EVALUATION REPORT FOR PhD THESIS "A SURVEY ON THE QUALITY OF OXYTOCIN AND MISOPROSTOL AS WELL AS ACCESSIBILITY OF MEDICINES IN RWANDA"

Thesis submitted for the fulfilment of the requirements for the award of a Ph.D. Degree in Pharmaceutical Sciences, to the School of Medicine and Pharmacy, College of Medicine and Health Sciences, University of Rwanda

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April, 2022

Declaration

I declare that this thesis contains my own work, except where specifically acknowledged, and it has been passed through the anti-plagiarism system and found to be compliant and this is the approved final version of the thesis:

"A survey on the quality of oxytocin AND misoprostol as well as prices, availability and affordability of medicines in Rwanda"

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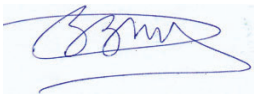
Date: April 14th 2022 Signature: 

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Date: 07. June 2022 Signature: 

Dedication

This thesis is dedicated to

Germaine Mukasibo

Melissa Pillar Cyuzuzo

Blaise Praise Meic Ganza

Paula Meica Gwiza

Gabriella Pius Keza.

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Abstract

Background

The third Sustainable Development Goal (SDG3.1) of the United Nations (UN) urge all countries to reduce the maternal mortality ratio at less than 70 maternal deaths per 100,000 live births by 2030. Oxytocin and misoprostol are used as the first and second choices, respectively, in the prevention and management of post-partum hemorrhage, which is the leading cause of maternal deaths. The SDG3.1 will not be achieved if full access to quality assured oxytocin and misoprostol is not assured. The main objective of this PhD thesis entitled "A survey on the quality of oxytocin AND misoprostol as well as prices, availability and affordability of medicines in Rwanda" was to measure the accessibility to 18 medicines including oxytocin and misoprostol, and investigate on the quality and stability of oxytocin and misoprostol used in health facilities of Rwanda.

Methods

Data on prices for 18 medicines were collected and analyzed using a standardized method developed by the Department of Medicine Policy and Standards of the WHO (WHO-DMPS) in partnership with the Health Action International (HAI), as updated in 2016. Data on storage conditions and samples of oxytocin and misoprostol were collected from 40 selected (simple random) health facilities and six wholesalers and government stores of Rwanda. Stability testing and quantitative analysis were performed according to the 2016 United States Pharmacopoeia (USP) for oxytocin injectables and the 2017 International Pharmacopoeia for misoprostol tablets.

Results

In Rwanda, the 18 surveyed medicines were affordable but poorly available in both the public and the faith- based sectors. Prices for generic medicines in the public and faith-based health facilities were low, but these prices were very high in the private pharmacies. The government procurement agency was found with low prices, compared to the international prices, with the MPR less than one for 16 out of 18 surveyed medicines. Affordability of medicines was better in the public and faith-based sectors than in the private sector. Oxytocin, the first choice in the prevention and management of post-partum hemorrhage, was poorly available in the private sector and its availability was less than the target of 80 % in the public and faith-based sectors.

Storage of oxytocin and misoprostol was found to follow the manufacturers' recommendations. However, quality problems were identified on the 9/57 samples of oxytocin and 10/25 samples of misoprostol collected from the Rwandan health facilities for the survey on quality. One brand of oxytocin showed contents in oxytocin exceeding the acceptable limit and an undeclared preservative. Two brands of misoprostol contained less than 50 % of the declared content in misoprostol and they released less then 50% of the declared content in misoprostol during the dissolution test. These brands were neither WHO-prequalified nor manufactured in countries with Stringent Regulatory Authority (SRA).

Chlorbutanol showed a high stabilizing effect on oxytocin preparations. All four samples/brands of oxytocin passed the accelerated stability testing according to the International Coucil on Harmonization (ICH) in partnership with the WHO. Damage to the primary packaging resulted in a decrease of 52.5 % of the content in misoprostol (from 100.7% to 48.2 %) after a 6 months storage at 40°C +/- 2°C and 75% +/- 5% RH, but no significant effect was found after a 6 months storage at 25°C +/- 2°C and 60% +/- 5% RH.

Conclusion

In Rwanda, important steps to ensure accessibility to medicines have been made, but more efforts are needed to ensure full access to quality assured oxytocin and misoprostol to expect the achievement of the SDG3.1 and reach the target of maternal mortality ratio by 2030.

Key words: Prices, Availability, Affordability, Quality, Oxytocin, Misoprostol, Post-partum haemorrhage.

List of acronyms and abbreviations

AAAQ:	Availability, Accessibility, Acceptability and Quality of services
CHAM:	Christian Health Association of Malawi
DH:	District Hospital
DTCs:	Drug Therapeutic Committees
EPMM:	Ending Preventable Maternal Mortality
EWEC:	Every Woman Every Child
FEFO:	First to Expire First Out
HAI:	Health Action International
HPLC:	High Performance Liquid Chromatography
ICH:	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
IU:	International Unit
LMICs:	Low and Middle-Income Countries
MMR:	Maternal Mortality Ratio
MoH:	Ministry of Health
MPD:	Medical Procurement and Distribution
MPR:	Median Price Ratio
MSH:	Management Sciences for Health
NRA:	National Regulatory Authority
PATH:	Program for Appropriate Technology in Health
PGE:	Prostaglandin
PPH:	Postpartum haemorrhage
RH:	Referral Hospital
SDG:	Sustainable Development Goal
SMO:	Severe Maternal Outcome
SRA:	Stringent Regulatory Authority
UHC:	Universal Health Coverage
UNCoLSC:	United Nations Commission on Life-Saving Commodities for Women and Children
UNIDO:	United Nations Industrial Development organization

UNFPA: United Nations Population Fund

UNICEF: United Nations Children's Fund

USAID: United States Agency for International Development

US $: United States Dollar

USP: United State Pharmacopoeia

WHO: World Health Organization

WHO-DMPS: Department of Medicine Policy and Standards of the WHO

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Chapter two, three, four and five have been published in scientific journals as peer-reviewed journal articles.

Chapter one: General introduction

1.1. Background

Estimates show 295,000 maternal deaths worldwide in 2017 (1). During the same period (2017), Rwanda has registered 900 maternal deaths, resulting in a maternal mortality ratio (MMR) of 248 deaths per 100,000 live births (1). The UN, in its SDG3.1, encourages all countries to achieve an MMR of less than 70 maternal deaths per 100,000 live births and not more than 140 maternal deaths per 100,000 live births by 2030 (2). Post-partum hemorrhage (PPH) is defined as excessive bleeding (more than 500 ml) of the mother after child delivery and, in 2007, was mentioned by WHO as the leading cause of maternal deaths (3). Since 1997, oxytocin injectable 10 UI/ml vial of 1 ml was on the WHO model list of essential medicines and misoprostol tablet 200 mcg has been added on this list in 2011 (4,5). The 2019 and 2017 "Model list of essential medicines" of WHO included oxytocin 10 IU 1 ml injectable ampoule as the first choice and misoprostol 200 mcg tablet as the second choice in the prevention and treatment of PPH (6,7).

Despite the addition of oxytocin and misoprostol on the WHO model list of essential medicines, a study conducted in Nigeria in 2016 listed PPH as the second cause of Severe Maternal Outcome (SMO) after pre-eclampsia/eclampsia (8), while in another study conducted in Rwanda in 2017, PPH was identified as the first cause of SMO (9). In the report published by the WHO in 2019 under the "Ending Preventable Maternal Mortality (EPMM)", which is the first target of the SDG3.1, the number of cases of maternal mortality in Low and Middle-Income Countries (LMICs) was more than 40 times higher than in developed countries (1). Post-partum hemorrhage is considered as an avoidable cause of maternal deaths, by ensuring the availability, accessibility, acceptability and quality (AAAQ) of services; therefore, full access to quality assured oxytocin and misoprostol (2).

Availability and affordability of good quality 13 life-saving commodities defined by the "United Nations Commission on Life-Saving Commodities for Women and Children" (UNCoLSC) would prevent 230,000 maternal deaths worldwide in five years (10). The 13 life-saving health commodities are: oxytocin, misoprostol, magnesium sulfate, injectable antibiotics, antenatal corticosteroids (ANCs), chlorhexidine, resuscitation devices, amoxicillin, oral rehydration salts (ORS), zinc, female condoms, contraceptive implants and emergency contraception. However, the number of women who die from causes related to pregnancy and childbirth is still big (11). It is frustrating and regrettable to see a health professional who is unable to stop the excessive bleeding of a woman who loses her life because of stock-out or poor-quality oxytocic medicines, after delivery of a normal child. Literature data showed that the quality of essential oxytocin and misoprostol in low income countries does not meet pharmacopoeial requirements, and consequences on individuals, families, national health systems and the economy are significant (10,12–15).

In Low- and Middle-income countries (LMICs), there is an issue of availability of data and/or accuracy of available data. The common three main causes which hinder access to quality essential medicines in low-income countries are: the insufficient supply of high quality health commodities, the inability to effectively regulate these high-quality commodities, including their prices, and the lack of knowledge on how, why and when to use the medicines (10). In 2013, Yoonjoung Choi1 and Paul Ametepi compared "reported availability and observed availability" of essential medicines at the health facility level in five sub-Saharan African countries, including Rwanda. The authors have found that "reported availability" was higher than "observed availability" across all five countries. The difference between reported availability and observed availability was on average 6%, ranging from 3 % in Rwanda to 8 % in Namibia (16).

1.1.1. Accessibility to quality essential medicines in LMICs

In 2002, the results from the 2001-2002 pilot country surveys using the "WHO/HAI Medicine Prices – a new approach to measurement" method and tools to measure prices, availability, affordability and price components for essential medicines were published. In 2008, HAI in partnership with the WHO-DMPS published the second edition of the manual to collect and analyze medicines prices, under the title "Measuring medicine prices, availability, affordability and price components" (17). One of the advantages of using this standardized methodology and tools in Rwanda, was that this study contributed to the dataset of WHO, and results can be compared to the ones from similar studies in other LMICs.

This method, after converting local prices into United States Dollars (US $), compares local prices to the international reference prices published by the Management Sciences for Health (MSH) (18). WHO recommends to use the prices published by MSH as reference prices, since most of these prices are collected from not-for-profit suppliers of developing countries, not including insurance or transportation charges. Prices of selected medicines are collected in local currency, then converted into US $ to ease comparison, and finally, they are expressed as ratios in terms of Median Price Ratio (MPR). To calculate MPR for a medicine, the local price in US $ is divided by its corresponding MSH reference price. A medicine with an MPR less than or equal to one is considered as being cheap (17). This thesis used the MSH reference prices edition 2015 published in 2016 (19) since they were the most recent.

Individual medicine is considered available if at least one usable (good quality according to the results of visual inspection) unit (e.g. tablet, vial, bottle) is found at the data collection site at the time of data collection. First, the number of health facilities where the individual medicine is available is determined, divided by the total number of health facilities included in the study and then expressed as a percentage (19). To serve as a reference, the target 9 of the WHO stated a voluntary target availability of affordable essential medicines of 80 % by 2025 (20,21).

Accessibility is a term that combines both availability and affordability. To evaluate

affordability for an individual medicine, the cost for a course of treatment in local currency is divided by the daily wage of the government's employee who is paid the lowest salary. This results in the number of days during which the lowest-paid unskilled government's employee needs to work to buy the entire course of treatment of this medicine. Affordability should not be greater than one (17).

With reference to the survey entitled "Baseline assessment of WHO's target for both availability and affordability of essential medicines to treat non-communicable diseases", conducted in 2016 by Margaret Ewen et al. published in 2017, availability was found to be lower than the target of 80 % and generic medicines were more available and more affordable than their originator brands (21). Reduced availability of essential medicines may be associated with the unavailability of funds and the low power to purchase medicines.

In some LMICs, the source of funds to procure medicines plays a big role in the availability of essential medicines. Health Programs' medicines such as antimalarials, anti-tuberculosis medicines, antiretrovirals, etc. which are provided with financial support, are generally more affordable than other essential medicines such as antibiotics, anti-inflammatory medicines, etc. In 2017, Felix Khuluza and Lutz Heide found that in Malawi, the availability of artemether/lumefantrine and sulfadoxine/pyrimethamine, which were provided with financial support from international donors, was high in public and the Christian Health Association of Malawi (CHAM) facilities (93 % and 100 %, respectively), but the availability of amoxicillin was 40 % in public health centers (22).

Unfortunately, a third of the population does not have access to essential medicines in the region of Sub- Sahara, and in Rwanda, stock-outs for some essential medicines can go up to 10.5 months per year (23). This may lead to treatment failures and a high number of deaths in most LMICs, where local manufacturing is still low, and the procurement agencies rely mainly to imported essential medicines.

However, since 2010, the UN and its partners had raised awareness on the suffering of women and children around the world, caused by the lack of access to life-saving commodities and, created the UNCoLSC (10). The overall goal of this commission which is part of the "Every Woman Every Child" (EWEC) movement, was to increase access to 13 life-saving commodities (including oxytocin and misoprostol) in 50 of the world's poorest countries (10). It was also planned that by the end of 2015, the WHO, the United Nations Children's Fund (UNICEF), United Nations Population Fund (UNFPA), national and international regulators, private sector actors and other partners should have jointly reviewed the quality of the most commonly used life-saving commodities. According to the commission, at least three manufacturers per commodity would have been selected to manufacture quality-certified and affordable products (10).

Reference made to the database of WHO accessed on August 2, 2021 available at https://extranet.who.int/pqweb/medicines/prequalified-lists/finished-pharmaceutical-products, there were four WHO pre-qualified manufacturers for oxytocin and four WHO pre-qualified manufacturers of misoprostol (3 for misoprostol alone and 1 for misoprostol + mifepristone). Unfortunately, some countries may not use this database to select their brands, which increases the risk of procuring poor-quality oxytocin and misoprostol and leads to high MMR observed in most LMICs.

Local manufacturing of pharmaceutical products influences positively accessibility to quality-ensured and affordable oxytocin and misoprostol (24). It is easier to monitor and enforce the quality and the prices of essential medicines with local pharmaceutical production than when pharmaceutical products come from abroad. According to the United Nations Industrial Development Organization (UNIDO), local pharmaceutical production should be accelerated in order to ensure a "Sustainable supply of safe, effective, quality, and affordable essential medicines and vaccines (as a contribution to SDG target 3.8) – with a focus on quality production and inclusive and sustainable industrialization" by 2030, especially in LMICs (25). Across Africa, UNIDO programs have contributed to improving quality, accessibility and affordability of essential medicines, through the UNIDO's "Partnership Mentoring Program" (26). The findings presented in this thesis do not contain any made-in-Rwanda medicines; all oxytocin and misoprostol brands found in the Rwandan health facilities were manufactured outside the country. There is a risk of long lead times causing unavailability for purchased medicines, and the probability of finding high price and poor-quality oxytocin and misoprostol on the Rwandan market is more than zero.

In 2007, the bulletin of the WHO published results on the availability of 32 selected essential medicines for chronic diseases in six LMICs from South America, Asia, and Africa, with both the public and the private sectors medicines outlets being represented. Only less than 7.5 % of the 32 medicines were available, except in two countries where 28 % and 30 % of the selected medicines were available (27).

In December 2008, Cameroon A. et al. published a review of medicine prices in 36 developing and Middle-income countries, totalizing 45 national and subnational surveys done using the WHO/HAI methodology. According to the authors, the average public sector availability of generic medicines ranged from 29·4 % to 54·4 % across WHO regions (28), far below the WHO recommended minimum target of 80 % by 2025 (20,29).

In 2011, WHO in collaboration with HAI, published their first working paper in the series of "WHO/HAI project on medicine prices and availability", in which it was highlighted that "The high price of medicines is a major concern for policy-makers, insurers, and patients" (30). Without strong regulations on medicine prices, speculations may render essential medicines unaffordable, and, therefore inaccessible. Consequently, it would be difficult to achieve the

SDG3.1 of the UN with Universal Health Coverage (UHC) by 2030.

The WHO/HAI method was used in China in 2012, where the mean availability in the public sector was found to be very low if compared to the minimum desired by WHO: 7.1 % for originator brands and 20.0 % for generic medicines (31). Public sector patient prices were found to be lower than private-sector patient prices, but availability was higher in the private sector than in the public sector, for generic medicines (31).

The standardized method to measure prices, availability and affordability was used also in Ghana by Edmund Mohammed Nyanwura and Reuben K. Esena in 2013, to assess availability and affordability. Their study revealed that the mean availability of 29 essential medicines was 64.1 %. Affordability was estimated at 1.67 days' wage for adult conditions and 0.78 days' wage for a child's disease conditions. Also, prices were higher in the private sector than in the public sector (32).

It is essential to measure prices, availability and affordability to know where countries are standing toward achieving the UN goals by 2030, especially in LMICs. UHC requires full access to health services and medicines. In this thesis, the 18 selected medicines were: Oxytocin injection 10IU/ml vial 1 ml, misoprostol tablet 200 mcg, Salbutamol 100mcg/dose inhaler 200 doses, Metformin tablet 500mg, Captopril tablet 25mg, Simvastatin tablet 20mg, Amitriptyline tablet 25mg, Ciprofloxacin tablet 500mg, Co-trimoxazole suspension 8+40mg/ml, Amoxicillin capsule 500mg, Ceftriaxone injection vial, Diclofenac tab 50mg, Paracetamol suspension 24mg/ml 60ml, Omeprazole capsule 20mg, Tranexamic acid injection 100mg/ml, Metronidazole tablet 250mg and Levonorgestrel tablet 1.5mg. Fifteen of them were selected from the 14 medicines defined as the core list in the standardized methodology of WHO/HAI, second edition, updates of 2016; one medicine was not found on the Rwandan essential medicines list, edition 2015. In addition, five medicines used to manage maternal and child health conditions were added as the supplementary list, including oxytocin used as the first choice and misoprostol used as the second choice in the prevention and management of PPH.

1.1.2. Quality of medicines in LMICs

The problem of poor quality essential medicines has been often reported and seems common in LMICs (33). Poor manufacturing procedures and inadequate storage conditions contribute to poor quality medicines and therefore, to the high treatment failure rates observed in LMICs (34). Some lifesaving medicines require strict observation of recommended storage conditions, which is a real challenge in LMICs that rely on imported medicines. The role of the national pharmaceutical quality control laboratory is a key in the assurance of patient's pharmaceutical safety.

In 2002, the WHO's "Expert Committee on Specifications for Pharmaceutical Products" published the guidelines entitled "WHO Good practices for national pharmaceutical control

laboratories", adopted in 1999 (35). The aim of the establishment of these guidelines was to internationally harmonize laboratory procedures to generate comparable analytical results both at the national and international levels and boost cooperation and mutual recognition between laboratories in all WHO regions. Since then, surveys on the quality for essential medicines have been performed, except for biological products such as vaccines and blood products, then, the WHO has made a data bank. However, until 2015, the problem of poor quality pharmaceutical products was still a global health and economic threat (36).

There may be a close relationship between the quality of pharmaceutical products and their country of origin, depending on the maturity and level of performance of the National Regulatory Authority (NRA) in place. Samples manufactured in countries with a Stringent Regulatory Authority (SRA) or WHO-prequalified showed low rates of substandard essential medicines in different studies (37). In 2012-13, levomethorphan, with the same molecular formula as the cough medicine dextromethorphan and five times stronger than morphine, was found in locally produced cough medicines that claimed to contain only dextromethorphan, and caused deaths of adults in Pakistan and hospitalization of children in Paraguay (12). In both two cases, the active pharmaceutical ingredient was imported from India (12), a non-SRA country. In addition, results from a survey on the quality of essential medicines in Africa and Asia published by Petersen A. et al. in 2017 revealed that 12 out of 869 samples did not contain the stated active pharmaceutical ingredient(s), and the highest proportions of substandard and falsified medicines were found in African countries (37).

Quality of oxytocin in LMICs

Oxytocin is the first-line medicine in the prevention and treatment of PPH. But, it is known to be very sensitive to environmental conditions – it degrades at high temperatures (38). Even though some brands of oxytocin carry a label stating a storage at room temperature, in 2018, a consortium of the "Reproductive Health Supplies Coalition, the United States Agency for International Development (USAID) and the Program for Appropriate Technology in Health (PATH)" has issued an advocacy messaging framework with the title "Buy quality oxytocin, keep it cool", and recommended that all oxytocin products should be stored at 2-8 °C irrespective of the manufacturer's storage recommendations (39,40). It would be beneficial if health authorities in Rwanda and elsewhere consider this recommendation.

Only a few authors have published stability study results showing that oxytocin preparations can be stored at 30 °C for one month or 40 °C for 1 week but then use them immediately or discard (41,42). In case oxytocin is not stored properly, it can lose its potency. This may contribute to higher maternal mortality rates, caused by the failure to prevent or manage postpartum hemorrhage, especially in LMICs.

In 2017, 94 % of all maternal deaths occurred in LMICs and 196,000 maternal deaths out of 295,000 maternal deaths worldwide (66.4 %) occurred only in sub-Saharan Africa (1). From a

systematic review of the quality of oxytocin preparations available in LMICs published by Torloni et al. in 2016, 45.6 % of oxytocin samples failed quality tests (15). In 2020, Torloni et al. conducted another review of published papers, and the new prevalence of failure to comply with quality requirements for oxytocin was nearly 40 % (43). In 2018, Anyakora et al. analyzed 159 samples of oxytocin collected from health facilities of Nigeria, using High-Performance Liquid Chromatography (HPLC). The authors found that 74.2 % oxytocin samples failed the assay test (44). In 2020, Nhomsai H. et al. published results on the quality of 65 oxytocin samples collected from health facilities in Malawi, showing failure rates of 11 % (45).

To overcome the challenges with the storage of oxytocin in resources limited countries, a heat-stable formulation of the oxytocin analogue called carbetocin has recently been added to the WHO Essential Medicines List, and in future it may become a further treatment option for post-partum hemorrhage for use in facilities where storage at 2-8 °C is problematic.

Quality of misoprostol in LMICs

Misoprostol tablet is used as the second choice in the management of PPH, but it is also sensitive to environmental conditions, and it has to be protected from humidity (46–48). Before tableting, misoprostol has to be prepared as a dispersion of 1 % in hydroxypropyl methylcellulose to protect it from being degraded (49). After tableting, misoprostol tablets have to be packed into a double aluminum blister to be protected from moisture and humidity (46). According to Kahsay, misoprostol can undergo dehydration resulting into misoprostol A. Misoprostol can also undergo epimerization at its carbon C8 resulting in 8-epi misoprostol, or isomerization of misoprostol A resulting into misoprostol B (50).

In 2016, findings published in the WHO report showed a failure rate of 45 % in the assay test of 119 samples of misoprostol (48). In 2018, Anyakora et al. analyzed 166 samples of misoprostol collected from health facilities of Nigeria using High-Performance Liquid Chromatography (HPLC), and they found that 33.7 % of samples of misoprostol failed the assay test (44). In 2020, Nhomsai H. et al. published results on the quality of 30 samples of misoprostol tablet 200mcg, collected from health facilities in Malawi, showing a failure rate of 17 % (45).

As the second choice in the management of PPH, the quality of misoprostol should be monitored and ensured, to contribute to the SDG3.1 of the UN.

