone	other nervous system drugs	30 days
03	Propranolol	beta-blocker	45 days
03	Captopril	antihypertensive renin angiotensin antagonist	45 days
03	Midazolam	psycholeptics	48 days

04	Dexamethasone	corticosteroid	60 days
04	Hydrochlorothiazide	diuretic	60 days
04	Methyldopa	antihypertensive	60 days
05	Furosemide	diuretic	90 days
05	Nifedipine	calcium channel blocker	90 days
06	Potassium Chloride 6%	mineral supplementation	180 days

The composition of the pharmaceutical adaptations was then analyzed (Table 3). It was found that simple syrup is used as the vehicle and carboxymethylcellulose (CMC) as the viscosifying/dispersing agent. These have a shelf life of 180 days and are prepared in the hospital pharmacy itself, being incorporated into the adapted formulations according to production..

Table 3. Excipients used in adapted formulations (N=14).

Excipient	*Function*	*Use in formulations (%)*
Distilled/purified water	Thinner	7 (50%)
Syrup	Viscosifier/dispersant	13 (92,8%)
Carboxymethylcellulose	Viscosifier/dispersant	5 (35,7%)
Citric acid	pH adjustment/preservative	6 (42,8%)

Table 3 shows that in half of the adaptations selected for the study, distilled water was added as a diluent for the drug in the syrup. The use of simple syrup in extemporaneous formulations is a positive factor both for the taste of pediatric patients, as it is able to mask the taste of some drugs, and to promote their greater stability, due to its hypertonic characteristic capable of inhibiting bacterial growth.[20]

In addition, citric acid is an input used to acidify the medium, which can provide stability to the drug or promote its dissolution in the formulation, especially for the adaptation of captopril, being able to act as a stability promoter, as described by. No preservatives or flavor adjuvants are used.

Microbiological analysis - The total count of mesophilic microorganisms, after analysis of the times proposed methodologically, obtained the results that can be seen in Table 4, considering the microbial limits for non-sterile products in the total count of fungi/yeasts of 20 UFC/g or mL and for the total count of aerobic bacteria the limit of 200 UFC/g or mL of product.[19]

Table 4. Total count of mesophilic microorganisms in Most Probable Number (MPN) per g or mL of product (N=16).

Drug/ Excipient	*Result mesophile count in MPN per g or mL of product*

	Zero time	*½ life*	*Expiration date*
Clobazam	27*	64*	>1100*
Sildenafil	15	11	>1100*
Spiranolactone	1100*	75*	43*
Losartan	20	6,1	>1100*
Methadone	95*	23*	160*
Propranolol	95*	38*	150*
Captopril	9,3	29*	11
Midazolam	19	16	11
Dexamethasone	28*	1100*	35*
Hydrochlorothiazide	290*	15	460*
Methyldopa	290*	>1100*	290*
Furosemide	-	-	>1100*
Nifedipine	15	-	6
Potassium Chloride 6%	6,1	6,1	3
Simple syrup	-	-	3
Carboxymethylcellulose	>1100*	14	-

*Above the permitted microbial limit, non-sterile products have a total fungal/yeast count of 20 CFU/g or mL and a total aerobic bacteria count of 200 CFU/g or mL. MPN= most probable number.

Based on the results shown in Table 4, together with the limits determined in the literature, of the 16 samples analyzed at time zero, 8 (50.0%) showed non-compliant results. Among these, clobazam, methadone, propranolol and dexamethasone did not comply with the limits stipulated for fungi/yeasts, while spironolactone, hydrochlorothiazide, methyldopa and carboxymethylcellulose did not meet the limits for aerobic bacteria.

In the half-life analysis of the formulations, 7 (43.75%) showed non-compliant results, with clobazam, spironolactone methadone, propranolol and captopril being outside the stipulated limits for fungi/yeasts and dexamethasone and methyldopa being outside the limits for aerobic bacteria.

In the analyses referring to the final deadline, 10 (62.5%) of the formulations did not comply with the established parameters. Spironolactone, methadone, propranolol and dexamethasone were found to be outside the permitted limit of CFU/g or mL of product and the samples of clobazam, sildenafil, hydrochlorothiazide, methyldopa and furosemide failed to meet the permitted limit for aerobic bacteria.