1.1.3. Role of national regulatory authorities in ensuring accessibility to good quality essential medicines

A key element in ensuring access to quality essential medicines is the presence of a well-functioning NRA, which is a unit of the Ministry of Health (MoH). The primary role of a NRA extends from commodities security to ensuring consumers' safety and proper use of resources (51). However, in most cases, this role passes unseen and undervalued by political leaders (24).

National leaders' political will also plays an important role in establishing a strongly effective and efficient NRA by creating a strong legal framework and enforcing laws.

In LMICs, NRAs are invisible and under-resourced (24). Advocacy and more investment in NRAs are needed. This should be a national responsibility for sustainable financing and to move towards the Universal Health Coverage (UHC) by 2030 (51). International cooperation and partnerships should be negotiated in harmonization and/or support to national efforts.

It was recommended that when importing essential medicines, national procurement agencies, even though they are not obliged, should prioritize the WHO-prequalified manufacturers or select brands manufactured in countries with an SRA (39,51).

1.1.4. Consequences of inaccessibility to quality essential medicines

One of the consequences of a weak NRA is inaccessibility to good quality essential medicines observed through high prices, unavailability, unaffordability, and the presence of falsified and substandard essential medicines in the country. This leads to complication of diseases, emergence of new strains of resistant micro-organisms and increased mortality rate. Patients may suffer from side effects caused by the unprescribed wrong active pharmaceutical ingredients (APIs) or additives, stay untreated because of the weak concentration, or develop toxicity effects because of high concentration of the APIs or excipients (52).

LMICs, by selecting the cheapest medicines, may select substandard/ falsified brands and therefore, instead of saving their economies, loose too much money (52). Upon failure of the treatment, patients lose confidence in their health systems in LMICs. Rich people prefer paying health care services from rich countries with SRA, which worsens the economic situation in poor countries (13).

According to the WHO in its report published in 2017 under the title “A study on the public health and socioeconomic impact of substandard and falsified medical products”, the treatment of malaria using substandard and falsified antimalarials caused an additional 529 deaths per 1 million malaria cases seeking treatment, only in the sub-Sahara region (where Rwanda is located), as a result of the reduced effectiveness (13,52).

1.1.5. Risks associated with the use of poor-quality Oxytocin and misoprostol as life-saving medicines

When poor quality life-saving medicines are used to treat a condition, the patient risks death as the consequence of treatment failure and disease complications. The country may register increased morbidity and mortality, loss of confidence in health workers and systems, adverse effects from incorrect ingredients or incorrect amount, and increased resistance in case of

antibiotics (53). Beside health risks, socio-economic risks are also associated with poor-quality life-saving medicines (13,53).

1.2. Problem statement

Rwanda is one of the nine countries have achieved a reduction of at least 75 % in maternal mortality ratio from 1990 to 2015, out of 95 countries with a maternal mortality ratio which were more than 100 maternal deaths per 100,000 live births in 1990 (54). In 2017, 295,000 maternal deaths were estimated worldwide (1), and scientists estimated that access to good quality oxytocin would save 1.4 million lives in the following ten years (39). Otherwise, an equal number of lives would be lost.

In Rwanda, the estimated maternal mortality ratio per 100,000 live births was 275 in 2015 and 248 in 2017 (1,55). Even though Rwanda is on the right track, more actions are still needed to maintain and sustain these efforts to achieve the UN goal of less than 70 maternal deaths per 100,000 live births by 2030. Increasing access to quality, affordable oxytocin and misoprostol would reduce the number of women who die during or after child delivery (10).

Research and reports at the national level should focus on the availability of data to take appropriate decisions about how to improve accessibility to good quality medicines. So far, no results have been published about prices, availability, affordability and quality AND stability of oxytocin and misoprostol in Rwanda. There was a need to analyze the situation to update decision-makers and stakeholders about how the quality and accessibility of oxytocin and misoprostol are in Rwanda.

1.3. Objectives

1.3.1. General objective

The main objective of this thesis was to measure the accessibility to oxytocin and misoprostol, and investigate on the quality and stability of different brands of oxytocin and misoprostol being used in health facilities of Rwanda as a contribution to achieving the SDG 3.1 of the UN by 2030.

1.3.2. Specific objectives

1. To assess the accessibility in terms of prices, availability and affordability of 18 selected medicines including oxytocin and misoprostol available in Rwanda using a standardized methodology.
2. To determine the stability of oxytocin preparations collected from the Rwandan wholesalers with regards to the storage conditions as per ICH

3. To determine the stability of misoprostol brands collected from the Rwandan wholesalers with regards to the storage conditions as per ICH.
4. To determine the quality of oxytocin and misoprostol preparations used at different levels of the health system in Rwanda with reference to Pharmacopoeia.

1.3.3. Contribution of the study

Findings from this study serve as a contribution to the following:

1. Drug Therapeutic Committees (DTCs) find in this thesis evidence to shape a continuous monitoring in order to improve and/or maintain accessibility to life-saving medicines (chapter 2: prices, availability and affordability of medicines in Rwanda);
2. This thesis provides additional data which raise awareness of storekeepers about the stability of oxytocin in the storage conditions available in Rwanda (chapter 3: Stability of Oxytocin Preparations in Malawi and Rwanda: Stabilizing Effect of Chlorobutanol);
3. These findings help storekeepers to improve the storage of misoprostol tablet in order to remain within its shelf-life specification (chapter 4: Stability of misoprostol tablets collected in Malawi and Rwanda: Importance of intact primary packaging)

Current findings serve as a baseline for the quality of oxytocin and misoprostol used in the prevention and treatment of postpartum hemorrhage in Rwanda (chapter 5: Quality of oxytocin and misoprostol in health facilities of Rwanda).

1.4. Thesis outline

The focus of this thesis was the two treatments of post-partum haemorrhage in Rwanda, oxytocin and misoprostol. Three main aspects were explored: accessibility in terms of prices, availability and affordability of treatments; stability of oxytocin and misoprostol in available storage conditions; and quality of procured brands of oxytocin and misoprostol as the first and second choices in the treatment of PPH. In order to cover all of these aspects, this thesis has been organized in six chapters.

The first chapter called “General introduction”, describes the background of PPH as the problem of interest, states the problem and defines the study objectives. This chapter also review the scientific knowledge on post-partum haemorrhage, accessibility to quality assured essential medicines in terms of prices, availability and affordability, the quality of oxytocin from previous studies, the role of the national regulatory authorities in ensuring accessibility to good quality essential medicines, consequences of inaccessibility to quality essential medicines,

and risks associated with the use of poor-quality oxytocin and misoprostol as life-saving medicines. It provides a holistic picture of factors influencing high MMR in resources-limited countries.

The second chapter named “Prices, availability and affordability of medicines in Rwanda” presents findings on the accessibility of the Rwandan population to 18 selected survey medicines including oxytocin and misoprostol. Accessibility to medicines was described by three measurements: prices, availability and affordability. Both originator brands and the lowest generic brands were surveyed, collecting data from the three main categories of health facilities found in Rwanda: public/government, faith-based and private. Even though the target was on oxytocin and misoprostol used as the first and the second choices in the management of PPH, the survey included 16 more medicines, not only to make the survey scientifically relevant, but also to have a picture of how medicines are accessible to the Rwandan population in general.

Chapter three named “Stability of Oxytocin Preparations in Malawi and Rwanda: Stabilizing Effect of Chlorbutanol” shows how different brands of oxytocin, collected from the wholesales of Malawi and Rwanda, are stable at different temperatures, and the strength of different stabilizing agents used in these preparations. This chapter provides results which help to understand results on the quality of oxytocin preparations collected from health facilities. In fact, oxytocin degrades at high temperatures, and this may occur during storage at the health facility or at a stage prior to the storage at the health facility level. Samples of oxytocin were collected from the main wholesales of Rwanda and Malawi, in a shared study, and were analysed in the same conditions, as described by the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) (56).

The fourth chapter is named “Stability of misoprostol tablets collected in Malawi and Rwanda: Importance of intact primary packaging” and presents results which have been published in a scientific journal. It provides data which help to understand problems of quality of misoprostol samples collected from health facilities. Samples of misoprostol were collected from wholesales of Rwanda and Malawi in a shared study.

The fifth chapter entitled “Quality of oxytocin and misoprostol in health facilities of Rwanda” presents results of the study conducted on samples of oxytocin and misoprostol collected from selected health facilities of Rwanda.

The sixth chapter is named “Discussion, conclusion and recommendations” and discusses in general the findings presented in this thesis. It also draws a general conclusion with regards to the general objective of his thesis, with a few recommendations to different decisionmakers.

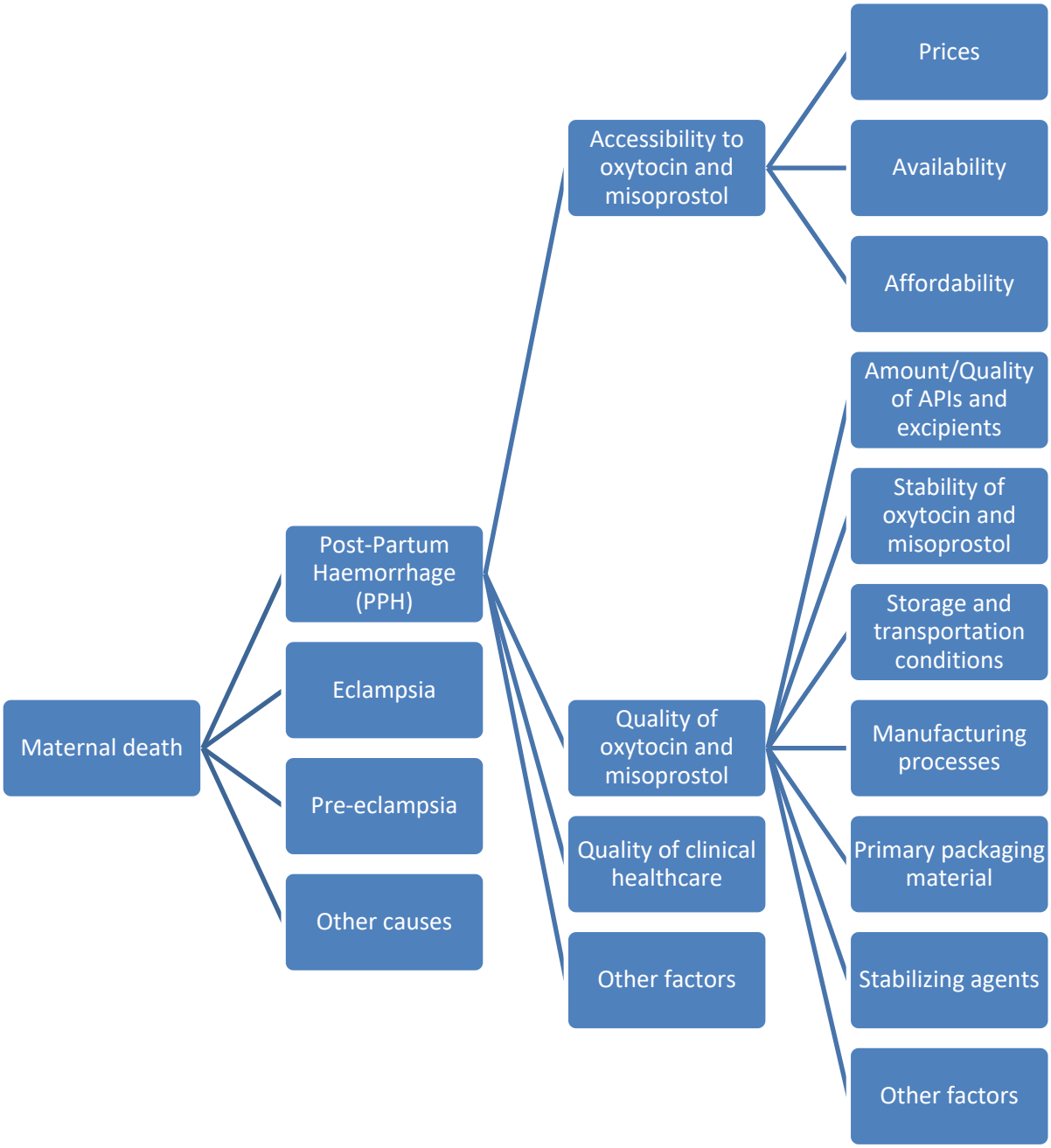
1.5. Limitations

This thesis was limited in time and space. The study on prices, availability and affordability covered only 18 selected medicines. According to WHO/HAI methodology, 50 medicines are recommended, including the 14 medicines of the core list also included in this thesis. This study and the study on the quality of oxytocin and misoprostol covered only Rwanda, while the stability studies covered Rwanda and Malawi. Due to the constraint of time, the stability studies were limited to the six months accelerated stability studies as defined by WHO/ICH. This thesis does not report on the causes of substandard samples of oxytocin and misoprostol, and related substances were not fully documented. Brands of oxytocin that found substandard during the pre-screening step were excluded from the stability study; but their stability profile would also be scientifically interesting.

1.6. Delimitations

Samples of oxytocin and misoprostol were collected from medicines outlets selected by simple random, and 8 district out of 30 districts of Rwanda were chosen by convenience and grouped into six regions. Probably a stratified selection would be more representative of the territory of Rwanda. In this thesis, only the stability studies covered both Rwanda and Malawi. This thesis covered only PPH as the main cause of maternal death, and did not cover the quality of clinical healthcare and other factors that may influence post-partum haemorrhage.

1.7. Conceptual framework for maternal deaths and its dependent variables



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Chapter two: Prices, availability and affordability of medicines in Rwanda

Abstract

Background

Access to affordable and good quality medicines is a key to meeting Sustainable Development Goal No. 3 by the year 2030. Prices, availability and affordability of essential medicines have been studied in many developing countries, but no such information has been published about Rwanda yet. This study aimed at providing data on prices, availability and affordability of medicines in different health facilities of Rwanda.

Methods

A survey was carried out on availability, prices and affordability of 18 medicines in Kigali City and five districts of Rwanda. 44 health facilities were surveyed, including public and faith-based hospitals, public and faith-based health centers and private pharmacies. The standardized methodology developed by WHO and Health Action International (HAI) was used to collect and analyze the data.

Findings

Prices for generic medicines in public and faith-based health facilities were remarkably low, with median price ratios (MPRs) of 1.0 in comparison to the international procurement prices published by Management Sciences for Health. In private pharmacies, prices were twice as high (MPR = 1.99 for generics). Availability of medicines fell short of the of 80% target set by WHO, but was better than reported from many other developing countries. Availability of medicines was highest in the private sector (71.3%) and slightly lower in the faith-based (62.8%) and public (59.6%) sectors. The government procurement agency was found to work efficiently, achieving prices 30% below the international procurement price given in the International Medical Product Price Guide. Affordability of medicines was better in the public and faith-based sectors than in the private sector.

Conclusion

In Rwanda, medicines are affordable but poorly available in both the public and the faith-based sectors. Further improvements of the availability of medicines in the public and the faith-based health facilities represent the most important key to increase accessibility and affordability of medicines in Rwanda.

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No data were changed, only minor edit adjustments were made to fit the paper into the Microsoft word format and accommodate comments from panelists.

2.1. Introduction

Access to quality essential medicines is part of the Sustainable Development Goal (SDG) No. 3 of the United Nations (UN) and a key to achieving Universal Health Coverage (UHC) by 2030 [1,2]. It is one of the five crucial policy areas identified by the "Lancet's Commission on Essential Medicines Policies" in 2016 [3]. To achieve sustainable access to essential medicines for all people, affordability of essential medicines should be ensured especially in low and middle-income countries. The Lancet's commission estimated that between US$13 and US$25 per capita (total US$ 77 to US$152 billion) is required annually to finance a basic package of 201 essential medicines (378 dosage forms) in all Low- and Middle-Income Countries (LMICs) [3]. In its "Global Action Plan for the Prevention and Control of Non-Communicable Diseases 2013-2020" , the 66th World Health Assembly has formulated the voluntary global target: an 80% availability of the affordable basic technologies and essential medicines, including generics, required to treat major non-communicable diseases in both public and private facilities [4]. The "UN Commission on Life-Saving Commodities for Women and Children" estimated that improved access to and proper use of 13 life-saving commodities (for reproductive, maternal, newborn and child health) in 50 of the world's poorest countries would prevent more than 6 million deaths of women and children in five years [5].

In 2003, the first edition of a standardized method for the measurement of medicine availability, prices and affordability was published by World Health Organization (WHO) in collaboration with Health Action International (HAI). The second edition of this methodology was published in 2008, and the global core list of included medicines was updated in May 2016 [6]. Surveys have been conducted using this methodology in many LMICs, frequently reporting low availability, high prices and correspondingly poor affordability of essential medicines [7].

With all these efforts at international and national levels, so far in Rwanda only a single, small study has been published on medicine availability. It investigated stock-out times of 10 essential medicines in 15 rural health centers in a single district of Rwanda in the year 2013, and reported that on average these medicines were out of stock for 2.7 months; quinine tablets were out of stock even for 10.5 months [8]. Beyond that report, no further results have been published about availability, prices and affordability of medicines in Rwanda. Therefore, in the present study we investigated availability, prices and affordability of 18 important medicines in public, private and faith-based health facilities in Kigali city and in five further districts of Rwanda, using the methodology published by WHO and HAI [6]. The results provide the first

comprehensive picture of medicine availability and prices in Rwanda, and may help to identify the priorities for a further improvement of access to medicines in this country.

2.2. Methods

2.2.1. Ethical approval

This study was authorized by the Ministry of Health (Approval Notice No. 20/1361/DGPHFIS/2018) after ethical approval by the Institutional Review Board of the College of Medicine and Health Sciences of the University of Rwanda (Approval Notice No. 026/CMHS IRB/2018).

2.2.2. Medicines included

Data were collected on prices, availability and affordability of 18 medicines (Table 1). 13 of these medicines are included in the WHO and HAI global core list [6]. Five supplementary medicines used in maternal and child health, especially in the management of post-partum hemorrhage, were also surveyed as this is a priority in Rwandan health policies. Of the 18 survey medicines, one (tranexamic acid injection 100mg/ml) was not included in the 2015 Rwanda Essential Medicines List (REML) [9,10], but has recently been recommended by WHO for post-partum hemorrhage (PPH) in addition to standard care [11]. Originator brands registered in Rwanda were identified using the list of medicines authorized in Rwanda which is published by the Ministry of Health [12]. According to this list, originator brands were registered in Rwanda for only eight of the 18 medicines included in this study (Table 1).

Table 1: Medicines included into this survey

	Survey medicines	Included in WHO/HAI Global Core List	Originator brand registered in Rwanda	Availability in the health system according to the 2015 REML		Daily dose [units or ml]	Treatment duration [days]	Number of units or ml for one course of treatment
				District hospital	Health center			
Antibiotics								
1	Amoxicillin capsule 500mg	Yes	-	+	+	3	7	21
2	Ceftriaxone injection 1g	Yes	-	+		1	1	1
3	Ciprofloxacin tablet 500mg	Yes	-	+	+	2	7	14
4	Co-trimoxazole suspension 8+40mg/ml	Yes	Yes	+	+	10 ml	7	70 ml
5	Metronidazole tablet 250mg	-	Yes	+	+	6	7	42
Medicines against Non-Communicable Diseases (NCDs)								
6	Amitriptyline tablet 25mg	Yes	-	+	+	3	30	90
7	Captopril tablet 25mg	Yes	-	+	+	2	30	60
8	Diazepam tablet 5mg	Yes	Yes	+	+	1	7	7
9	Diclofenac tablet 50mg	Yes	-	+	+	2	30	60
10	Metformin tablet 500mg	Yes	Yes	+	+	3	30	90
11	Omeprazole capsule 20mg	Yes	-	+		1	30	30
12	Salbutamol inhaler 100mcg/dose	Yes	Yes	+	+	As needed	As needed	200 doses
13	Simvastatin tablet 20mg	Yes	Yes	+		1	30	30
Medicines for Maternal AND Child Health								
14	Levonorgestrel tablet 1.5mg	-	Yes	+	+	1	1	1
15	Misoprostol tablet 200mcg	-	Yes	+		4	1	4
16	Oxytocin injection 10IU/ml	-	-	+	+	1 ml	1	1 ml
17	Paracetamol suspension 24mg/ml	Yes	-	+	+	15 ml	3	45 ml
18	Tranexamic acid injection 100mg/ml	-	-	Not included in the 2015 REML		5 ml	1	5 ml

The Global Core List of survey medicines has been published by WHO and HAI [6] and updated in 2016 [7].

2.2.3. Districts and regions surveyed

Six survey regions (administrative districts) were deliberately chosen with the purpose to represent the four provinces and the capital city of Rwanda: Karongi district in the Western province, Muhanga and Kamonyi districts in the Southern province, Musanze district in the Northern province, Bugesera district in the Eastern province, and Kigali city.

2.2.4. Sectors surveyed

Availability of medicines, and prices which patients had to pay for these medicines, were recorded in health facilities/pharmacies in the public sector (outpatient only), in the private sector and in the faith-based sector. Furthermore, public procurement prices, i.e. prices paid by the "Rwanda Biomedical Center/ Medical Production, Procurement and Distribution Division (MPDD)" in its medicine purchases for the government health system, were collected from the central medical store, in accordance with the WHO/HAI manual.

2.2.5. Health facilities surveyed

For each or the six survey regions, a list of all active health facilities was obtained from Ministry of Health files, and then organized into five categories: district hospitals, public health centers, faith-based health centers, private clinics and private pharmacies. For each survey region, two health facilities from each category were selected by simple random, using the RND function of Microsoft Excel, provided two or more facilities were available. If there was only a single facility of the category in the region, that facility was selected.

Initially 57 health facilities were selected: 9 district hospitals, 12 public health centers, 12 faith-based health centers, 12 private clinics and 12 private pharmacies. One of the selected district hospitals had to be excluded from the study because it was found to be a specialized orthopedic hospital which was not stocking the surveyed medicines. Furthermore, according to Ministry of Health regulations [13], the selected private clinics were only authorized to keep emergency medications, not the survey medicines. Medicines prescribed in private clinics are dispensed from private pharmacies. However, in this study, ceftriaxone was found in 3 of the 12 private clinics, and oxytocin in one private clinic. Other private clinics may stock the survey medicines, but did not share this information with the investigator (as stocking these medicines may constitute a violation of the Ministry of Health rules). It was decided to exclude all 12 private clinics from data analysis. Therefore, a total of 44 health facilities were included in the data analysis (Table 2).

Table 2: Districts and health facilities from which data were collected for this study

District	Public facilities		Faith-based facilities		Private facilities	Total
	Hospital	Health Centre	Hospital	Health Centre	Pharmacies	
Kigali City	2	2	0	2	2	8
Muhanga	0	2	1	2	2	7
Musanze	1	2	0	2	2	7
Karongi	0	2	2	2	2	8
Bugesera	0	2	1	2	2	7
Kamonyi	0	2	1	2	2	7
Sub-total	3	12	5	12	12	44
	15		17		12	

2.2.6. Data collection

Data were collected from February-April 2019, entered into the automated Microsoft Excel workbook developed by WHO and HAI [6] and double-checked by the investigator to ensure accuracy of data. Following the WHO and HAI methodology [6], for each medicine data were collected on the originator brand (if registered in the country) and their generic equivalent. If several generic medicines were found in an outlet for a survey medicine, price data were recorded for the generic with the lowest unit price.

2.2.7. Data analysis

Summary results such as percent availability, median price ratios and cost for one treatment course were calculated using the default settings of the automated Microsoft Excel workbook developed by WHO and HAI [6], with a single exception: the Excel workbook allowed to differentiate between district hospitals and health centers only in case of public facilities, not in case of faith-based health facilities. Since in Rwanda, the same rules about medicine availability apply to public and faith-based facilities, the availability of misoprostol, omeprazole, ceftriaxone and simvastatin (expected to be available at hospital but not at health center level) in faith-based facilities was calculated manually, only considering their availability at the five faith-based district hospitals. Further analysis and generation of tables and figures were also done using Microsoft Excel.

2.2.7.1. Measurement of availability

Following WHO and HAI methodology [6], medicines availability was calculated by sector as the percentage of facilities which had at least one unit of each medicine in stock at the time of the visit. Mean availability, across the survey medicines, by sector was also calculated.

As shown in Table 1, according to the REML misoprostol, omeprazole, ceftriaxone and simvastatin are expected to be available at hospitals but not at health center level. This rule applies to both public and faith-based health facilities. Accordingly, for these four medicines availability in the public and faith-based sectors was calculated considering only their availability in hospitals (3 public hospitals, 5 faith-based hospitals).

2.2.7.2. Measurement of prices

Price data were only collected if the medicine was physically in stock at the time of the visit. International reference prices were used as external benchmarks to assess Rwandan prices. The source of the reference prices was the 2015 Management Science for Health International Medical Product Price Guide (MSH-IMPPG), which was the most recent version available [14]. These prices are medians of procurement prices offered by for-profit and not-for-profit suppliers to governments or large not-for-profit "Non-Governmental Organizations" for generics, and are therefore relatively low priced and represent efficient bulk procurement prices. The reference prices, given in US$, were converted to local currency (1 US$ = 902.1530 Rwandan francs). Median values of the observed local medicine prices were compared to the international procurement prices published in the MSH-IMPPG, resulting in the Median Price Ratios (MPRs). Prices were expressed in Rwandan francs (Rwf) and as MPR:

$$\text{Medicine Price Ratio (MPR)} = \frac{\text{Median unit price in Rwf}}{\text{International reference unit price in Rwf}}$$

MPRs were calculated only if at least four prices for the respective medicine were available from a health sector. For example, in the public sector no MPR for misoprostol was calculated as only two prices were obtained for the generic and also the originator brand. In the private sector, no MPR for tranexamic acid (generic) was calculated because there was only a single price obtained.

2.2.7.3. Assessing treatment affordability

Following the WHO and HAI methodology [6] , for each medicine affordability was assessed as the number of day's wages needed by the lowest paid unskilled government worker to purchase a course of treatment based on standard treatment regimens. Treatment courses requiring more than 1 day's wages are considered unaffordable by WHO and HAI. Based on the information given by Minimum-Wage.org [15], one day's wage of the lowest paid unskilled government worker in Rwanda was 1,000 Rwf. The number of tablets/units of each medicine required for a course of treatment is shown in Table 1. For medicines from the WHO and HAI global core list, the number of units for a course of treatment are defined in the WHO and HAI

manual [6]. For the five medicines on the supplementary list, the “Rwanda Gynecology and Obstetrics Guidelines” and the WHO Model Formulary 2008 were used to define the daily dose and treatment duration for each medicine [16,17].

2.3. Results

2.3.1. Medicine availability

Availability of generic medicines (Fig 1a) was, on average, 64.8% in the private pharmacies, 59.3% in the faith-based health facilities, and 55.2% in the public sector. Of the eight originator brands (Fig 1b), three were not available in any of the investigated facilities. Mean availability of these eight originator brands was 29.2% in the private pharmacies, 11.7% in the public sector, and 7.9% in the faith-based health facilities.

For salbutamol inhaler and misoprostol tablets, several public and faith-based health facilities were found to stock only the originator brand but not a generic medicine. The prices of these two originator brand medicines were not very different from the prices of the respective generic medicines.

Fig 1c gives the availability of generic and originator combined for each medicine by sector. Availability of all investigated medicines was highest in the private sector (71.3%) and slightly lower in the faith-based (62.8%) and public (59.6%) sectors. For the investigated antibiotics, this combined availability was, on average, 93.3% in the private sector, and 64.2% and 61.3% in the faith-based and the public sector, respectively. Notably, amoxicillin 500mg tablets/capsules showed low availability in public and faith-based facilities, and were not in stock even in several of the hospitals. However, other strengths (such as 250mg tablets/capsules) may have been in stock.

Availability of the medicines used to treat NCDs in private, faith-based and public facilities was 75.0%, 68.8% and 64.2%, respectively, therefore not very different from the results for antibiotics. Simvastatin was unavailable in all the sampled public and faith-based hospitals, despite its inclusion into the REML.

Oxytocin, a life-saving medicine against post-partum hemorrhage, showed availabilities in public (73.3%) and faith-based health facilities (82.4%) close to the 80% target set by the WHO [4,18], while private pharmacies (8.3%) rarely stocked oxytocin injection. Misoprostol is used for induction of labor, against post-partum hemorrhage and for abortions, as well as for the prevention and treatment of stomach ulcers. According to the REML, it should be available in hospitals, and indeed was found in stock in all hospitals sampled in this study. It was also found in one public health center, one faith-based health center, and in five of the twelve private pharmacies. Tranexamic acid is not included in REML, and correspondingly was found only in

a single facility, which was a private pharmacy. The contraceptive levonorgestrel is included in REML and should be available both in health centers and in hospitals. In this study, however, it was only found in private pharmacies.

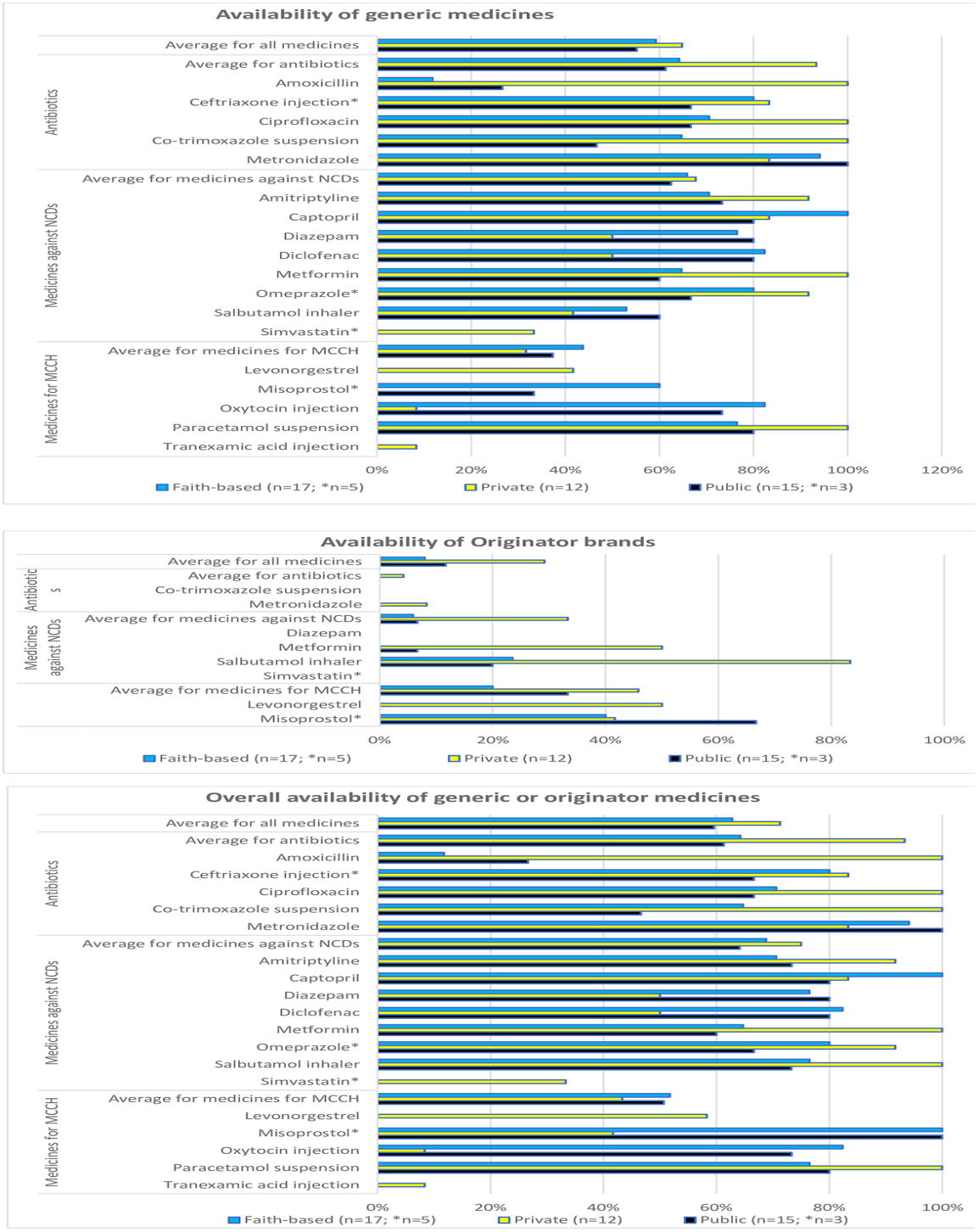

Figure 1: Availability of the investigated medicines in public health facilities, faith-based health facilities, and private pharmacies

a) Availability of generic medicines. b) Availability of originator brands. c) Overall availability of the medicines (generic or originator)

* Medicines allowed at hospital level only.

2.3.2. Medicine prices

For lowest priced generics in faith-based and public health facilities (Fig 2a), median MPRs were 1.0 in both cases. In private pharmacies, the median MPR was twice as high (MPR = 1.99).

Overall, government procurement was efficient for lowest priced generics, with a median MPR of 0.7, i.e. prices 30% below the international reference prices. For 16 out of 18 surveyed medicines, the MPR was less than one. The two medicines where the government procurement price exceeded the international reference price were oxytocin injection (MPR 1.76) and ceftriaxone injection (MPR 1.55).

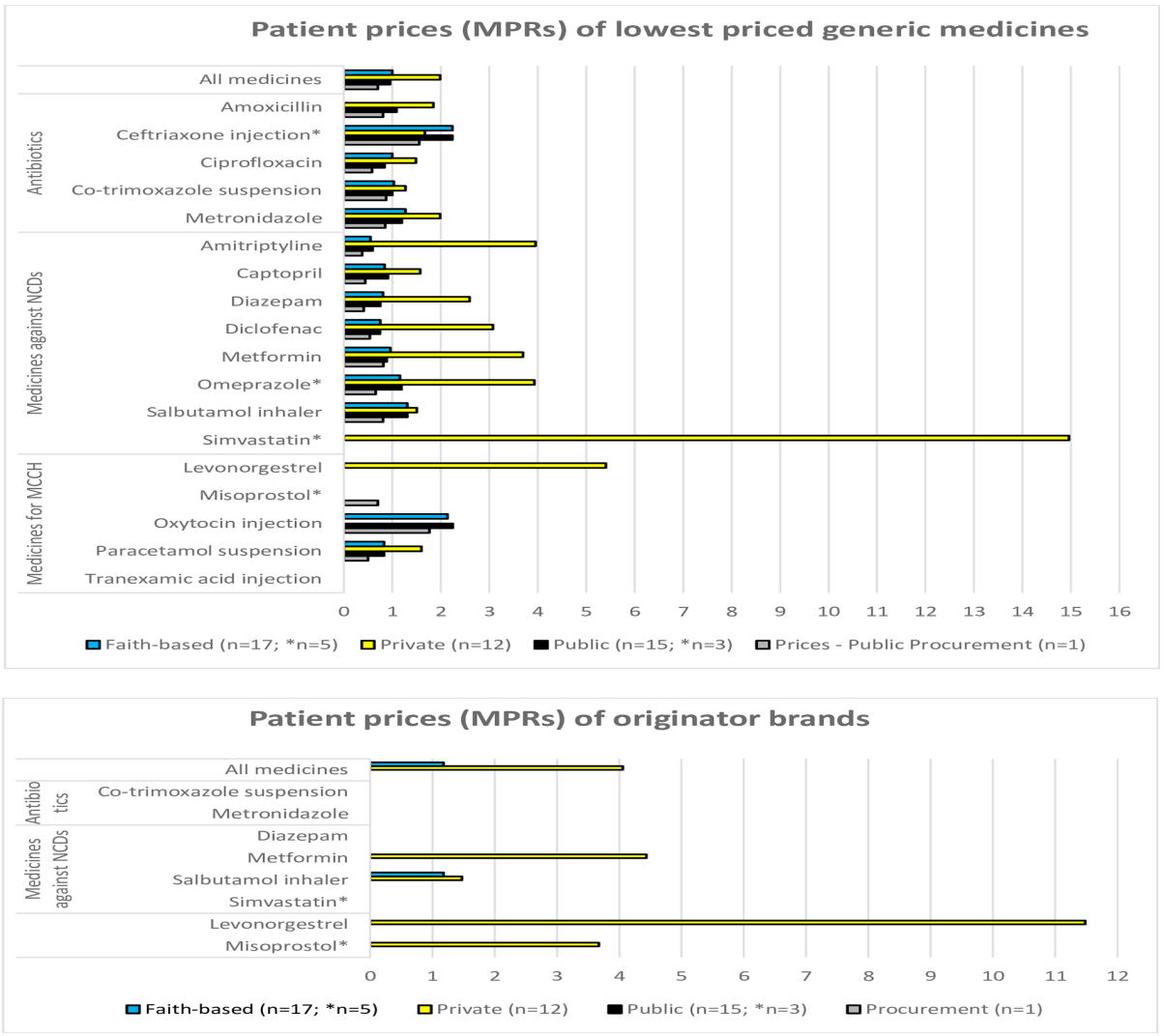

Figure 2: Median price ratios of the investigated medicines in public health facilities, faith-based health facilities, private pharmacies, and in public procurement.

a) Lowest-priced generic medicines; b) Originator brands.

2.3.2.1. Patient prices of individual medicines

In public and faith-based health facilities, MPRs for lowest priced generics used to treat NCDs were slightly lower than those for antibiotics. Conversely, in private pharmacies the MPRs for generic medicines used to treat NCDs were higher than those for antibiotics (Fig 2a). Levonorgestrel and simvastatin generics were only found in private pharmacies, not in public or faith-based health facilities. This lack of competition translated into remarkably high prices in the private sector, reaching an MPR of 15 for lowest priced generics of simvastatin.

As mentioned above, originator brands were rarely found in public and faith-based health facilities, and even in private pharmacies, the required number of four prices for the calculation of an MPR was only reached for four medicines (Fig 2b). The overall MPR for those four brands in the private sector was 4.05, ranging from 1.48 for salbutamol to 11.48 for levonorgestrel.

A paired analysis of patient prices for lowest priced generics and originator brands in each sector was not possible due to the few originator brands where an MPR was calculated.
For salbutamol inhalers in the private and faith-based sectors (Figs. 2a and 2b) and for misoprostol tablets in the faith-based sector (S2 file, Supplementary Information), the median prices of the originator brands were not dissimilar from the median prices of the respective lowest priced generics (note: the misoprostol data was based on few data points).
S2 Table (Supplementary Information) gives the median prices in local currency.

2.3.2.2. Cross sector price comparisons

Based on a paired analysis of lowest priced generics:

- patient prices in the public health facilities were 30% higher than the government procurement prices (14 medicines);
- patient prices in private pharmacies were 103% higher than in public facilities (13 medicines);
- Patient prices in faith-based facilities were 10% higher than in public sector facilities (13 medicines); and
- Patient prices in faith-based facilities were 47% lower than in private pharmacies (12 medicines)

There was insufficient data to do across sector paired analyses for originator brands.

2.3.3. Medicines affordability

As explained in the Methods section, affordability was expressed as the number of day's wages of the lowest paid unskilled government worker required to purchase a course of treatment based on standard treatment regimens [6]. For chronic diseases, the cost for 30 days of treatment were used in this calculation.

According to WHO/HAI, treatments are considered unaffordable if they require more than one day's wage. As shown in Fig 3, antibiotic treatments with lowest priced generics were affordable, even when purchased from private pharmacies. It should be noted that for ceftriaxone, the treatment regimen was for a single dose of 1g. If the "WHO Model Prescribing Information" for pneumonia were followed [19], a course of treatment would consist of 1-2g per day for 7 days, therefore treatment costs of ceftriaxone would result 7-14 times higher than shown in Fig 3.

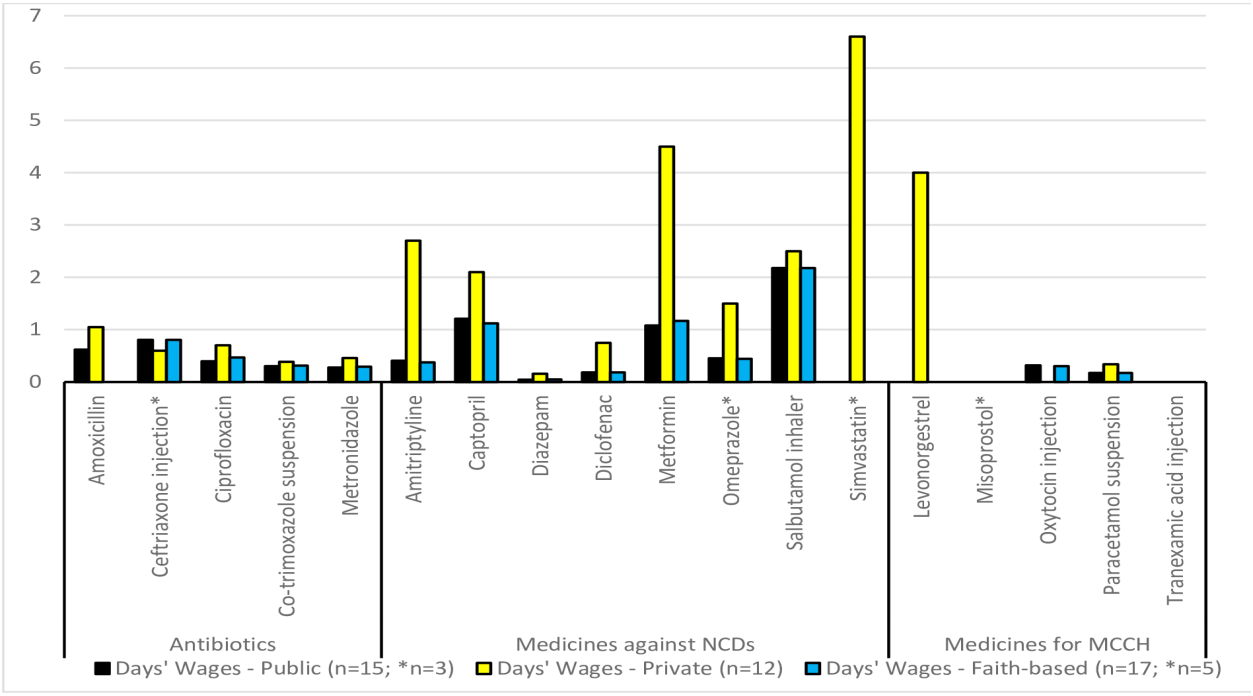

Figure 3: Number of day's wages required to purchase a course of treatment of the lowest-priced generic medicine

Of the eight medicines for chronic diseases, six required more than one day's wage if lowest priced generics were purchased in a private pharmacy, with simvastatin being the least affordable treatment (6.6 days wages for 30 tablets). Oxytocin, the most important life-saving medicine for post-partum hemorrhage, was found affordable at 0.3 days wages.

All four treatments with originator brands purchased from private pharmacies were found to be unaffordable, ranging from 2.4 – 8.5 days' wages. S3 Table gives the treatments, their median patient price in Rwf, and the number of day's wages required to purchase the treatment.

In Rwanda, 92% of the population are covered by the nation's health insurance schemes [20]. By far the largest part of the population, including the people in the informal sector of rural Rwanda, is covered by the Community Based Health Insurance (CBHI), also commonly known as Mutuelle de Santé. Public servants and employees of the formal sector are covered by the Medical Insurance Scheme (MIS) provided by the Rwanda Social Security Board (RSSB), and members of the armed forces are insured by the Medical Military Insurance (MMI) [21]. The CBHI covers (at least) 90% of the total cost of health care services, and this includes 90% of the cost for essential medicines in the public and faith-based sectors [22, 23].

This provision renders all treatments with lowest-price generic medicines in the public and faith-based sectors affordable. However, the problem is that the availability of medicines in the public and faith-based sectors was often much lower than the 80% availability target set by WHO for NCDs medicines. Therefore, patients have to revert to the private sector to obtain these medicines, and since CBHI does not cover the cost of medicines from the private sector, patients have to pay 100% of the costs, which renders many treatments for chronic diseases unaffordable (Fig 3).

2.4. Discussion

Access to quality-assured essential medicines has been defined as a priority by WHO in the struggle to meet the Sustainable Development Goal No. 3 of the United Nations [1,2]. The results of the present study on the availability, prices and affordability of important medicines is a contribution to assessing progress towards this goal in Rwanda.

Median public procurement prices for 16 out of 18 generic medicines were lower than the international supplier price given in the 2015 MSH-IMPPG [14]. This shows efficient procurement procedures in the Rwandan public sector. This also translated into remarkably low patient prices in the public and faith-based sectors, due to proper regulation of the prices in the Rwandan public health system [21]. As usual, patient prices in the private sector were clearly higher; for example, the patient price for amitriptyline tablets 25mg in private pharmacies was more than 6-times its government procurement price. Obviously, higher prices in the private sector can be justified by price components in the supply chain such as salaries, rent for premises, transportation means when importing medicines, etc. Nearly all previous studies in LMICs found that prices were higher in the private sector than in the public and the faith-based sectors [24]. An exception is a study from Malawi which found prices of antibiotics and antimalarials in the faith-based sector to be higher than in the private sector [25].

According to WHO, availability of essential medicines for NCDs should be at least 80% [4,18]. However, in the present study the availability of most of the essential medicines in public and faith-based health facilities in Rwanda was found to fall short of this WHO benchmark. This situation is unfortunately quite common in many LMICs. E.g. an authoritative review published in “The Lancet” reported that the average availability of generic medicines in public health facilities of 36 LMICs ranged from 29.4% to 54.4% [24]. In Rwanda, the availability of the 18 essential medicines investigated in this study was mostly higher than in the 36 countries investigated in that review, but lower than e.g. the availability of 20 essential medicines in clinics and health centers in Ghana in 2013 [26]. On the other hand, the availability of generic medicines in the public and private sectors of Shaanxi Province, Western China [27] was lower than found in the present study for Rwanda.

The results on medicine affordability obtained in this study have to be placed in the context of the Rwandan health system and the organization of its pharmaceutical sector. The Medical Production, Procurement and Distribution Division (MPDD) of the Rwanda Biomedical Center

is the most important supplier of medicines to all public institutions. Faith-based health facilities obtain their medicines also from MPDD, or from the Bureau des Formations Médicales Agréées du Rwanda (BUFMAR), while private pharmacies purchase from private wholesalers.