These data show that a large proportion of the formulations submitted for analysis, during the period of the deadline, do not comply with the microbiological limits, either in terms of aerobic bacteria or fungi/yeasts. Studies such as the one by Mugoyela (2010), show that the

main contaminants of these non-sterile formulations are aerobic bacteria such as *Bacillus spp* and fungi such as *Candida spp* and *Aspergillus spp*.

In the results obtained from the captopril and hydrochlorothiazide formulations, variations were observed between their approval and disapproval, according to the limits stipulated by the pharmacopoeia, during the study period. Possible reasons for this include handling errors, environmental contamination during analysis or even a decline in the cell viability of the microorganism during storage.[21]

The pharmaceutical adaptations that maintained acceptable microbial limits throughout the study period were midazolam, nifedipine and potassium chloride 6%, representing 18.75% of the adapted drug samples. When analyzing the Standard Operating Procedure (SOP) for these adaptations, we found that the adaptation was carried out using sterile medication (ampoules), except in the case of nifedipine, which was made using slow-release tablets.

All the pharmaceutical adaptations complied with the legal requirement for the absence of the following pathogens: *Escherichia coli, Salmonella sp., Pseudomonas aeruginosa* and *Staphylococcus aureus*.

DISCUSSION

During the survey and classification of formulations that could be adapted in the pharmacy sector of the hospital where the study took place, it was found that the main pharmacological classes subjected to this process during the study period were: antihypertensives, antibacterials, psycholytics and diuretics. Other studies, such as the one by García-López (2020), using the same ATC classification, show that the main classes of *off-label* drugs are those for the central nervous system, gastrointestinal tract and respiratory system. The prescription profile is different to that of the hospital under study, except for psycholytics, which are classified as drugs that act on the central nervous system.

With regard to the composition of the formulations, it can be seen that no preservatives were used, which can largely contribute to the microbiological growth observed in the results obtained from the mesophilic microorganism count, where 13 (81.25%) samples showed a total number of mesophilic microorganisms above the permitted limits for fungi/yeasts or aerobic bacteria.[17]

Microbial contamination above the permitted limits can easily compromise the health of pediatric patients, because in some cases the presence of microorganisms alone is not harmful to health, but the toxins they produce can trigger symptoms such as diarrhea, acute gastroenteritis, stomach pain or even, in severe cases, death. [23]

Only 3 (18.75%) formulations were found to be within acceptable microbial limits during analysis: midazolam, nifedipine and potassium chloride 6%. These results show the need for training and adequate infrastructure for the manufacture of these products so that patient safety can be ensured. [8,21]

When it came to testing for pathogens, all the pharmaceutical adaptations were free of *Escherichia coli, Salmonella sp., Pseudomonas aeruginosa* and *Staphylococcus aureus*, thus within the limits recommended by the pharmacopoeia. It is therefore interesting to understand that in the case of testing for *S. aureus*, when not producing enterotoxins, and *E. coli* are important markers for assessing the hygiene of the hands of the handler and the workbench during the process.[24,25]

Pseudomonas aeruginosa is described as an important human pathogen, it has the ability to contaminate non-sterile products due to its versatility to grow in unfavorable environments and its intrinsic and acquired resistance to antimicrobials, its absence in the analysis is a good marker in monitoring the quality of water and food, as well as the absence of Salmonella sp., being especially dangerous for children and immunocompromised, and can lead to fatal dehydration. [26,27]

The scarcity of commercial formulations suitable for the pediatric public, considering differences in physiological and metabolic response, makes it necessary to use *off-label* preparations. Therefore, in order to ensure their quality, rigorous safety assessment is necessary, considering the possibility of adverse reactions, toxicity and especially physicochemical and microbiological stability.

Therefore, effective microbiological quality control is essential to increase the safety of manipulated medicines, guiding pharmacists to improve their practices through appropriate procedures and access to crucial information on the stability, compatibility, bioavailability and safety of these products

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