The medicine pricing and reimbursement system of Rwanda has been described in detail in a study by WHO in 2016 [21]. As mentioned above, most of the population of Rwanda is covered by the Community Based Health Insurance (CBHI) scheme. CBHI is managed by the Rwanda Social Security Board (RSSB), which is under supervision of the Ministry of Finance and Economic Planning. CBHI covers costs for health services and medicines in public or faith-based health facilities in Rwanda.

The patient prices of medicines in the public and faith-based health facilities are regulated by ministerial instruction No. 20/1658/PTF/2007 of 15 June 2007, allowing a maximum mark-up of 20% over the procurement prices [21]. No national medicine pricing policy exists for the private sector; for patients covered by the Medical Insurance Scheme of RSSB, this insurance allows a maximum profit margin of 40% over the private wholesalers' prices [21].

For patients covered by the Community Based Health Insurance (CBHI), the annual insurance fees per person as well as their co-payments for health services and medicines depend on the socio-economic class of the family, known as "Ubudehe" category. Persons in categories 1 and 2 (24% of the total population) do not pay any fees or co-payments for health services. Persons in the higher categories 3-6 pay annual insurance fees as well as a flat fee of 200 RWF at the health center level, and 10% of each bill (consultation, diagnosis and medicines) at the hospital level as co-payments. At the end of the month, each partner health facility submits an invoice to the Rwanda's CBHI for payment of the uncovered fees, for treated patients during the month [22]. The historical development of this community-based health insurance in Rwanda has been described in a study by the University of Rwanda and Management Sciences for Health [23].

Considering the excellent health insurance coverage in Rwanda [21], treatments with the medicines investigated in this study were affordable in the public and faith-based sectors, setting a positive example for other LMICs in the strive for UHC. However, in the private sector treatments for chronic diseases, such as amitriptyline, captopril, metformin, salbutamol inhaler and simvastatin, were largely unaffordable. The same situation was found in the private sector

of 36 low- and middle-income countries [24]. This is a serious financial problem for the patients who have to take these medicines for long time periods, usually for life.

This study identified a key issue for patients in Rwanda, that is, that the availability of medicines in the public and faith-based sectors was often low, forcing them to buy in the private sector at far higher prices and no CBHI scheme coverage. The reasons for this sub-optimal availability needs to be identified, and actions taken to improve the situation. In addition, systems need to be put into place for regularly monitoring the availability, prices and affordability of key essential medicines in the public, private and faith-based sectors of Rwanda.

Limitations of the study

The study included a small range of medicines, few of which had originator brands registered in the country. It was originally intended to include also private clinics into this study. However, in Rwanda private clinics are not supposed to store medicines except for few emergency medications, therefore private clinics had to be excluded from the study. Medicine prices in Rwanda were collected in 2019, but the latest available version of the MSH-IMPPG was for 2015. In addition, this survey was conducted at one point in time only, so it does not take into account availability and prices changes over time.

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Supporting information

S1 Table: Availability of surveyed medicines

Class of medicines/ indication	Surveyed medicines		Originator Brand			Lowest Price generic			Originator and lowest price generic Combined		
	Medicine Name	**Medicine list**	**Public (n=15)**	**Private (n=12)**	**Faith-based (n=17)**	**Public (n=15)**	**Private (n=12)**	**Faith-based (n=17)**	**Public (n=15)**	**Private (n=12)**	**Faith-based (n=17)**
Medicines for MCCH	Tranexamic acid injection	Supplementary				0.0%	8.3%	0.0%	0.0%	8.3%	0.0%
	Paracetamol suspension	Global				80.0%	100.0%	76.5%	80.0%	100.0%	76.5%
	Oxytocin injection	Supplementary				73.3%	8.3%	82.4%	73.3%	8.3%	82.4%
	Misoprostol	Supplementary	66.7%	41.7%	40.0%	33.3%	0.0%	60.0%	100.0%	41.7%	100.0%
	Levonorgestrel	Supplementary	0.0%	50.0%	0.0%	0.0%	41.7%	0.0%	0.0%	58.3%	0.0%
	Average for medicines for MCCH		33.3%	45.8%	20.0%	37.3%	31.7%	43.8%	50.7%	43.3%	51.8%
Medicines against NCDs	Simvastatin	Global	0.0%	0.0%	0.0%	0.0%	33.3%	0.0%	0.0%	33.3%	0.0%
	Salbutamol inhaler	Global	20.0%	83.3%	23.5%	60.0%	41.7%	52.9%	73.3%	100.0%	76.5%
	Omeprazole	Global				66.7%	91.7%	80.0%	66.7%	91.7%	80.0%
	Metformin	Global	6.7%	50.0%	0.0%	60.0%	100.0%	64.7%	60.0%	100.0%	64.7%
	Diclofenac	Global				80.0%	50.0%	82.4%	80.0%	50.0%	82.4%
	Diazepam	Global	0.0%	0.0%	0.0%	80.0%	50.0%	76.5%	80.0%	50.0%	76.5%
	Captopril	Global				80.0%	83.3%	100.0%	80.0%	83.3%	100.0%
	Amitriptyline	Global				73.3%	91.7%	70.6%	73.3%	91.7%	70.6%
	Average for medicines against NCDs		6.7%	33.3%	5.9%	62.5%	67.7%	65.9%	64.2%	75.0%	68.8%
Antibiotics	Metronidazole	Supplementary	0.0%	8.3%	0.0%	100.0%	83.3%	94.1%	100.0%	83.3%	94.1%
	Co-trimoxazole suspension	Global	0.0%	0.0%	0.0%	46.7%	100.0%	64.7%	46.7%	100.0%	64.7%
	Ciprofloxacin	Global				66.7%	100.0%	70.6%	66.7%	100.0%	70.6%
	Ceftriaxone injection	Global				66.7%	83.3%	80.0%	66.7%	83.3%	80.0%
	Amoxicillin	Global				26.7%	100.0%	11.8%	26.7%	100.0%	11.8%
	Average for antibiotics		0.0%	4.2%	0.0%	61.3%	93.3%	64.2%	61.3%	93.3%	64.2%
	Average for all medicines		11.7%	29.2%	7.9%	55.2%	64.8%	59.3%	59.6%	71.3%	62.8%

S2 Table: Medicines prices expressed as Median Price Ratios (MPRs)

Class of medicine	Medicine name	Originator brand				Lowest-price generic			
		Govt. Procurement MPR	Patient MPR			Govt. Procurement MPR	Patient MPR		
			Public (n=15)	Private (n=12)	Faith-based (n=17)		Public (n=15)	Private (n=12)	Faith-based (n=17)
Antibiotics	Amoxicillin capsule 500mg					0.81	1.09	1.85	
	Ceftriaxone injection 1g					1.55	2.24	1.67	2.24
	Ciprofloxacin tablet 500mg					0.58	0.84	1.49	1.00
	Co-trimoxazole suspension 8+40mg/ml					0.88	1.00	1.27	1.03
	Metronidazole tablet 250mg					0.85	1.19	1.99	1.27
Medicines against NCDs	Amitriptyline tablet 25mg					0.38	0.59	3.96	0.55
	Captopril tablet 25mg					0.44	0.91	1.58	0.84
	Diazepam tablet 5mg					0.41	0.75	2.60	0.81
	Diclofenac tablet 50mg					0.54	0.75	3.08	0.75
	Metformin tablet 500mg			4.43		0.81	0.89	3.69	0.96
	Omeprazole capsule 20mg					0.66	1.19	3.93	1.16
	Salbutamol inhaler 100mcg/dose			1.48	1.18	0.81	1.31	1.51	1.31
	Simvastatin tablet 20mg							14.96	
Medicines for MCH	Levonorgestrel tablet 1.5mg			11.48				5.40	
	Misoprostol tablet 200mcg			3.67		0.70			
	Oxytocin injection 10IU/ml					1.76	2.25		2.14
	Paracetamol suspension 24mg/ml					0.50	0.83	1.60	0.83
	Tranexamic acid injection 100mg/ml								
Median	All medicines			4.05	1.18	0.70	0.96	1.99	1.00

S3 Table: Medicines affordability expressed as amount of local currency (Rwf) and number of day's wages required for one course of treatment

Surveyed medicines		Originator brand						Lowest-price generic					
		Public (n=15)		Private (n=12)		Faith-based (n=17)		Public (n=15)		Private (n=12)		Faith-based (n=17)	
Class of medicines	Treatment	Median Treatment Price (Rwf)	Days' Wages	Median Treatment Price (Rwf)	Days' Wages	Median Treatment Price (Rwf)	Days' Wages	Median Treatment Price (Rwf)	Days' Wages	Median Treatment Price (Rwf)	Days' Wages	Median Treatment Price (Rwf)	Days' Wages
Antibiotics	Amoxicillin capsule 500mg x21							617.4	0.6	1050.0	1.1		
	Ceftriaxone injection 1g x1							803.5	0.8	600.0	0.6	803.8	0.8
	Ciprofloxacin tablet 500mg x14							394.8	0.4	700.0	0.7	470.4	0.5
	Co-trimoxazole suspension 8+40mg/ml 70ml							303.8	0.3	385.0	0.4	312.2	0.3
	Metronidazole tablet 250mg x42							275.9	0.3	459.9	0.5	294.0	0.3
Medicines against NCDs	Amitriptyline tablet 25mg x90							405.0	0.4	2700.0	2.7	375.4	0.4
	Captopril tablet 25mg x60							1210.5	1.2	2100.0	2.1	1122.0	1.1
	Diazepam tablet 5mg x7							45.5	<0.1	157.5	0.2	49.0	<0.1
	Diclofenac tablet 50mg x60							181.5	0.2	750.0	0.8	183.0	0.2
	Metformin tablet 500mg x90			5400.0	5.4			1080.0	1.1	4500.0	4.5	1170.0	1.2
	Omeprazole capsule 20mg x30							452.9	0.5	1500.0	1.5	440.9	0.4
	Salbutamol 100mcg/dose 200 dose inhaler			2450.0	2.5	1954	2.0	2178.0	2.2	2500.0	2.5	2178.0	2.2
	Simvastatin tablet 20mg x30									6600.0	6.6		
Medicines for MCH	Levonorgestrel tablet 1.5mg x1			8500.0	8.5					4000.0	4.0		
	Misoprostol tablet 200mcg x4			2400.0	2.4								
	Oxytocin injection 10IU/ml x1ml							316.0	0.3			300.9	0.3
	Paracetamol suspension 24mg/ml x45ml							175.0	0.2	337.5	0.3	174.2	0.2
	Tranexamic acid injection 100mg/ml												

Chapter three: Stability of Oxytocin Preparations in Malawi and Rwanda: Stabilizing Effect of Chlorobutanol

ABSTRACT

Oxytocin is used for the prevention and treatment of postpartum hemorrhage, the leading cause of maternal mortality in low- and middle-income countries. Because of the high instability of oxytocin, most products are labeled for storage at 2–8°C. Some other products are on the market which are labeled for non-refrigerated storage, but independent evaluations of their stability hardly exist.

In the present study, seven brands (nine batches) of oxytocin were purchased from wholesalers and medical stores in Malawi and Rwanda and investigated by accelerated stability testing according to the ICH/WHO guidelines. Two oxytocin brands approved by a stringent regulatory authority (SRA) or by the WHO Prequalification of Medicines program and purchased in Europe were used as comparison.

All investigated brands which were either produced in countries with SRAs, or were WHO-prequalified products, were labeled for storage at 2–8°C, and all of them passed stability testing with very good results. Even exposure to 25°C or 30°C for several months hardly affected their oxytocin content. However, two other investigated brands were labeled for non-refrigerated storage, and both of them had been produced in countries without SRAs. These two preparations showed not higher but lower stability than the brands labeled for storage at 2–8°C, and, for both of them, noncompliance with pharmacopoeial specifications was found after accelerated stability testing. At 40°C, and in forced degradation studies at 80°C, chlorobutanol showed a remarkable stabilizing effect on oxytocin, which may deserve further investigation.

The results of the present study support the policy "Buy Quality Oxytocin, Keep It Cool."

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No data were changed, only minor edit adjustments were made to fit the paper into the Microsoft word format and accommodate comments from panelists.

3.1. INTRODUCTION

Oxytocin is listed in the essential medicines list of the WHO[1] and is used for the prevention and treatment of postpartum hemorrhage (PPH).[2] Postpartum hemorrhage is the leading cause of maternal mortality in low-income countries.[2] One of the sustainable development goals of the United Nations is "to reduce the global maternal mortality ratio to less than 70 per 100,000 live births by 2030."[3] To achieve this, prevention and treatment of PPH with oxytocin is an essential step.

Several previous studies have shown that the quality of oxytocin, especially in low- and middle-income countries (LMICs), is often poor.[4–13]This may result from poor manufacturing (e.g., inappropriate formulation or packaging), from poor storage and/or transportation conditions, or from a combination of these factors. The nonapeptide oxytocin is known to be very sensitive to high temperatures.[4,14–17] The typical degradation mechanisms are shown in Figure 1. If oxytocin is not stored properly, then it may lose its potency, which can result in higher mortality rates of PPH.

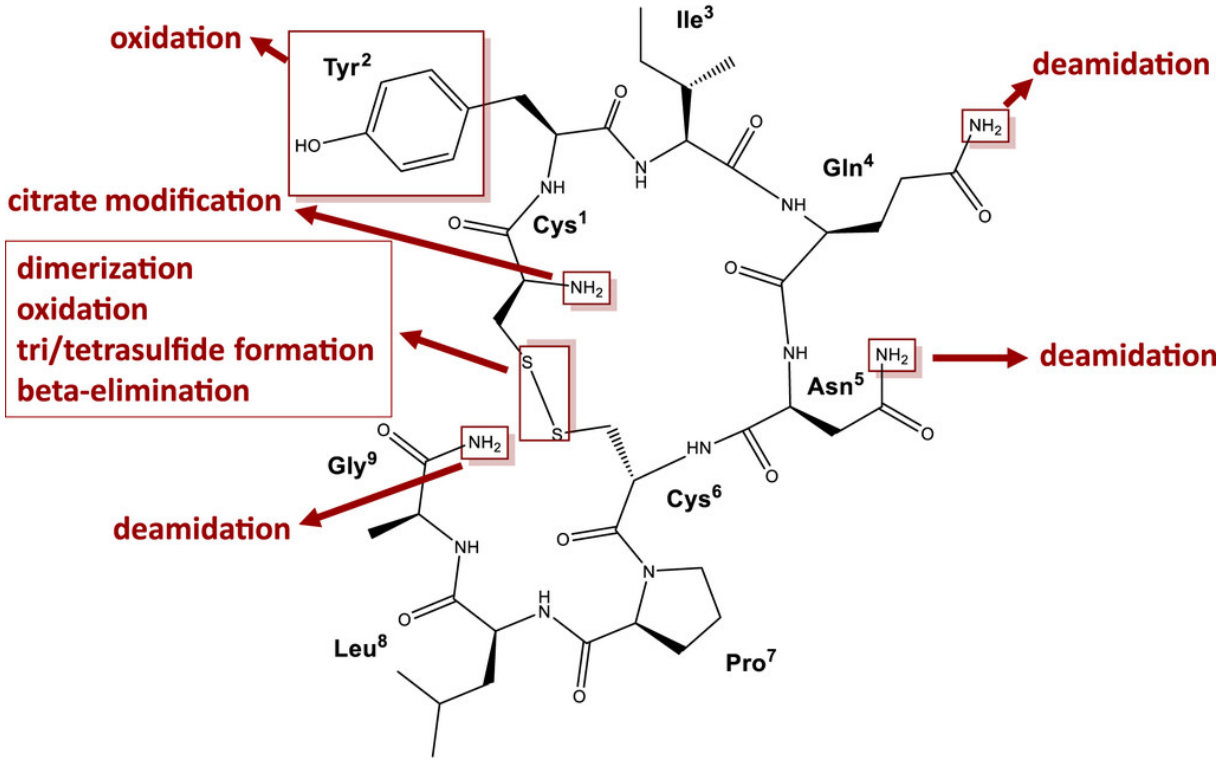

Figure 1. Structure of oxytocin and its typical degradation mechanisms. Modified from Avanti et al.[39]

Most of the currently marketed oxytocin products have to be stored at 2–8°C according to the labeling information. Maintaining this storage temperature can present a challenge, especially

in rural health facilities in LMICs.[6,9,13,18,19] Several oxytocin preparations are now on the market which are labeled for non-refrigerated storage. However, according to a report of the Reproductive Health Supplies Commission, there are nearly 300 different oxytocin products, offered by at least 100 manufacturers, and there is considerable confusion about the storage and labeling requirements for these.[20] Similar concerns were raised by the WHO in a report on the quality of medicines for maternal and child health, which concluded for oxytocin injections: "It would be useful to verify to which extent manufacturers' instructions for higher storage temperatures were based on reliable stability studies."[11] The Promoting the Quality of Medicine Program of the U.S. Pharmacopeia stated a need to conduct "systematic stability studies […] in accordance with the ICH guidelines […], to reassess the specifications for storage and shelf life of oxytocin injections."[21] It was with these recommendations in mind that we decided to investigate the stability of selected oxytocin preparation according to the ICH guidelines (see Methods).

The stability of pharmaceutical products must be investigated and documented by the manufacturers, and National Medicines Regulatory Authorities (NMRAs) usually do not confirm these data independently. Countries with the so-called stringent regulatory authorities (SRAs) are usually trusted to ensure complete and correct stability testing by the manufacturers. Stringent regulatory authorities are NMRAs who are members, observers, or associates of the International Council of Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) and currently comprise the national regulatory authorities of the EU member states, the United States, Japan, Switzerland, Canada, Australia, Iceland, Liechtenstein, and Norway.[22] In addition, the WHO prequalification of medicines program (now called "WHO Prequalification Team: medicines") prequalifies finished pharmaceutical products for certain indications, including reproductive and maternal health, based on information submitted by manufacturers and on inspections of the corresponding manufacturing sites.[23,24]

However, many medicines circulating in LMICs are neither approved by a stringent regulatory authority (SRA) nor WHO prequalified. National regulatory authorities of LMICs often have limited capacity for independent evaluation and analysis[25,26] and need to rely only on the manufacturers' data when registering medical products. Independent scientific confirmations of manufacturers' claims, for example, on heat-stability of oxytocin preparations, are therefore important.

The ICH has formulated guidelines for stability testing of pharmaceutical products (Q1A-Q1F).[27] Testing a product's stability over its entire shelf life is very time consuming, and it was not applicable in the context of this study. Therefore, accelerated stability studies for a shorter time, but at higher temperature and relative humidity (RH), that are also permissible and frequently used for the prediction of shelf life and storage requirements, were used to fit the context of this particular study. The precise conditions for accelerated stability studies depend on the intended storage recommendation, as well as on the climatic zone of the country where the medicine will be registered, as specified by the ICH[28] and by the WHO[22] guidelines. Malawi is currently assigned to WHO climatic zone II (subtropical and Mediterranean) and Rwanda to zone IVa (hot and humid).[29]

The so-called forced degradation studies[30] are carried out at even higher temperatures. They were used in the current study to investigate the influence of excipients such as buffers and the widely used bacteriostatic agent chlorobutanol on the stability of oxytocin.

3.2. Materials and Methods

3.2.1. Study design and ethical approval

This study followed the WHO guidelines on the conduct of surveys of the quality of medicines[31] and the MEDQUARG guidelines,[32] where applicable. Ethical clearance to conduct this study was received from the College of Medicine Research and Ethics Committee in Malawi (Reference no. P.07/27/2215). Approval was also granted by the Malawi National Regulatory Agency (Pharmacy, Medicines and Poisons Board [PMPB]), as well as by the Ministry of Health, Rwanda (Reference no. 20/1361/DGPHFIS/2018).

3.2.2. Sample collection

In Malawi and Rwanda, all brands of oxytocin available during the time of sample collection (February–March 2018) at government and faith-based medical stores (i.e., at the Central Medical Stores Trust in Malawi, the Rwanda Biomedical Center/Medical Production, Procurement and Distribution Division of Rwanda, and the Bureau des Formations Médicales Agréées du Rwanda in Rwanda) and from all pharmaceutical wholesalers located in Blantyre and Lilongwe (Malawi) and in Kigali (Rwanda) were purchased by local researchers (F. K. and T. B.). For comparison, additional samples were purchased from the pharmacy of the university hospital in Tübingen, Germany, and from an international wholesaler (Imres B.V., Lelystad, The Netherlands).

A mystery shopper approach was used when ordering from commercial wholesalers. An overt approach was used when samples were collected in person from government or faith-based medical stores. For each brand, 100 vials of oxytocin were purchased. If different batches of a certain brand were available, then samples from each batch were purchased. After purchase, samples were stored according to the manufacturers' specifications, and temperature loggers were kept with all samples until their placement in stability chambers.

Samples were hand-carried by the investigators from Malawi or Rwanda to Germany via airplane, with traveling time of less than 24 hours. At the Pharmaceutical Institute of Tübingen University, samples were pretested for assay and pH according to the U.S. Pharmacopeia. All samples that were within specifications at the time of pretesting were included into the stability study.

3.2.3. Storage in stability chambers

Samples were stored in four different stability chambers (Table 1) for 6 months (April–October 2018) at Alpha-Pharma-Service GmbH, Heilbronn, Germany. Conditions were chosen in

accordance with the ICH and WHO guidelines for stability testing of pharmaceutical products.[22,28] At different time points (months 0, 1, 2, 3, and 6), samples were withdrawn from the chambers and analyzed at the Pharmaceutical Institute at Tübingen University, Germany.

Table 1: Conditions for accelerated stability testing and controls

		ICH/WHO* stability testing conditions	
Temperature (°C)	**Relative humidity (%)**	**For refrigerated products**	**For non-refrigerated products**
5 ± 3	n. def.	Long term	–
25 ± 2	60 ± 5	Accelerated	Long term, zone II
30 ± 2	65 ± 5	Accelerated, more severe*	Long term*, zone IVa, intermediate
40 ± 2	75 ± 5	–	Accelerated

n. def. = not defined. According to the ICH/WHO,[22,28] long-term testing should cover the proposed shelf life or a minimum of 12 months at the time of submission. Stability testing under accelerated or intermediate conditions should cover a minimum of 6 months.

*In addition to the conditions listed in the ICH guidelines, the WHO guidelines list also more severe conditions for products that are intended to be marketed in climatic zones III–IV. Malawi is assigned to climatic zone II and Rwanda to climatic zone IVa.[29]

3.2.4. Sample analysis.

At the Pharmaceutical Institute of Tübingen University, Germany, samples were visually inspected, and tested for identity, assay, and pH value according to the monograph of the U.S. Pharmacopoeia (USP 40, oxytocin injections). Methods were validated according to USP 40. Analysis was conducted via high-performance liquid chromatography (HPLC, Agilent Infinity 1260 II with a binary pump, a variable wavelength detector, a refrigerated autosampler, and an integrated column compartment; Agilent Technologies, Santa Clara, CA). Solvents were HPLC grade.

For analysis, a gradient system of mobile phase A (0.1 M NaH_2PO_4 buffer) and mobile phase B (ACN: H_20 1:1 V/V) with the following gradient was used: 0 minutes, 30% B; 10 minutes, 40% B; 17.5 minutes, 65% B; 20.5 minutes, 65% B; 23.5 minutes, 30% B; 26 minutes, 30% B; flow rate, 1.5 mL/minute; injection volume, 70 µL; column with guard from Dr. Maisch GmbH,

Ammerbuch, Germany (Reprospher 100: 12.5 cm × 4.6 mm, 5 µm C18); and detection at 220 nm. The U.S. Pharmacopoeia Reference Standard (batch N° F3K133) was obtained from Merck KGaA, Darmstadt, Germany. pH values were tested with a pH-combination microelectrode (N 6000 BNC) from SI Analytics GmbH, Mainz, Germany. From each sample, three vials were analyzed.

Five-point calibration curves were prepared at each time point (i.e., months 0, 1, 2, 3, and 6) to assure linearity. Intermediate precision[33] was calculated from the data of the calibration curves of the reference standards, which showed relative SDs (RSDs) of less than 2.1% for the reference solution corresponding to 100% of the declared content (Supplemental Table S3).

3.2.5. Forced degradation study.

Both the ICH and WHO guidelines mention forced degradation studies (sometimes referred to as stress testing); however, they do not fix specific conditions under which forced degradation studies should be performed. In the present study, the conditions described by Blessy et al.[30] in 2013 were used. The commercial preparations of oxytocin were investigated in their original glass vials, and the commercial preparation of oxytocin from Hexal with added chlorobutanol (5 and 1.5 mg/mL) was investigated in tightly sealed flasks. Furthermore, solutions prepared from solid synthetic oxytocin (Sigma-Aldrich/Merck, Taufkirchen, Germany; Batch N° 103H05241; purity ≥ 97 % by HPLC), in a concentration of 10 IU/mL in either distilled water or 0.2 M sodium acetate buffer pH 4.6, both either with or without addition of chlorobutanol (1.5 or 5 mg/mL), were prepared and investigated.

All samples were stored for 5 days at 80 °C. After 24, 72, and 120 hours, one sample of each formulation was removed from the heat chamber and stored at 4 °C until analysis. For each formulation, a sample stored at 4 °C was used as control. Analysis was carried out as described previously.

3.2.6. Registration status of medicine brands.

The PMPB of Malawi was contacted to inquire the registration status of the medicines collected in Malawi. For the preparations by Ningbo Pharma Biotech Co. Ltd., Ningbo, Zhejiang, China, Umedica Laboratories Pvt. Ltd., Mumbai, India, and Ciron Drugs and Pharmaceuticals Pvt. Ltd., Mumbai, India, it was confirmed that they were registered, but registration could not be confirmed for the product by Biologici Italia Laboratories S.r.l., Masate, Italy. The Rwanda Food and Drug Authority (RFDA) was contacted to inquire the registration status of the medicines collected in Rwanda. However, the RFDA's process of full registration of medicines

had not yet been in effect at the time of sample collection. It could not be confirmed which of the collected preparations were preregistered according to the previous procedures.

The preparations of Biologici Italia Laboratorie S.r.l., of Rotexmedica GmbH, Trittau, Germany and of AS Grindeks, Riga, Latvia, were also registered in European countries, as was the preparation of Hexal AG, Holzkirchen, Germany. However, no oxytocin preparation by Laboratoires Sterop, Brussels, Belgium, was found in the medicine registers of the European Heads of Medicines Agencies (https://mri.cts-mrp.eu/Human/) and in the national medicines register of Belgium (https://banquededonneesmedicaments.afmps-fagg.be/#/query/human/), indicating that the Sterop preparations collected in Rwanda are manufactured for export only.

3.2.7. Statistical analysis.

Statistical evaluation was carried out using JMP 14.2 (SAS GmbH, Heidelberg, Germany). Significance of differences between active pharmaceutical ingredient (API) content and pH values at different conditions and months were calculated using uni- and multivariate analysis of variance and Student's ***t***-test.

3.2.8. Information of national authorities and stakeholders.

This article was shared with the PMPB of Malawi, with the RFDA, and with the WHO Rapid Alert System. In addition, the results were presented to the PMPB, the Malawi Central Medical Stores Trust, the Ministry of Health and national and international stakeholders during a meeting in Lilongwe, Malawi, on September 4, 2019. First results of this study were also presented in a lecture by L. H. during a visit to the RFDA on December 4, 2018.

3.3. RESULTS

3.3.1. Overview of investigated oxytocin samples.

Nine brands of oxytocin, that is, five brands in Malawi and four in Rwanda, were collected. Two of the purchased brands had to be excluded from the subsequent investigation: oxytocin inj. 5 IU/mL from MACIN Remedies Ltd., Moga, India, collected in Malawi, was not available in a sufficient amount for stability testing. Oxytocin inj. 10 IU/mL from Jiangxi Xierkangtai Pharmaceutical Co. Ltd., Pingxiang, Jiangxi, China, collected in Rwanda, showed a large peak of an undeclared substance (later identified as the commonly used preservative benzyl alcohol[34]) in HPLC analysis, which precluded precise quantitative analysis of oxytocin because of lack of baseline separation of the closely adjacent HPLC peaks of benzyl alcohol and oxytocin. Therefore, seven brands collected in Malawi and Rwanda were included into the subsequent stability testing. For two of these brands, two different batches were offered at the time of collection, and, in each case, both batches were collected and investigated.

As a comparison, two further oxytocin preparations were purchased in Europe: one preparation which is commonly used in Germany, purchased through the pharmacy of the University Hospital Tübingen, and one of the two oxytocin preparations which had been prequalified by the WHO at the time of sample collection; the latter preparation was purchased from the manufacturer in Latvia via Imres B.V. because it was not registered in Germany. A different batch of the latter brand had also been purchased in Rwanda.

Therefore, as shown in Table 2, eight brands (total 11 batches) were included into the stability testing. Four of these brands had been produced in Belgium, Germany, or Italy, that is, in countries with an SRA.[22] As mentioned, one further brand was produced in Latvia and represented a WHO-prequalified product.[35] The other three brands were from India and China, that is, from countries without an SRA. Six of the eight brands were labeled for storage at 2–8 °C, whereas two brands, produced in China and India, were labeled for storage “below 25 °C” and “not exceeding 30 °C,” respectively (Table 2).

The declared shelf life of the different products varied between 2 and 4 years, and all samples remained within their shelf life during the entire duration of the study. Pretesting for assay and pH showed that 10 of the 11 samples were clearly within USP 40 specifications at the beginning of the stability study. One sample (Ningbo batch no. 160802) showed an assay value of 89.0 %. Given the RSD of this measurement (RSD = 1.41 %; Supplemental Table S1), this deviation

from the pharmacopoeial limits (90–110%) was not statistically significant ($\boldsymbol{P} = 0.236$); therefore, this preparation was not classified as being out of specifications.

Table 2: Investigated oxytocin samples

Collected in:	Stated manufacturer (and brand name, if not marketed under INN name)	Country of manu-facture	Expiry date	Batch number	Stated shelf life (years)	Stated storage requirements (°C)	Stated (or detected) excipients								Pre-qualification status
							Water for inj.	Chlorobutanol (mg/ml)	Sodium acetate	Acetic acid	Sodium hydroxide	Sodim chloride	Citric acid	Ohters	
Malawi	Ningbo Pharma Biotech Co., Ltd (WW-Oxy 10)	China	August 19	160802	3	Less than 25	+	-	-	-	-	-	-	+*	none
			January 19	160183											
	Umedica Laboratories PVT. LTD	India	January 20	JA802	2	not exceeding 30	+	1.5†	-	-	-	-	-	+*	none
	Ciron Drugs AND Pharmaceuticals Pvt. Ltd.	India	August 19	7EA01228	2	2-8	+‡	5†	-	-	-	-	-	-	none
	Biologici Italia Laboratories S.r.l.	Italy	March 19	UF602ON	3§	2-8	+	-	+	+	+	-	-	-	SRA
Rwanda	Laboratoires STEROP	Belgium	August 19	160269	3	2-8	+	5	-	-	-	+	+	-	SRA
	Laboratoires STEROP (Steroxine 10 IU/1ml)‖		January 19	160042											
	Rotexmedica GmbH Arzneimittelwerk	Germany	September 20	70779A	3	2-8	+	-	+	+	-	+	-	-	SRA
	AS GRINDEKS	Latvia	November 20	37711116	4	2-8	+	-	+	+	+	+	-	-	WHO-PQ
Europe	AS GRINDEKS		September 21	38910917											
	Hexal AG	Germany	Junuary 20	HC0075	3¶	2-8	+	-	-	+	-	+	-	-	SRA

WHO-PQ = WHO-prequalified product; SRA = produced in a country with stringent regulatory authority. All samples represented 1 mL vials with a stated content of 10 IU oxytocin/mL.

*High-performance liquid chromatography (HPLC) analysis showed additional peaks, indicating additional, unidentified ingredients.

†Chlorobutanol not declared but detected in HPLC analysis.

‡Water for inj. not explicitly declared.

§Manufacturing date not stated on the packaging; information received from the websites of the British Medicines and Healthcare products Regulatory Agency (http://www.mhra.gov.uk) and the Irish Health Products Regulatory Authority (http://www.hpra.ie/).

‖One branded and one unbranded generic oxytocin product of Laboratoires STEROP were found in Rwanda, with identical composition.

¶Manufacturing date not stated on the packaging but received from the manufacturer.

3.3.2. Accelerated stability studies of oxytocin

Table 2 also shows the stated (or detected) excipients in the investigated preparations. For optimal stability of oxytocin, USP 40 specifies that the pH value must be between 3.0 and 5.0. This is usually achieved by the inclusion of, for example, acetate or citrate buffers.[36–39] For the WHO-prequalified preparation, and for the four preparations produced in countries with an SRA, the presence of such buffering substances was correctly declared on the packaging. According to the label information, four of these five preparations also contained sodium chloride (usually included for tonicity). For the Sterop preparation, in addition, the presence of 5 mg/mL of the bacteriostatic agent chlorobutanol was declared.

In clear contrast, for the three preparations from China and India, no excipients were stated on their packaging beyond water for injection (for the Ciron preparation, not even water was stated). Nevertheless, their pH value was found to be in the correct range of 3.0–5.0, and remained so during stability testing, which may indicate the presence of undeclared buffering agents. Furthermore, HPLC analysis showed the (undeclared) presence of 1.5 and 5.0 mg/mL chlorobutanol in the preparations by Umedica and Ciron, respectively. Chlorobutanol can be readily detected in the HPLC analysis.

3.3.3. Accelerated stability studies of oxytocin.

All eight brands (11 batches) listed in Table 2 were subjected to accelerated stability testing according to the current WHO guidelines for stability testing of finished pharmaceutical products.[22] Stability chambers used, operated by a commercial company specialized in pharmaceutical stability testing, also complied with the specifications for RH of the WHO guidelines, although this is not relevant for the investigated oxytocin preparations which were packaged as aqueous solutions in sealed glass vials which are considered to be moisture impermeable.[22]

The evaluation criteria for stability testing are described in full detail in the mentioned WHO guidelines,[22] but may be summarized as follows: pass/fail decisions of accelerated stability testing are made on the basis of two criteria: 1) for 6 months, the sample must remain within specification, that is, in the present study, oxytocin samples still had to show an API content between 90 % and 110 % of the declared amount (as specified by the USP, International Pharmacopeia, and British Pharmacopeia), and a pH value between 3.0 and 5.0; 2) no

significant change of the API content must occur within 6 months, that is, no change of the initial content of the API by 5 % or more.[22,28]

Obviously, the temperature conditions which the oxytocin preparations must be able to withstand in accelerated stability testing are related to the storage recommendations which the manufacturer states on the label[22]: products labeled for refrigerated storage (2–8 °C) must demonstrate their stability for 6 months at 25 °C or 30 °C, and the decision for either of these two temperatures should be "based on a risk-based evaluation." As described in the following text, in the present study, results obtained at 25 °C and at 30 °C were similar. Products labeled as "do not store above 25 °C," such as the oxytocin preparation by Ningbo (Table 2), must demonstrate their stability for 6 months at 30 °C ("intermediate" condition, tested in case they failed at 40 °C). And products labeled as "do not store above 30 °C," such as the oxytocin preparation by Umedica (Table 2), must demonstrate their stability for 6 months at 40 °C.

Figure 2 shows the oxytocin content of all eight brands listed in Table 2 after 6 months of storage at 5 °C, 25 °C, 30 °C, or 40 °C. Clearly, the WHO-prequalified preparation as well as the four preparations produced in countries with an SRA passed this stability test, showing oxytocin contents between 97.4 % and 109.3 % of the declared amount after 6 months at 30 °C. Supplemental Table S1 lists the individual assay values determined for each preparation at each time interval (0, 1, 2, 3, and 6 months); none of the mentioned five brands showed a significant change of the API content over 6 months at 30 °C; that is, all passed the stability testing also by this criterion. Supplemental Table S2 shows all pH values determined in this study. In all five mentioned preparations, the pH value remained well within specifications, that is, within the 3.0–5.0 range at 30 °C.

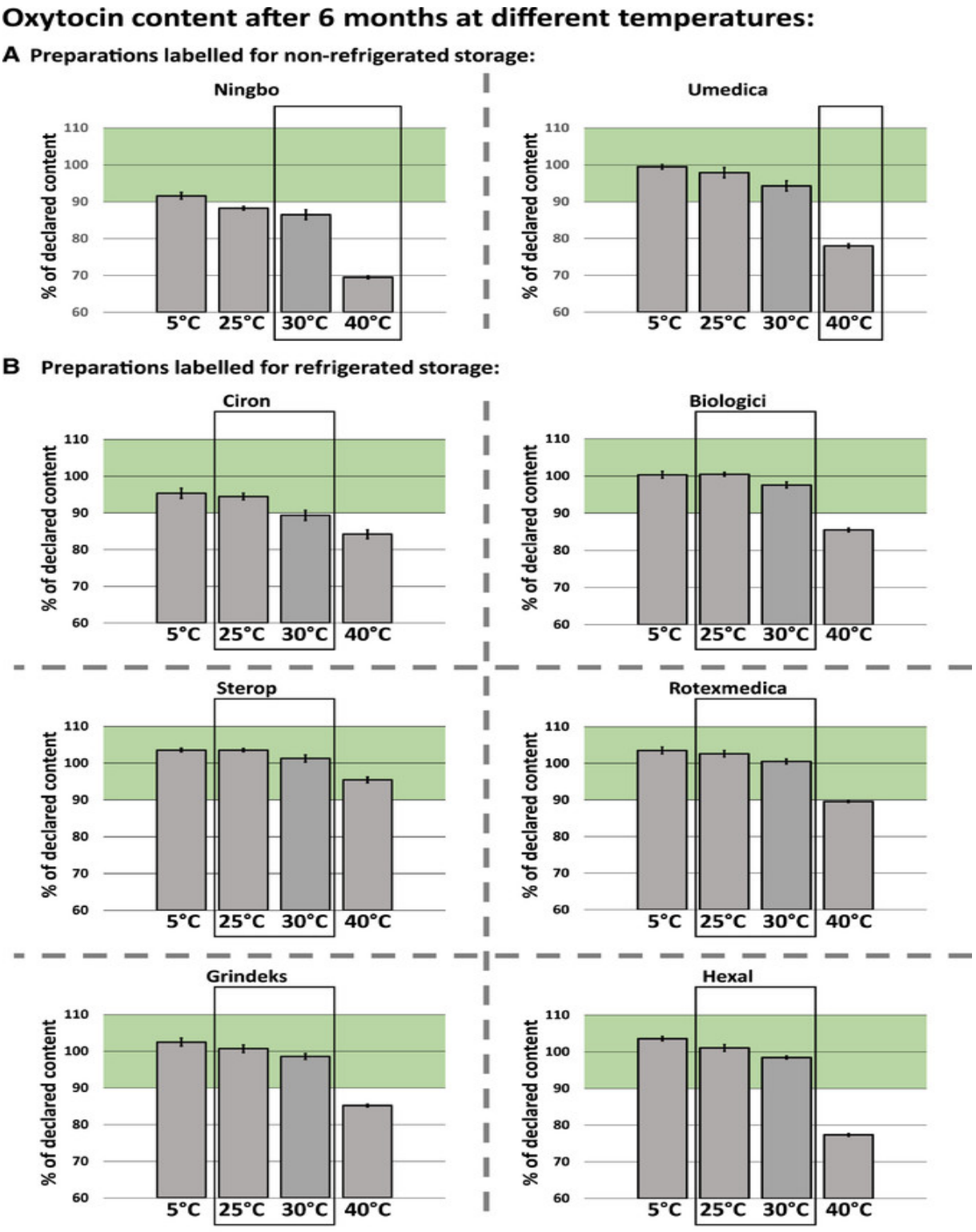

Figure 2: Accelerated stability testing of commercial oxytocin preparations: oxytocin content after 6 months at different temperatures. Full names of the manufacturers, and further details of the investigated preparations, are listed in Table 2. Results shown here were obtained with Ningbo batch N° 160802, Sterop batch N° 160269, and Grindeks batch N° 37711116 (see Table 2). The U.S. Pharmacopoeia specifies that the oxytocin content must be between 90 % and 110 % of the declared amount. This range is marked in the diagrams. Error bars show SD.

For the preparation by Ciron, an API content of 89.3 % of the declared amount was determined after 6 months at 30 °C. Given the RSD of this measurement (RSD = 1.55 %, Supplemental Table S1), this deviation from the pharmacopoeial limits (90–110 %) is not statistically significant (***P***= 0.459); therefore, this preparation was not classified as failing stability testing. The change of the API content of this preparation, and its pH value, was within the permitted limits.

A different picture emerged for the preparations by Ningbo and Umedica, that is, for the two preparations in this study which were labeled by their manufacturers as not requiring refrigerated storage. As immediately obvious from Figure 2, their stability at 30 °C and 40 °C was not better but rather lower than that of the preparations labeled for refrigerated storage. The product by Umedica was labeled for storage "not exceeding 30 °C" and, by WHO guidelines, therefore had to remain within specifications for 6 months at 40 °C. It clearly failed this test, showing a final API content of only 78.0 % of the declared amount, far outside of the 90–110 % range specified by the pharmacopoeias. Furthermore, it showed a 21 % loss of its API content over 6 months at 40 °C, grossly exceeding the permitted 5 % change. The preparation by Ningbo was labeled for storage "below 25 °C." Clearly, both investigated batches failed accelerated stability testing at 40 °C (final API content 69.5 % and 74.6 %, respectively; Figures 2 and 3 and Supplemental Figure S1) and, by WHO guidelines, therefore had to be tested at "intermediate conditions" of 30 °C. After 6 months at 30 °C, the API content of batch 160802 was found to be 86.5 % of the declared amount and was therefore out of specification, even though the change of the API content was 3 % and therefore within the permitted limit of 5 %. Batch 160183 showed an API content of 90.7 % after 6 months at 30 °C and therefore remained in specifications.

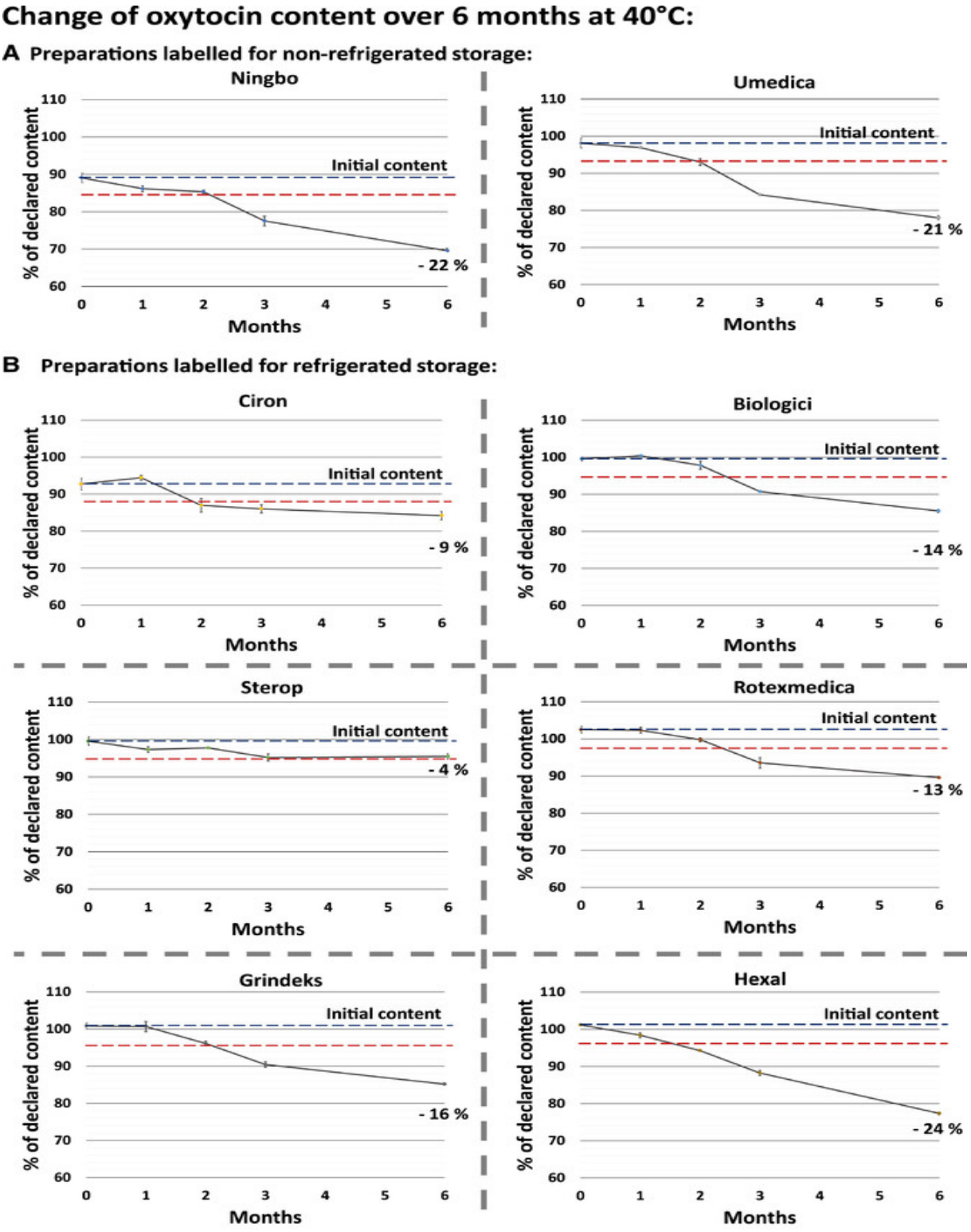

Figure 3: Accelerated stability testing of commercial oxytocin preparations: change of oxytocin content over 6 months at 40°C. Full names of the manufacturers, and further details of the preparations, are listed in Table 2. Results shown here were obtained with the same batches as shown in Fig. 2. In stability testing, a "significant change" of the API content is defined as a 5 % change of the initial content.[22,28] Initial content and initial content minus 5 % are shown as dashed lines. Error bars show SD. Final loss of content after 6 months is calculated relative to the initial content.

The different stability of the investigated preparations under temperature stress conditions is clearly visible in Figure 3, which depicts the time course of the loss of the API content over 6 months at 40 °C. This temperature is a more extreme condition than recommended for stability testing of preparations labeled for storage at 2–8 °C; therefore, the data depicted in Figure 3 are not directly relevant for pass/fail decisions for most of the preparations, but are still interesting in terms of the stabilizing effect of certain excipients. The most striking observation from these data is that the highest stability of oxytocin was shown by the two products which contained 5 mg/mL of the bacteriostatic agent chlorobutanol. However, in clear contrast to the other investigated products, these two preparations showed marked changes of their pH values, reaching a final value of 2.8 in case of the Sterop product and 3.0 in case of the Ciron product (Supplemental Table S2).

As mentioned earlier, for three of the investigated oxytocin brands, two different batches had been collected, with different manufacturing and expiry dates. Supplemental Figure S1 shows the results of the stability testing for these additional three batches, depicted in the same way as in Figures 2 and 3. Notably, API losses measured for the two different batches of the same brand were very similar in all three cases, indicating that the observed differences in the stability of different brands were primarily because of differences in manufacturing and pharmaceutical formulation, not to differences in age or in pre-sampling storage conditions of the investigated products. Individual measurements for all investigated batches are shown in Supplemental Tables S1 and S2.

3.3.4. Thermal forced degradation studies of oxytocin.

The markedly different stability of the investigated preparations in accelerated stability testing (Figures 2 and 3) stimulated our interest to additionally conduct a forced degradation study at higher temperature, that is, at 80 °C for 5 days.[30] For two of the preparations (Umedica and Biologici; Table 2), the number of remaining vials was insufficient for this experiment, but for the other six brands, the results are shown in Figure 4A. In remarkable similarity to the results at 40 °C (Figure 3), the four preparations by Rotexmedica, Grindeks, Ningbo, and Hexal showed decreasing stability in that order, with API losses between 55.9 % and 67.5 % within 5 days. However, in sharp contrast to the results obtained at 40 °C, the highest losses in the API content after 5 days at 80 °C were shown by the two preparations by Ciron and Sterop, that is, the preparations containing 5 mg/mL chlorobutanol (API losses of 75.2 % and 82.4 %,

respectively). Conversely, after 1 day, at 80 °C, these two preparations still had clearly higher API contents than the other four (Figure 4A).

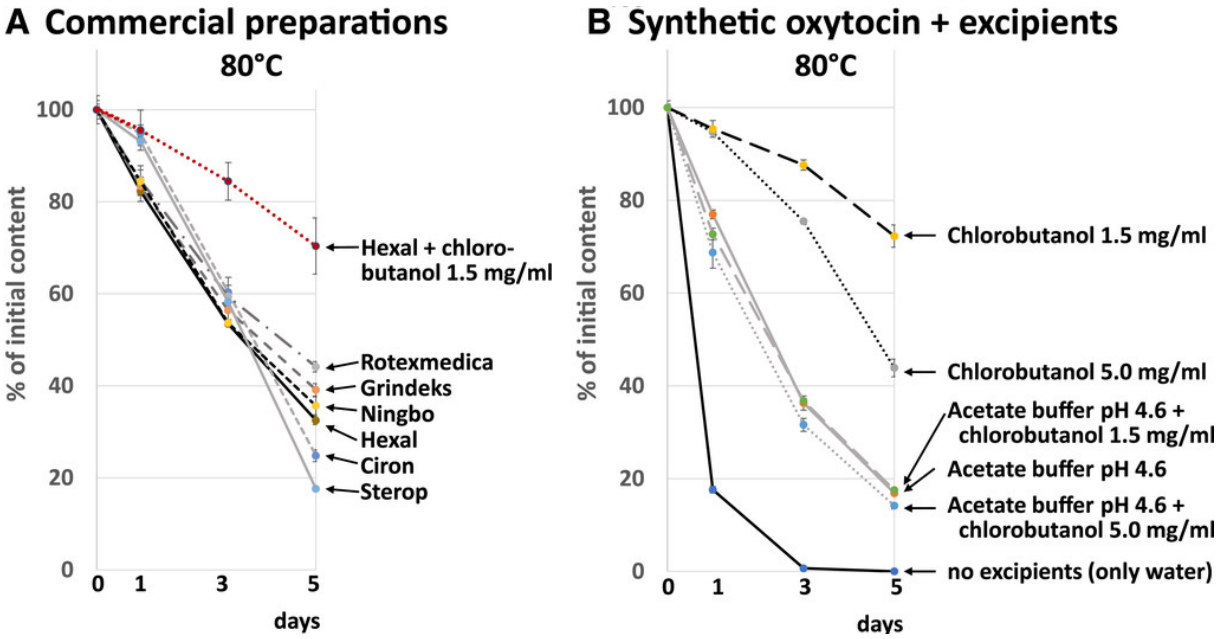

Figure 4: (**A**) Forced thermal degradation studies of commercial oxytocin formulations. Details of the investigated preparations are given in Table 2 and in Supplemental Tables S4 and S5. (**B**) Forced thermal degradation studies of solutions of oxytocin (Sigma-Aldrich/Merck; 10 IU/mL) in the presence of different excipients.

Chlorobutanol is known to undergo hydrolysis under elevated temperature, forming hydrochloric acid and other acidic reaction products.[40]This is likely to explain the change of pH of the preparations containing 5 mg/mL chlorobutanol, which had been observed already at 40 °C (Supplemental Table S2). As may be expected, this effect was even more pronounced at 80 °C, with both preparations showing a pH of 2.0 after 5 days, far out of the stability optimum of oxytocin at pH 4.5.[14]

To further investigate the effect of excipients on oxytocin stability, pure synthetic oxytocin in the solid form was purchased from Sigma-Aldrich/Merck and dissolved to a concentration of 10 IU/mL in water, with and without the addition of acetate buffer pH 4.6 (200 mM, i.e., as approximately isotonic solution) and/or chlorobutanol in concentrations of 1.5 mg/mL (8.5 mM) or 5 mg/mL (28 mM). These solutions were subjected to forced degradation at 80 °C, and the result is shown in Figure 4B. In pure water, oxytocin degraded completely, falling below the limit of detection within 5 days. This degradation is much more rapid than that observed for any of the investigated commercial preparations (Figure 4A). That further indicates that all commercial preparations may have contained buffering or stabilizing agents, even if not

declared on the label. The inclusion of acetate buffer had a stabilizing effect on oxytocin solution (83.1 % API loss within 5 days). Notably, the inclusion of 1.5 mg/mL chlorobutanol had an even stronger stabilizing effect: only 27.7 % of the API amount was lost over 5 days at 80 °C; the final pH value was determined as 2.8. Inclusion of chlorobutanol in a concentration of 5 mg/mL was less effective for stabilization (56.1 % API loss). In this case, the final pH value was found to be 2.2, which is more unfavorable for oxytocin stability.

The stabilizing effects of acetate buffer and chlorobutanol were not additive: in acetate buffer without chlorobutanol, oxytocin degraded with similar velocity as in acetate buffer with added chlorobutanol (1.5 or 5 mg/mL). In the latter experiments, the pH values of the solutions after 5 days at 80 °C were 4.6 and 4.5, respectively, indicating that the buffer capacity of 200 mM acetate buffer pH 4.6 was sufficient to compensate for the effect of any acidic hydrolysis products of chlorobutanol.

In a final experiment, chlorobutanol was added to the commercial oxytocin preparation of Hexal, and stability was investigated for 5 days at 80 °C. As shown in Figure 4A, the addition of 1.5 mg/mL greatly increased the stability of oxytocin under these conditions (29.6 % API loss, compared with 67.5 % in the absence of chlorobutanol; final pH 2.8). Adding 5 mg/mL chlorobutanol to the Hexal preparation was less effective for stabilization (API loss 68.4 %; Supplemental Table S4), consistent with an observed drastic change of the pH value (final pH 2.0; Supplemental Table S5). These results are similar to the ones observed with the synthetic oxytocin from Sigma-Aldrich/Merck (Figure 4B).

The HPLC analyses of the samples taken during forced degradation studies showed a quantitative effect of chlorobutanol on the velocity of oxytocin degradation and a striking qualitative effect (Figure 5). In the absence of chlorobutanol, concomitantly to the decrease in the oxytocin peak (retention time 8.2 minutes), several peaks of degradation products with higher retention time (15.1–19.3 minutes) appeared (Figure 5A). This is similar to the HPLC observations of Avanti et al.,[39] who identified these degradation products as oxytocin trisulfides and tetrasulfides and as various disulfide-linked oxytocin dimers, some of these showing deamidation of one or more of three amidated carboxyl groups of oxytocin (Figure 1). Notably, in the present study, these decomposition products were not observed in water containing 1.5 mg/mL chlorobutanol (Figure 5B), and the same observation was made for 5

mg/mL chlorobutanol, as well as for the preparations of Ciron and Sterop which each contained 5 mg/mL chlorobutanol. By contrast, all investigated solutions not containing chlorobutanol showed degradation products in the 15.1- to 19.3-minute range.

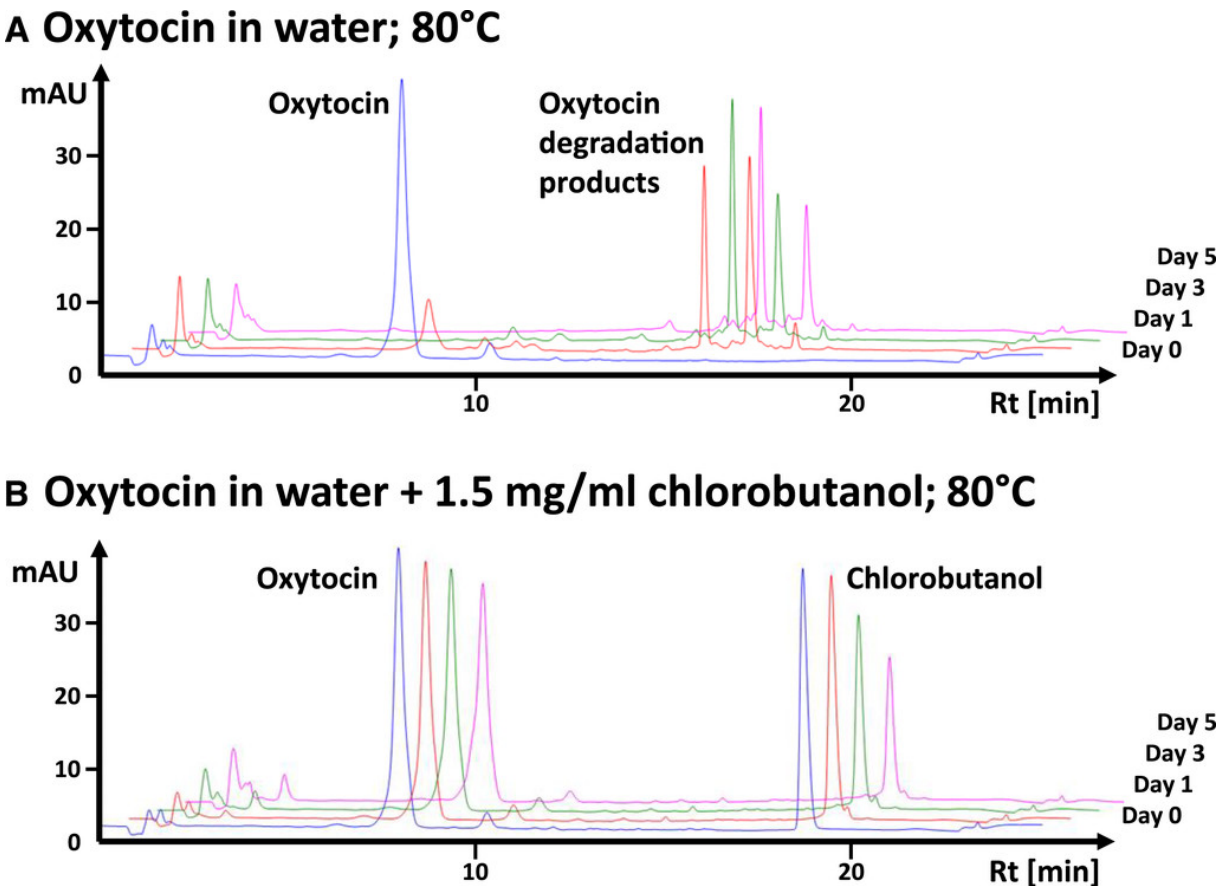

Figure 5: High-performance liquid chromatography chromatograms of oxytocin (Sigma-Aldrich/Merck; 10 IU/mL) in water under forced degradation at 80 °C, in the absence and presence of chlorobutanol 1.5 mg/mL.

In the accelerated stability studies nor in the forced degradation studies, visible quality defects such as particles, precipitations, or color changes were observed in any investigated products.

3.4. DISCUSSION

An encouraging result from the present study is that six of the eight investigated oxytocin brands passed the accelerated stability testing conducted according to the WHO/ICH.[22,28] This is even more encouraging as the products were not purchased ex-factory but from the supply chains in Malawi and Rwanda (and in the case of the Hexal product in Germany). Therefore, the extended storage before testing, possibly even at less than optimal storage conditions, had not affected the quality and stability of these products regarding the tested criteria.

However, two other samples were found not to comply with specifications after accelerated stability testing, and these were the two brands labeled for non-refrigerated storage. The Umedica product carried a storage recommendation "not exceeding 30 °C" and therefore had to be tested at 40 °C. It failed at this temperature (Figures 2 and 3), and only complied with the stability testing at 30 °C, appropriate for products labeled for refrigerated storage. By contrast, batch 160802 of the Ningbo product even failed assay testing after 6 months at 30 °C, and at 40 °C, it showed the poorest result of all eight tested brands. This Ningbo batch's initial API content (at month 0) was 89.0 % of the declared amount. Although it may be argued that the low initial content could have been caused by improper storage before sample collection, it is noteworthy that also two oxytocin samples stating Ningbo as the manufacturer name had been investigated in a survey conducted by the WHO, and both samples failed assay testing because of the insufficient API content.[11] In the present study, the second batch of Ningbo showed an initial API content of 94.9 % of the stated amount. It also failed testing at 40 °C, although it passed at 30 °C.

While this study was in progress, Nguyen et al.[17] published an investigation of the stability of five oxytocin products purchased ex-factory. Three of these products, labeled for storage at 2–8 °C, have also been investigated in the present study, that is, the products by Grindeks, Biologici, and Rotexmedica. The results obtained by Nguyen et al. after 1, 2, and 3 months of storage at 30 °C and 40 °C are in excellent agreement with those of our study. Nguyen et al. did not test for the full period of 6 months; therefore, results at that time point cannot be compared, but there is no evidence pointing at any relevant differences. Nguyen et al. also investigated one European oxytocin brand labeled for storage at ≤ 25 °C, as well as one Argentinian product which had formerly been labeled for storage at ≤ 25 °C but had been relabeled for refrigerated storage just before their study. They reported that, at 30 °C and 40°C, these two preparations exhibited very similar stability profiles as the three products labeled for refrigerated storage. Only after 4 months at 40 °C, and especially in a forced degradation study

at 50 °C, the European product labeled for storage at ≤ 25 °C showed much higher API losses than the others, concomitant with a sharp decrease in the pH value. That preparation contained 5 mg/mL chlorobutanol. The authors concluded that of the products tested, those designated for storage at ≤ 25 °C provided no stability benefit over those labeled for refrigerated storage.

The present study investigated six oxytocin brands labeled for storage at 2–8 °C, as well as two brands labeled for storage at ≤ 25 °C and at ≤ 30 °C. The two latter products had been manufactured in China and India, respectively. The present study's data are therefore complementary to the data provided by Nguyen et al.[17] in regard to the range of tested products. Our study results further support the conclusion that products designated for storage at ≤ 25 °C do not provide stability benefits over those labeled for refrigerated storage. In addition, our study shows that some of the former products may even fail the relevant WHO/ICH specifications for the stability of finished pharmaceutical products.

Five of the eight oxytocin brands investigated in this study passed stability testing with excellent results, with final API contents not more than 2.6 % below the amount stated on the label after 6 months at 30 °C (Supplemental Table S1). Notably, all these five brands were WHO prequalified and/or produced in countries with SRAs. By contrast, of the three preparations produced in India and China, that is, in countries without SRAs, two showed noncompliance with pharmacopoeial specifications after accelerated stability testing, and one passed testing with just borderline results. These poor stability outcomes must not be generalized to all manufacturers and medicines from non-SRA countries. There are certain manufacturers (usually larger companies), for example, in India and China, whose products are as good as generic medicines produced in countries with SRAs.[41] However, there are also companies (usually smaller ones) in these two countries whose products show a large rate of substandard medicines.[42] It should be both an ethical and an economic interest of the authorities in India and China to address and eliminate this problem.

For the prequalified WHO brands and/or produced in countries with SRAs, excipients were correctly declared. By contrast, for the three preparations without WHO prequalification and produced in countries without SRAs, the excipients were not declared correctly, which is a violation of registration requirements in most countries.

The present study results strongly support the maxim “Buy Quality Oxytocin, Keep It Cool” advocated by the Reproductive Health Supplies Coalition and other international stakeholders.[21,43–46] Given the lack of consistent evidence that products labeled for non-refrigerated storage show better temperature stability than those labeled for storage at 2–8 °C, and given the clear evidence that manufacturers’ storage recommendations of “below 25 °C” and even of “below 30 °C” cannot be reliably complied with in many facilities in LMICs,[13] procurement of oxytocin injections should be limited to brands labeled for refrigerated storage, and national medicines regulatory agencies should consider to only register oxytocin products with this storage recommendation.[43,44] This will also help to avoid confusion of the personnel in supply chains and health facilities by different storage recommendations for different oxytocin brands.[13,18,47,48] All efforts should be made to maintain the cold chain for oxytocin from the manufacturer up to the patient. The WHO/UNICEF recommendation to allow storage of oxytocin in the ubiquitous cold chain facilities of the Expanded Program on Immunization should be implemented as widely as possible for this purpose.[49]

Given the current shortcomings of the cold chain facilities in LMICs, however, it may still not be possible to avoid short-term exposures of oxytocin to ambient temperatures. In this context, the data depicted in Figure 2 may be of interest, showing that all products labeled for storage at 2–8 °C were remarkably stable at 25 °C in our stability study. Even at 30 °C, only very moderate losses of the API content were observed over the investigated period. This indicates that accidental storage of such oxytocin preparations outside of the cold chain for limited periods may not necessitate the immediate disposal of the products, and this is consistent with the information given, for example, in the leaflet of the Grindeks product (Table 2), stating that it “may be stored up to 30 °C for 3 months, but must then be discarded.”

According to Hawe et al.,[14] degradation of oxytocin in a 10-IU/mL solution of pH 4.5 has an activation energy of 128 ± 3.8 kJ/mol and follows (pseudo-) first-order kinetics. Using the Arrhenius equation,[14] we calculated that a temperature increase just from 25 °C to 30 °C would increase the degradation reaction rate by 2.3. Thakral et al.[21] extrapolated rate constants for oxytocin degradation at 25–40 °C from experimentally obtained rate constants at higher temperatures, assuming that the mechanism of reaction remains unchanged. Thereby, they calculated the time required for a 10 % loss of oxytocin content at temperatures of 25 °C, 30 °C, and 40 °C as 183 days, 82 days, and 17 days, respectively, at pH 4.5. Figures 2 and 3 of the

present study clearly show that the degradation rates observed experimentally at these temperatures for commercial oxytocin products were much lower, indicating different reaction mechanisms at lower temperatures and/or the presence of stabilizing agents.

Attempts to devise more heat-stable formulations of oxytocin have been described extensively in the literature,[14,36–39,50], mostly focusing on including specific buffers and divalent metal ions. However, to the best of our knowledge, the striking effect of chlorobutanol, a bacteriostatic agent widely used as a preservative in pharmaceuticals,[40] both on the velocity on oxytocin degradation (Figure 4) and on the type of degradation products formed (Figure 5) has not been described in the literature in any detail up to now. This stabilizing effect has only been very briefly mentioned in abstract by Liu et al.,[51] and a molecular dynamics computer simulation by Xu et al.[52,53] suggested that chlorobutanol can reduce the number of hydrogen bonds between oxytocin and water and prevent oxytocin molecules from aggregating. Evidence has been presented that divalent metal ions may lead to a change of the conformation of oxytocin in certain buffers, thereby shielding the intramolecular disulfide bond (Figure 1) and diminishing degradation reactions which involve that site of the molecule.[36–39] It is tempting to speculate that chlorobutanol may similarly affect oxytocin. Possibly, 200 mM acetate buffer may prevent this conformational change, explaining the lack of a stabilizing effect of chlorobutanol observed in this buffer (Figure 4B).

Chlorobutanol itself degrades at higher temperatures, as shown in Figure 5 which shows 47.5 % degradation of chlorobutanol in 5 days at 80 °C under the used conditions. The degradation leads to acidic degradation products[40] and lowers the pH value, which then may strongly deviate from the oxytocin stability optimum (pH 4.5), especially when a high concentration of chlorobutanol (i.e., 5 mg/mL) is used in weakly buffered solutions. The detrimental effect of the progressive lowering of the pH value would then compete with the stabilizing effect of chlorobutanol on oxytocin, and this may offer a plausible explanation for the observation depicted in Figure 4A (Ciron and Sterop products) that chlorobutanol 5 mg/mL first increased and later on lowered oxytocin stability at 80 °C. The lowering of the pH and the resulting decrease in oxytocin stability was also observed in the forced degradation experiment by Nguyen et al.,[17] similar to our results. However, this effect may not be relevant at actual storage temperatures of oxytocin because the hydrolysis of chlorobutanol only occurs at higher temperatures.

It may well be worthwhile to investigate the mechanism of the stabilization of oxytocin by chlorobutanol using appropriate methods, especially liquid chromatography-mass spectrometry (LC-MS) analysis for the identification of the degradation products[39] and nuclear magnetic resonance (NMR) spectroscopy to investigate conformational changes of oxytocin.[36] However, such investigations exceed the scope of the present study.

A heat-stable formulation of carbetocin,[54] an oxytocin analogue which is already widely used for the prevention of PPH after caesarean delivery, has very recently been added to the WHO Essential Medicines List for use in PPH.[1] If the announcement of its manufacturer is put into practice that the price of this product will be made comparable to that of oxytocin in LMICs,[55] then this medicine may become a further valuable option to ensure good-quality medication against PPH also in facilities where storage at 2–8 °C cannot be ensured.

Limitations of this study.

As mentioned earlier, in this study, oxytocin samples were investigated which had been purchased from wholesalers and medical stores in Malawi and Rwanda, that is, preparations which reflect the quality and stability of medicines circulating in these countries. It has to be considered that these samples had already been stored for extended periods after manufacturing, and that the conditions of this storage are not known. Samples were not purchased from retail outlets to minimize the influence of varying conditions during prior storage on the study results. Although the observed differences in the stability of the investigated samples are valid and important, the data provided here do not provide definitive proof that certain products did not comply with their stability requirements already at the time of manufacture.

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Supplemental figure and tables

SUPPLEMENTARY INFORMATION

Figure S1: Accelerated stability testing of commercial oxytocin preparations: results of additional batches

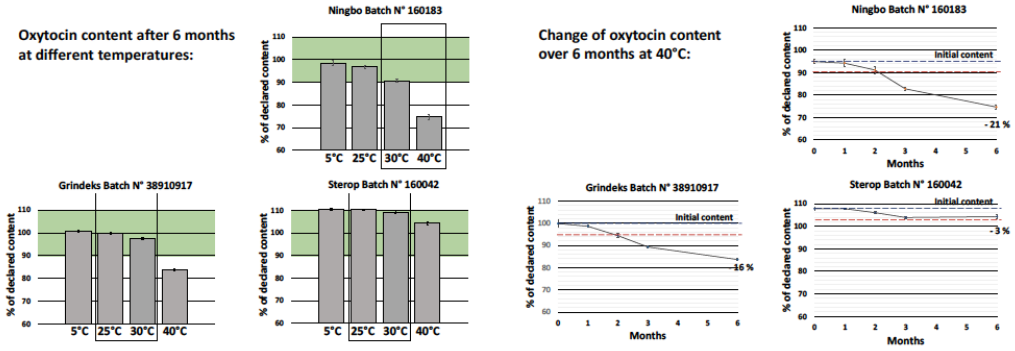

Table S1: Accelerated stability testing of commercial oxytocin preparations: change of oxytocin content over 6 months at different temperatures

		Oxytocin mean content (% of declared content)									
Condition	Sample	Month 0	RSD	Month 1	RSD	Month 2	RSD	Month 3	RSD	Month 6	RSD
Accelerated Ambient ICH 40 +/- 2° 75% RH +/- 5°	Ningbo 160802	89.0	1.41%	86.1	0.90%	85.3	0.48%	77.5	1.65%	69.5	1.05%
	Ningbo 160183	94.9	0.87%	94.2	1.69%	91.2	1.74%	82.8	0.76%	74.6	1.23%
	Umedica	98.1	1.29%	96.9	0.28%	93.0	1.06%	84.2	0.30%	78.0	0.70%
	Ciron	92.7	1.69%	94.4	0.70%	86.9	2.10%	86.0	1.34%	84.2	1.38%
	Biologici	99.5	0.53%	100.4	0.22%	97.8	1.15%	90.7	0.26%	85.5	0.54%
	Sterop 160269	99.6	1.11%	97.3	0.77%	97.8	0.19%	95.2	1.01%	95.4	0.80%
	Sterop 160042	107.8	0.62%	107.8	0.12%	106.1	0.45%	103.9	0.52%	104.3	0.77%
	Rotexmedica	102.4	0.89%	102.3	0.80%	99.8	0.53%	93.5	1.55%	89.6	0.29%
	AS Grindeks 37711116	100.9	0.82%	100.7	1.37%	96.1	0.66%	90.4	0.83%	85.2	0.42%
	AS Grindeks 38910917	99.9	1.65%	98.7	0.54%	94.5	0.99%	89.3	0.47%	83.6	0.59%
	Hexal	101.2	0.15%	98.4	0.69%	94.2	0.05%	88.2	0.82%	77.3	0.46%
Zone IVa; Accelerated Refrigerated ICH more severe conditions 30° +/- 2° 65% RH +/- 5%	Ningbo 160802	89.0	1.41%	90.3	0.87%	87.5	0.43%	86.1	0.64%	86.5	0.60%
	Ningbo 160183	94.9	0.87%	97.5	1.29%	98.0	1.78%	93.6	1.23%	90.7	0.80%
	Umedica	98.1	1.29%	100.0	0.47%	99.1	0.22%	93.1	0.39%	94.3	1.45%
	Ciron	92.7	1.69%	94.1	0.52%	92.5	1.70%	92.5	0.63%	89.3	1.55%
	Biologici	99.5	0.53%	102.4	0.38%	102.2	0.96%	95.8	0.52%	97.6	0.81%
	Sterop 160269	99.6	1.11%	99.6	0.11%	98.8	0.03%	97.0	0.21%	101.3	0.95%
	Sterop 160042	107.8	0.62%	108.7	0.32%	108.0	0.48%	104.9	0.08%	109.3	0.55%
	Rotexmedica	102.4	0.89%	104.2	0.16%	103.8	0.74%	99.6	0.45%	100.5	0.64%
	AS Grindeks 37711116	100.9	0.82%	101.9	0.57%	101.4	0.65%	97.8	0.89%	98.6	0.79%
	AS Grindeks 38910917	99.9	1.65%	101.0	0.54%	99.3	0.47%	95.6	0.46%	97.4	0.48%
	Hexal	101.2	0.15%	101.2	0.70%	100.5	0.40%	98.3	0.82%	98.4	0.38%

Zone II; Accelerated Refrigerated ICH 25° +/- 2° 60% RH +/- 5%	Ningbo 160802	89.0	1.41%	88.4	0.37%	90.2	1.06%	86.0	1.52%	88.2	0.44%
	Ningbo 160183	94.9	0.87%	100.2	1.35%	97.7	1.47%	96.9	0.75%	96.8	0.84%
	Umedica	98.1	1.29%	101.1	0.37%	100.3	0.37%	95.1	0.44%	97.9	1.40%
	Ciron	92.7	1.69%	94.3	1.63%	95.1	1.17%	92.8	1.20%	94.5	0.93%
	Biologici	99.5	0.53%	102.7	0.46%	102.8	0.96%	98.0	0.20%	100.5	0.48%
	Sterop 160269	99.6	1.11%	100.0	0.37%	98.5	0.33%	97.4	0.86%	103.5	0.35%
	Sterop 160042	107.8	0.62%	107.4	0.28%	108.1	0.39%	105.4	0.33%	110.3	0.32%
	Rotexmedica	102.4	0.89%	104.4	0.56%	104.0	0.69%	100.4	0.98%	102.6	0.83%
	AS Grindeks 37711116	100.9	0.82%	102.3	0.27%	102.0	0.93%	98.7	1.19%	100.7	0.99%
	AS Grindeks 38910917	99.9	1.65%	101.1	0.30%	100.0	1.24%	96.6	0.85%	99.9	0.44%
	Hexal	101.2	0.15%	101.7	0.87%	101.3	0.35%	99.5	0.90%	101.1	0.90%

Control long term testing conditions, refrigerated 5° +/- 3°	Ningbo 160802	89.0	1.41%	88.8	0.17%	90.9	1.12%	90.2	0.10%	91.6	1.45%
	Ningbo 160183	94.9	0.87%	97.4	0.08%	97.5	1.50%	96.0	1.67%	98.4	1.06%
	Umedica	98.1	1.29%	100.5	0.63%	100.7	0.39%	95.7	0.92%	99.5	0.65%
	Ciron	92.7	1.69%	97.5	0.74%	94.6	1.66%	94.4	0.40%	95.3	1.44%
	Biologici	99.5	0.53%	102.3	0.76%	103.6	0.16%	97.4	1.29%	100.3	0.91%
	Sterop 160269	99.6	1.11%	100.0	0.25%	99.4	0.42%	98.1	0.48%	103.6	0.46%
	Sterop 160042	107.8	0.62%	109.1	0.20%	108.5	0.29%	105.6	0.48%	110.5	0.53%
	Rotexmedica	102.4	0.89%	104.7	0.46%	105.5	0.83%	100.8	0.19%	103.5	0.87%
	AS Grindeks 37711116	100.9	0.82%	102.3	0.55%	103.4	0.75%	99.7	0.77%	102.5	1.06%
	AS Grindeks 38910917	99.9	1.65%	101.1	0.39%	101.6	0.45%	99.2	1.02%	100.7	0.48%
	Hexal	101.2	0.15%	101.9	0.92%	102.4	0.67%	100.4	0.55%	103.6	0.55%

RSD: relative standard deviation. ICH: International Conference on Harmonization. RH: relative humidity. Oxytocin content calculated as the mean of the results of three individual vials per sample.

Table S2: Accelerated stability testing of commercial oxytocin preparations: change of pH value over 6 months at different temperatures

		Oxytocin mean pH-values									
Condition	Sample	Month 0	RSD	Month 1	RSD	Month 2	RSD	Month 3	RSD	Month 6	RSD
Accelerated Ambient ICH 40 +/- 2° 75% RH +/- 5°	Ningbo 160802	3.9	0.63%	3.9	1.33%	4.3	1.28%	3.9	0.82%	4.2	1.04%
	Ningbo 160183	4.1	0.88%	4.0	0.37%	4.4	1.10%	4.0	0.65%	4.3	0.57%
	Umedica	4.2	1.24%	4.1	0.40%	4.5	1.62%	4.1	0.30%	4.3	0.74%
	Ciron	4.5	2.38%	3.5	1.69%	3.7	2.94%	3.1	1.16%	3.0	2.18%
	Biologici	3.8	0.95%	3.8	0.46%	4.1	2.29%	3.8	0.99%	4.0	0.45%
	Sterop 160269	4.0	0.57%	3.3	1.19%	3.5	2.82%	3.0	1.49%	2.8	1.35%
	Sterop 160042	3.8	0.22%	3.3	1.02%	3.5	2.37%	2.9	1.28%	2.8	1.45%
	Rotexmedica	4.0	0.47%	3.9	0.47%	4.3	1.90%	3.9	0.31%	4.1	0.30%
	AS Grindeks 37711116	4.1	0.64%	4.0	0.44%	4.4	1.93%	4.0	0.32%	4.2	0.32%
	AS Grindeks 38910917	4.1	0.46%	4.0	0.33%	4.4	1.69%	4.0	0.29%	4.2	0.33%
	Hexal	4.2	0.13%	4.1	1.20%	4.6	1.75%	4.3	0.46%	4.5	1.48%

Zone IVa; Accelerated Refrigerated ICH more severe conditions 30° +/- 2° 65% RH +/- 5%	Ningbo 160802	3.9	0.63%	3.9	0.77%	4.3	1.58%	3.9	1.15%	4.1	0.50%
	Ningbo 160183	4.1	0.88%	4.0	0.62%	4.4	1.42%	4.0	0.52%	4.2	0.38%
	Umedica	4.2	1.24%	4.1	0.43%	4.5	1.46%	4.1	1.79%	4.3	0.32%
	Ciron	4.5	2.38%	4.0	0.89%	4.4	1.74%	3.9	1.38%	3.8	1.45%
	Biologici	3.8	0.95%	3.8	0.47%	4.2	1.52%	3.8	0.57%	4.0	0.38%
	Sterop 160269	4.0	0.57%	3.7	0.79%	4.0	1.58%	3.6	0.63%	3.5	0.67%
	Sterop 160042	3.8	0.22%	3.6	0.79%	3.9	1.50%	3.4	0.75%	3.5	0.76%
	Rotexmedica	4.0	0.47%	3.9	0.85%	4.3	1.40%	3.9	0.44%	4.1	0.36%
	AS Grindeks 37711116	4.1	0.64%	4.0	0.53%	4.4	1.24%	4.0	0.37%	4.2	0.28%
	AS Grindeks 38910917	4.1	0.46%	4.0	0.56%	4.4	1.22%	4.0	0.41%	4.2	0.29%
	Hexal	4.2	0.13%	4.1	1.11%	4.6	1.39%	4.2	0.36%	4.4	0.69%

Zone II; Accelerated Refrigerated ICH 25° +/ 2° 60% RH +/ 5%	**Ningbo 160802**	3.9	0.63%	3.9	0.54%	4.3	1.28%	3.9	1.03%	4.1	0.43%
	Ningbo 160183	4.1	0.88%	4.0	0.66%	4.4	1.95%	4.0	0.56%	4.2	0.85%
	Umedica	4.2	1.24%	4.2	1.67%	4.5	1.61%	4.1	0.47%	4.3	0.60%
	Ciron	4.5	2.38%	4.2	3.03%	4.5	2.22%	4.1	0.53%	4.3	5.76%
	Biologici	3.8	0.95%	3.8	0.74%	4.2	2.07%	3.8	0.49%	4.0	0.57%
	Sterop 160269	4.0	0.57%	3.8	0.80%	4.2	1.24%	3.8	0.55%	3.8	0.65%
	Sterop 160042	3.8	0.22%	3.6	1.11%	4.0	1.55%	3.6	0.87%	3.7	0.58%
	Rotexmedica	4.0	0.47%	3.9	0.70%	4.3	1.54%	3.9	0.25%	4.1	0.30%
	AS Grindeks 37711116	4.1	0.64%	4.0	0.48%	4.4	1.57%	4.0	0.26%	4.2	0.67%
	AS Grindeks 38910917	4.1	0.46%	4.0	0.46%	4.4	1.49%	4.0	0.26%	4.2	0.29%
	Hexal	4.2	0.13%	4.0	1.70%	4.6	1.59%	4.1	0.58%	4.4	0.40%
Control long term testing conditions, refrigerated 5° +/ 3°	**Ningbo 160802**	3.9	0.63%	3.9	1.10%	4.3	1.99%	3.9	1.52%	4.1	0.81%
	Ningbo 160183	4.1	0.88%	4.1	0.36%	4.4	1.13%	4.0	0.54%	4.2	0.74%
	Umedica	4.2	1.24%	4.1	0.71%	4.5	1.09%	4.1	0.33%	4.3	0.34%
	Ciron	4.5	2.38%	4.2	1.40%	4.8	3.84%	4.2	0.24%	4.4	1.28%
	Biologici	3.8	0.95%	3.8	0.47%	4.2	1.73%	3.8	0.57%	4.0	0.55%
	Sterop 160269	4.0	0.57%	3.8	1.01%	4.3	1.49%	3.9	0.88%	4.0	0.44%
	Sterop 160042	3.8	0.22%	3.7	0.40%	4.1	1.76%	3.7	0.66%	3.9	0.56%
	Rotexmedica	4.0	0.47%	3.9	0.52%	4.3	1.64%	3.9	0.26%	4.1	0.39%
	AS Grindeks 37711116	4.1	0.64%	4.0	0.37%	4.4	1.42%	4.0	0.46%	4.2	0.42%
	AS Grindeks 38910917	4.1	0.46%	4.0	0.42%	4.5	1.64%	4.0	0.41%	4.2	0.43%
	Hexal	4.2	0.13%	4.1	0.24%	4.5	1.60%	4.1	0.59%	4.3	0.59%

RSD: relative standard deviation. ICH: International Conference on Harmonization. RH: relative humidity. pH values calculated as the mean of the results of three individual vials per sample, each vial tested twice, yielding 6 measurements per sample.

Table S3: Determination of intermediate precision[1] of oxytocin assay, using data from five-point calibration curves

	Mean AUC (mAU*s) Month 0	*RSD*	Mean AUC (mAU*s) Month 1	*RSD*	Mean AUC (mAU*s) Month 2	*RSD*	Mean AUC (mAU*s) Month 3	*RSD*	Mean AUC (mAU*s) Month 6	*RSD*	**Mean AUC (mAU*s) all months**	**RSD**
USP Reference 11.5 IU/ml	795.37	*1.25%*	809.72	*0.09%*	818.59	*0.43%*	807.35	*0.23%*	770.90	*0.62%*	**800.39**	**2.31%**
USP Reference 10 IU/ml	697.92	*1.31%*	699.32	*0.24%*	708.83	*0.37%*	699.52	*0.29%*	670.62	*0.79%*	**695.24**	**2.08%**
USP Reference 7 IU/ml	478.30	*0.67%*	483.76	*0.86%*	495.49	*0.08%*	485.75	*1.02%*	468.38	*0.87%*	**482.33**	**2.07%**
USP Reference 5 IU/ml	333.61	*1.52%*	342.19	*0.28%*	344.07	*0.24%*	344.91	*1.73%*	322.33	*1.19%*	**337.42**	**2.83%**
USP Reference 2 IU/ml	123.83	*3.24%*	131.64	*0.92%*	130.95	*1.01%*	140.46	*2.71%*	120.89	*1.10%*	**129.56**	**5.89%**

AUC: area under the curve. RSD: relative standard deviation. IU: international units. Each mean AUC value was calculated from on three HPLC measurements, but each mean AUC value of USP Reference 10 IU/ml from five HPLC measurements.

[1]Reference: ICH, 2005. Validation of analytical procedures: Text and methodology Q2(R1). Available from: https://database.ich.org/sites/default/files/Q2_R1__Guideline.pdf.

Table S4: Forced thermal degradation studies of solutions of oxytocin (Sigma-Aldrich/Merck; 10 IU/ml) in the presence of different excipients, and of commercial oxytocin formulations: change of oxytocin content over 5 days at 80°C

	Oxytocin content (% of initial content)							
	Day 0	*RSD*	Day 1	*RSD*	Day 3	*RSD*	Day 5	*RSD*
10 IU/ml synthetic oxytocin in destilled water	100.0	*0.44%*	17.6	*4.00%*	0.7	*16.98%*	0.0	*0.00%*
10 IU/ml synthetic oxytocin in sodium acetate buffer pH 4.6	100.0	*0.04%*	77.0	*1.25%*	36.3	*4.21%*	16.9	*1.29%*
10 IU/ml synthetic oxytocin in destilled water containing 5mg/ml chlorobutanol	100.0	*0.00%*	94.7	*0.34%*	75.5	*0.72%*	43.9	*4.36%*
10 IU/ml synthetic oxytocin in destilled water containing 1.5mg/ml chlorobutanol	100.0	*0.75%*	95.4	*1.89%*	87.6	*1.27%*	72.3	*3.34%*
10 IU/ml synthetic oxytocin in sodium acetate buffer pH 4.6 containing 5mg/ml chlorobutanol	100.0	*0.36%*	68.7	*4.93%*	31.6	*4.45%*	14.2	*4.90%*
10 IU/ml synthetic oxytocin in sodium acetate buffer pH 4.6 containing 1.5mg/ml chlorobutanol	100.0	*1.46%*	72.7	*1.75%*	36.7	*1.47%*	17.5	*2.79%*
Hexal (batch HWZ7694[a])	100.0	*0.04%*	82.3	*2.72%*	53.5	*0.98%*	32.5	*2.86%*
Hexal (batch HWZ7694 [a]) containing 5mg/ml chlorobutanol	100.0	*0.76%*	93.9	*2.23%*	64.8	*6.51%*	31.6	*11.46%*
Hexal (batch HWZ7694 [a]) containing 1.5mg/ml chlorobutanol	100.0	*2.01%*	95.6	*4.57%*	84.4	*4.83%*	70.4	*8.68%*
Ningbo 160183	100.0	*3.07%*	84.5	*2.91%*	53.7	*1.37%*	35.7	*5.51%*
Ciron	100.0	*0.16%*	94.9	*1.89%*	60.3	*5.32%*	24.8	*5.19%*
Sterop 160269	100.0	*0.70%*	93.1	*0.97%*	58.3	*0.31%*	17.6	*1.91%*
Rotexmedica	100.0	*0.68%*	84.7	*3.70%*	59.4	*4.10%*	44.1	*2.58%*
AS Grindeks 38910917	100.0	*1.22%*	83.4	*2.45%*	56.4	*3.67%*	39.1	*3.47%*

IU: international unit. RSD relative standard deviation.

[a] Batch HWZ7694 had identical packaging information as batch HC0075 listed in Table 2, but expiry date June 2021.

Table S5: Forced thermal degradation studies of solutions of oxytocin (Sigma-Aldrich/Merck; 10 IU/ml) in the presence of different excipients, and of commercial oxytocin formulations: change of pH values over 5 days at 80°C

	pH values							
	Day 0	*RSD*	Day 1	*RSD*	Day 3	*RSD*	Day 5	*RSD*
10 IU/ml synthetic oxytocin in destilled water	NA	*NA*	NA	*NA*	NA	*NA*	NA	*NA*
10 IU/ml synthetic oxytocin in sodium acetate buffer pH 4.6	4.7	*0.15%*	NA	*NA*	NA	*NA*	NA	*NA*
10 IU/ml synthetic oxytocin in destilled water containing 5mg/ml chlorobutanol	5.6	*2.65%*	2.9	*0.24%*	2.4	*0.30%*	2.2	*0.65%*
10 IU/ml synthetic oxytocin in destilled water containing 1.5mg/ml chlorobutanol	6.4	*2.33%*	3.9	*0.18%*	3.1	*0.23%*	2.8	*2.00%*
10 IU/ml synthetic oxytocin in sodium acetate buffer pH 4.6 containing 5mg/ml chlorobutanol	4.6	*0.00%*	4.7	*0.30%*	4.5	*0.16%*	4.5	*0.16%*
10 IU/ml synthetic oxytocin in sodium acetate buffer pH 4.6 containing 1.5mg/ml chlorobutanol	4.7	*0.60%*	4.6	*0.15%*	4.6	*0.15%*	4.6	*0.30%*
Hexal (batch HWZ7694 [a])	4.2	*0.13%*	4.2	*0.50%*	4.6	*0.15%*	4.3	*0.16%*
Hexal (batch HWZ7694 [a]) containing 5mg/ml chlorobutanol	4.6	*0.76%*	NA	*NA*	2.3	*0.31%*	2.0	*0.35%*
Hexal (batch HWZ7694 [a]) containing 1.5mg/ml chlorobutanol	4.6	*0.77%*	3.8	*0.75%*	2.9	*0.24%*	2.8	*0.25%*
Ningbo 160183	4.1	*0.88%*	4.0	*0.18%*	4.4	*1.13%*	4.1	*1.38%*
Ciron	4.5	*2.38%*	2.7	*0.26%*	2.6	*1.66%*	2.0	*0.70%*
Sterop 160269	4.0	*0.57%*	2.7	*0.53%*	2.5	*1.14%*	2.0	*0.36%*
Rotexmedica	4.0	*0.47%*	3.9	*0.00%*	4.2	*1.17%*	3.9	*0.18%*
AS Grindeks 38910917	4.1	*0.46%*	4.0	*0.35%*	4.3	*0.82%*	4.0	*0.18%*

IU: international unit. RSD: relative standard deviation. NA: no data available.

[a] Batch HWZ7694 had identical packaging information as batch HC0075 listed in Table 2, but expiry date June 2021.

Chapter four: Stability of misoprostol tablets collected in Malawi and Rwanda: Importance of intact primary packaging

Abstract

Misoprostol is listed in the WHO essential medicines list and can be used for induction of labour, prevention and treatment of post-partum haemorrhage, and abortions. The compound is unstable, and substandard misoprostol preparations have been detected in low- and middle-income countries.

We now investigated the stability of misoprostol tablets according to the international guidelines for stability testing of pharmaceutical products. Three brands (four batches) of misoprostol tablets were collected in Malawi and Rwanda: the originator product, a WHO-prequalified product, and a generic product without WHO prequalification. A further batch of the originator product was collected in Germany. To investigate the effect of damage to the primary packaging, additional blister strips of one sample were intentionally damaged with a needle and investigated in parallel. Samples were placed in stability chambers for six months at 40 °C/75 % relative humidity (RH) and at 25 °C/60 % RH. After 0, 1, 2, 3 and 6 months, misoprostol content was determined according to the International Pharmacopeia.

At 40 °C/75 % RH, all samples showed a decline of misoprostol content. However, four of the batches still remained within the pharmacopeial specifications. In contrast, one of the two batches of the generic product without WHO-prequalification showed a final content of 86.2 % which is out of specifications. Damage to the primary packaging greatly decreased stability, resulting in a final content of only 48.2 % of the declared misoprostol amount. At 25 °C/60 % RH all samples remained in specifications for six months, even the sample with the damaged blister. Dissolution of misoprostol remained in specifications of the pharmacopoeia for six months for all batches, except for the sample with damaged blisters stored at 40 °C/75 % RH.

This study confirms that the stability of misoprostol tablets must be ensured by intact, good-quality primary packaging. Careful supplier qualification is required in the procurement process.

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No data were changed, only minor edit adjustments were made to fit the paper into the Microsoft word format and accommodate comments from panelists.

4.1. Introduction

Misoprostol (Fig 1), a prostaglandin analogue, is listed in the essential medicines list of the World Health Organization (WHO) [1] as a uterotonic and can be used for prevention and treatment of post-partum haemorrhage (PPH). PPH is the leading cause of maternal mortality in low-income countries. It is defined as a blood loss of 500 ml or more within 24 hours after giving birth [2]. Oxytocin, the medicine of first choice for prevention and treatment of PPH, is very sensitive to environmental conditions. It degrades at high temperatures and usually has to be stored at 2–8 °C [3–7]. This storage requirement can be challenging, especially in rural areas of low- and middle-income countries (LMICs) where infrastructure is poor and ambient temperatures are often high [8–12]. Indeed, several previous studies have shown that the quality of oxytocin, especially in LMICs, is often poor [3, 10–17].

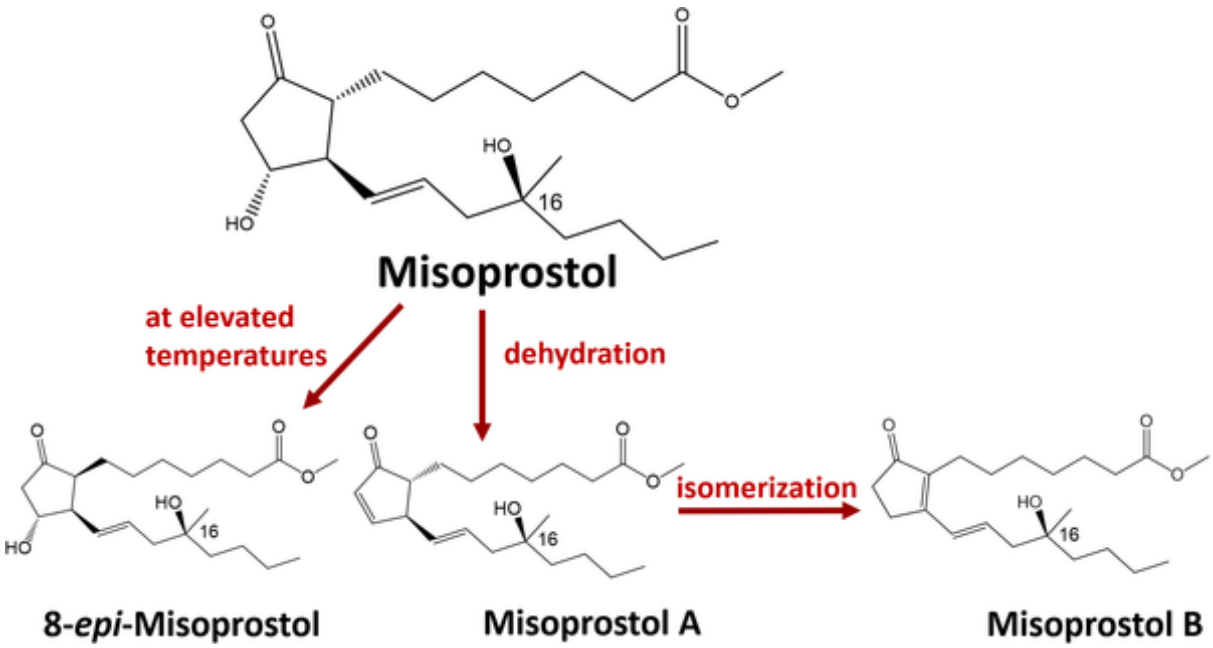

Fig 1. Structure of misoprostol and its typical degradation mechanisms. Commercial misoprostol is a mixture of the depicted structure at its epimer at C-16, as well as the enantiomers of both compounds. Corresponding stereoisomers are found for the degradation products.

Misoprostol is available in form of tablets which can be administered orally and which do not require refrigerated storage. Therefore, misoprostol tablets appear to offer an attractive alternative to oxytocin injections in the prophylaxis and treatment of PPH in places where no trained staff is available to administer medicines parenterally, or where appropriate storage conditions for oxytocin cannot be ensured [1, 18, 19]. Misoprostol is also used for the induction

of labour, for treatment and prevention of ulcers induced by non-steroidal anti-inflammatory drugs (NSAIDs) and for abortions [1, 20]. Concerns about its latter use have motivated some countries to restrict its availability.

The instability of misoprostol has been known since decades [21]. The most important degradation reaction is dehydration, leading to misoprostol A (Fig 1). Toledo-Vasquez et al. [22] described that in aqueous solutions, this reaction follows first-order kinetics, and at 60 °C and pH 7.66 results in a degradation of misoprostol with a half-life of only 8.8 hours. Dehydration is followed by the much slower isomerization to the resonance-stabilized misoprostol B [21, 22] (Fig 1). Epimerization of misoprostol at C-8 is a further degradation mechanism occurring at higher temperatures [21].

Pure misoprostol shows 50 % degradation within two weeks at 55 °C [22]. Fortunately, a dispersion of misoprostol in hydroxypropyl methylcellulose (HPMC) is much more stable, allowing the formulation of finished pharmaceutical products [21–24]. However, these misoprostol-HPMC dispersions have to be carefully protected from humidity, since the degradation velocity of misoprostol increases sharply when the water content of the dispersion exceeds 2 % [24], and tablets containing misoprostol-HPMC dispersions have been shown to absorb water very quickly when stored without protection [23]. Importantly, Hall [25] demonstrated that plastic-aluminium blisters which are frequently used for the packaging of pharmaceutical products are grossly inadequate for ensuring the stability of misoprostol tablets. This study investigated 215 samples of misoprostol tablets of different age after manufacture, collected in Asia, Africa and Latin America. When one year or more had elapsed after manufacture, all samples packaged in plastic-aluminium blisters showed a misoprostol content below the limit specified by the International Pharmacopeia (90 % of the declared amount). Double-sided aluminium blisters provided better protection, but one year or more after manufacture, even of those 28 % were reported to show misoprostol contents below the pharmacopeial limit [25].

The quality of misoprostol preparations circulating in LMICs has received much less attention in the scientific literature than the quality of oxytocin. Besides the above-mentioned study by Hall [25], only two reports have been published: Anyakora et al. [11] investigated 166 samples of misoprostol tablets collected in health facilities in Nigeria. 33.7 % of these were reported to fail the specifications of the International Pharmacopeia for misoprostol content, but the report did not specify how far these samples deviated from declared content. Our group recently

investigated the quality of oxytocin and misoprostol samples collected in Malawi's health facilities and drug outlets. Out of the 30 misoprostol samples, five (17 %) showed extreme deviations, containing only 12.7–30.2% of the declared content [12]. No systematic stability study of finished pharmaceutical products of misoprostol has been published so far in the scientific literature.

During the registration of medicines, National Medicines Regulatory Authorities (NMRAs) examine the stability data provided by the manufacturers, usually they do not carry out independent experiments to confirm these data. The NMRAs of the EU member states, USA, Japan, Switzerland, Canada, Australia, Iceland, Liechtenstein and Norway are currently considered as “Stringent Regulatory Authorities” (SRAs) [26], and medicines produced under supervision of an SRA are usually regarded with trust in regard to their manufacturers’ specifications for quality and stability. Similar trust is given to medicines which have received “WHO Prequalification” status, based on information submitted by manufacturers to WHO and on inspections of the respective manufacturing sites by experts mandated by WHO [27, 28]. Among the medicine types which are currently eligible for WHO prequalification status are medicines for reproductive and maternal health, including oxytocin injections and misoprostol tablets. However, only a small part of the medicines circulating in LMICs are approved by a SRA or WHO-prequalified [29]. In the present study, we investigated different batches of three misoprostol preparations: one preparation produced in a country with an SRA, another one that was a WHO-prequalified product, and one preparation produced in a non-SRA country and was not WHO-prequalified.

Rules for stability testing of pharmaceutical products have been devised by the International Council of Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH), most importantly guidelines Q1A-Q1F [30]. Obviously, the aim of testing is to confirm the stability of the product over the entire shelf-life, but testing durations of several years are difficult from an economic viewpoint. Therefore, so-called accelerated stability studies are permissible. They are carried out for shorter periods but at higher temperatures and relative humidities. These studies allow to predict the shelf life of the products under given storage conditions, and their results are used to decide about the label statements regarding shelf life and storage requirements for the respective products. Guidelines by ICH [31] and by WHO [26] specify the precise conditions for accelerated stability studies. Obviously, these conditions are related to the climatic zone of the country where the medicine

will be registered. Malawi is currently assigned to WHO climatic zone II (subtropical and Mediterranean), and Rwanda to zone IVa (hot and humid) [32].

In the present study, accelerated stability testing of misoprostol tablets collected in Malawi and Rwanda was carried out according to the ICH guidelines for stability testing of pharmaceutical products [31].

4.2. Materials and methods

4.2.1. Study design and ethical clearance

This study was designed to observe the recommendations contained in the WHO guidelines on the conduct of surveys of the quality of medicines [33], the MEDQUARG guidelines [34], and the ICH and WHO guidelines for stability testing of pharmaceutical products [26, 31]. The College of Medicine Research and Ethics Committee (COMREC) in Malawi gave ethical clearance to conduct this study (Reference No. P.07/27/2215), as well as the Ministry of Health, Rwanda (Reference No. 20/1361/DGPHFIS/2018). Approval was also granted by the Malawi National Regulatory Agency (Pharmacy, Medicines and Poisons Board, PMPB).

4.2.2. Sample collection

The brands of misoprostol tablets available at government medical stores and pharmaceutical wholesalers in Malawi and Rwanda during the time of sample collection (February-March 2018) were purchased by local researchers (F.K. and T.B.). If different batches of a certain brand were available, samples from each batch were purchased. From each brand and batch, 250 tablets were purchased. Samples were not collected from individual health facilities or private pharmacies, to minimize the influence of possibly incorrect storage conditions on the results of this study. Also, these facilities usually do not sufficient stock amounts of misoprostol tablets for stability testing. After purchase, samples were stored according to the manufacturers' specifications. Temperature data loggers were kept with the samples until these were placed into the stability chambers for testing. The investigators hand-carried the samples from Malawi or Rwanda to Germany via airplane; this transport required less than 24 hours. Samples were pre-tested for their misoprostol content according to International Pharmacopeia. Those samples which were within specifications at the time of pretesting were included into the stability study.

4.2.3. Storage in stability chambers

The samples were stored in their original primary packaging in stability chambers of Alpha-Pharma-Service GmbH, Heilbronn, Germany, for six months (April-October 2018). Conditions were chosen in accordance to ICH and WHO guidelines for stability testing of pharmaceutical products [26, 31]. Accelerated stability testing was carried out at 40 °C +/- 2 °C and 75 % +/- 5 % relative humidity (RH). Samples stored at 25 °C +/- 2 °C and 60 % +/- 5 % RH, i.e. at the ICH conditions for long-term testing of non-refrigerated products, climatic zone II, were investigated for comparison. After 0, 1, 2, 3, and 6 months, 20 tablets of each sample were

removed from both chambers and analysed at Tuebingen University, Germany (by N.H. and T.B.).

4.2.4. Sample analysis

Samples were visually inspected and subsequently tested for identity, assay and dissolution following the procedures described in the monograph for misoprostol tablets of the International Pharmacopeia 2017. Prior to the experiments, the methods were validated according to the International Pharmacopeia. Misoprostol was identified and quantified by high performance liquid chromatography (HPLC), using an Agilent Infinity 1260 II with binary pump and variable wavelength detector (Agilent Technologies, Santa Clara, CA, USA), with acetonitrile/water (45:55 V/V) as mobile phase, flow rate 1.5 ml/min, column ReproSil-XR 120 C18, 5 µm, 150 mm x 4.6 mm (Dr. Maisch GmbH, Ammerbuch, Germany), and UV detection at 200 nm. The injection volume was 100 µl for identity and assay, or 250 µl for dissolution. For identity and assay, five tablets were dissolved in 50 ml mobile phase; two independent experiments were carried out, and of the resulting solutions two aliquots each were analysed by HPLC, yielding four measurements for each sample. The HPLC autosampler was set to 4 °C to avoid misoprostol degradation. Samples showing additional peaks were also tested for related substances according to Kahsay et al. [35], using freshly prepared solutions from three tablets per sample and performing the tests without delay.

Dissolution was investigated with a dissolution tester PT-WS 610 (Pharma Test Apparatebau AG, Hainburg, Germany). Six tablets per sample were tested separately as described in the monograph for misoprostol tablets in the International Pharmacopeia, with 500 ml of water R as dissolution medium, a temperature of 37 °C, and a rotating paddle with 50 revolutions per minute. Samples were withdrawn after 30 min through an in-line filter. Misoprostol reference standard Ph. Eur. (batch N° 3.0) was obtained from the European Directorate for the Quality of Medicines (EDQM), Strasbourg, France.

At each time point during the stability testing, 5-point calibration curves were prepared to assure linearity. Intermediate precision [36] was calculated from the data of the calibration curves of the reference standards, (see S2 Table).

4.2.5. Statistical analysis

Statistical evaluation was done using JMP 14.2 (SAS GmbH, Heidelberg, Germany). Significance levels of differences were calculated using uni- and multivariate analysis of variance (ANOVA) and student´s t-test. Differences were considered significant when $p< 0.05$.

4.3. Results

4.3.1. Overview of investigated misoprostol samples

In this study, misoprostol samples were investigated which were offered by government medical stores and private wholesalers in Malawi and Rwanda, i.e. preparations which reflect the actual quality and stability of medicines distributed in these two African countries. In February and March 2018, the local investigators (F.K. and T.B.) enquired about the available brands and batches of misoprostol tablets. Samples were then purchased from private wholesalers using a mystery shopper approach (i.e. ordering with the help of a licensed pharmacy shop), and from government medical stores in an overt approach. If different batches of a certain brand were available, samples from each batch were purchased. In Malawi, certain brands of misoprostol tablets had recently been withdrawn from the market [12], and only one single brand was offered for sale at the time of sample collection, in form of two different batches; both batches were purchased. In Rwanda, three brands were offered at the time of sample collection (only a single batch of each brand), and each brand was purchased. However, one of these brands showed an insufficient API content already in pre-testing, and had to be excluded from the subsequent investigation. The Rwanda Food and Drug Authority (RFDA) was alerted about this substandard brand. Further investigations initiated by RFDA confirmed our findings, and effective from February 2019, RFDA recalled the substandard brand from the Rwandan pharmaceutical market. As a comparison, the originator brand (Cytotec®) was purchased through the pharmacy of the University Hospital Tuebingen. This is the brand most commonly used in Germany. A different batch of this originator brand had also been purchased in Rwanda.

Therefore, as shown in Table 1, three brands (total 5 batches) were included into the stability testing. The originator brand had been produced in the UK, i.e. in a country with an SRA. One further brand represented a WHO-prequalified product, manufactured in India. The third brand was not WHO-prequalified, and had also been produced in India.

Table 1. Investigated misoprostol samples

Collected in:	Collected at:	Stated manufacturer (and brand name)	Country of manufacture	Mfg. date / Exp. date	Batch number	Stated shelf life	Stated storage requirements	Primary packaging	Stated excipients / formulation	Pre-qualification status
Malawi	wholesaler	**Fourrts (India) Laboratories Pvt. Limited** (KONTRAC 200)	India	Jun 17/ May 19	E0571	2 years	Below 30°C in a dry place. Protect from light	alu/alu blister	none declared	none
	Central Medical Stores Trust			Feb 17/ Jan 19	D2205					
Rwanda	government district pharmacy	**Acme Formulation Pvt. Ltd.** (Ace Miso)	India	Sep 16/ Aug 18	ACE160963	2 years	Below 30°C protected from light	alu/alu blister	misoprostol as HPMC dispersion (1%), excipients q.s.	WHO-PQ
Rwanda	wholesaler	**Piramal Healthcare UK Limited** (Cytotec)	UK	Jul 17[1]/ Jun 20	B17173	3 years[1]	none	alu/alu blister	misoprostol-HPMC dispersion (1%), cellulose (microcrystalline), sodium carboxymethyl-amidon, hydrogenated castor oil	SRA
Germany	pharmacy of Tuebingen university hospital			Feb 17[1]/ Jan 20	B16131		Below 30°C protected from humidity		cellulose (microcrystalline), HPMC, sodium carboxymethyl-amidon, hydrogenated castor oil	

All samples represented tablets with a stated content of 200 µg/ tablet.

HPMC: hydroxypropyl methylcellulose. WHO-PQ: WHO-prequalified product. SRA: produced in a country with stringent regulatory authority.

[1]Information not stated on the packaging, but obtained from internet data bases [20, 37].

All samples represented tablets with a stated content of 200 µg/ tablet.

Surprisingly, packaging and leaflet of the originator brand collected in Rwanda did not state any storage requirements. In contrast, packaging and leaflet of the same brand collected in Germany stated that storage below 30 °C was required. The same storage temperature was stated by the manufacturers of the two other brands. The stated requirements for protection from light and humidity were somewhat different between the brands (Table 1). All samples had double-sided aluminium blisters as primary packaging. Pre-testing for the content of misoprostol showed that these samples were within the specifications of the International Pharmacopoeia at the beginning of the stability study.

The three manufacturers were contacted and were requested to confirm the authenticity of the samples. The two manufacturers from India confirmed that the label information and appearance of the samples conformed to their products. The latest response from Pfizer (the marketing authorization holder) / Piramal was that they were still investigating this matter. The declared shelf life of the investigated brands was either two or three years. While four of the five investigated batches remained within their shelf life during the entire duration of the study,

the WHO-prequalified product by Acme expired during the stability testing. Nevertheless, it was found to remain within specifications during the entire testing period, even after storage for six months at 40 °C and 75 % RH (see below).

Table 1 also shows the stated excipients for the investigated preparations. Notably, for the product Kontrac 200® which was neither SRA-approved nor WHO-prequalified, no excipients were declared at all.

4.3.2. Accelerated stability study

According to the current ICH/WHO guidelines for stability testing of finished pharmaceutical products [26, 31], accelerated stability testing of medicines which are labelled for storage below 30 °C has to be performed at 40 °C and 75 % RH for six months. All five collected batches of misoprostol tablets were tested under these conditions. The results are depicted in Fig 2a, and the exact misoprostol amounts determined at each time point, together with the standard deviation of the measurements, are listed in S1 Table. As visible in Fig 2a, both batches of the originator brand as well as the batch of the WHO-prequalified product remained in specifications over the entire six months testing period, i.e. their misoprostol content remained in the range of 90–110 % of the declared amount. However, of the two batches of the product which had been produced in a non-SRA country and which had not been WHO-prequalified, only one batch remained in specifications, while the other batch showed a final content of 86.2 % of the declared amount, which is out of specifications. The decrease of the misoprostol content of this sample was 14.5 % over 6 months, which is significantly more that the decrease observed in the originator and the WHO-prequalified samples (decrease over 6 months: 8.9 %, 7.4 % and 8.3 %, respectively; all $p < 0.0001$), and also significantly more than in the other investigated batch from the same manufacturer (7.5 % decrease over 6 months; $p < 0.0001$). Contrary to expectations, the failing batch was the one with the longer remaining shelf life (Table 1), indicating that its different stability was not due to the age of the sample, but possibly due to batch-to-batch differences in the manufacturing of this product, or due to different storage conditions of the two batches prior to sample collection.

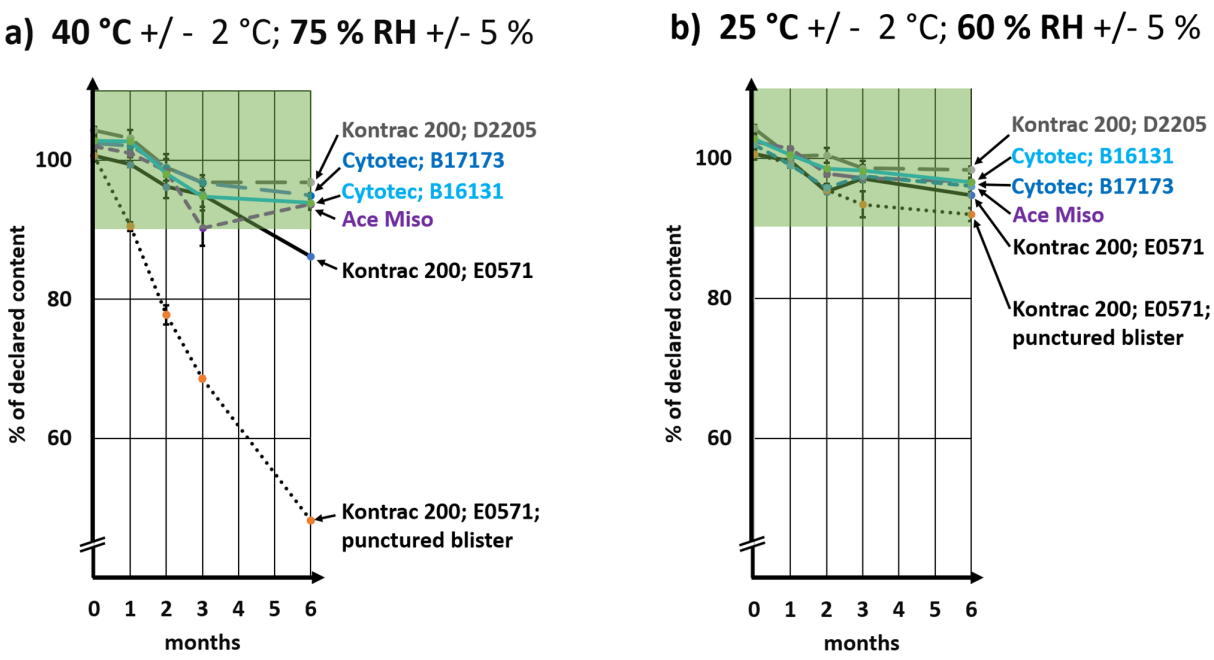

Fig 2. Change of misoprostol content in tablets store for 6 months a) at 40 °C and 75 % relative humidity (RH) and b) at 25 °C and 60 % RH. Error bars show standard deviation. The International Pharmacopeia requires a content of misoprostol between 90–110 % of the declared amount. This range is marked in the figure.

4.3.3. Effect of damaged blisters

All investigated products were correctly packaged in double-sided aluminium blisters. In order to investigate the importance of the intactness of the primary packaging for the stability of the misoprostol tablets, the blister strips of one sample were intentionally damaged by puncturing a single hole of approximately 1 mm diameter into each alveolus of the blisters (S1 Fig), allowing access of air and humidity to the tablets. The sample in these punctured blisters was investigated in parallel to the samples with the intact blisters. As expected, damage to the primary packaging had a strong detrimental influence on stability (Fig 2a and S1 Table): already after two months at 40 °C and 75 % RH, the misoprostol content was out of specifications, and after six months the remaining amount of misoprostol was as low as 48.2 % of the declared content. HPLC analysis of this sample for related substances according to Kahsay et al. [35] clearly showed the decrease of the misoprostol content and the concomitant increase of the typical degradation products of misoprostol, i.e. misoprostol A and, to a smaller extent, misoprostol B and 8-***epi***-misoprostol (Fig 3).

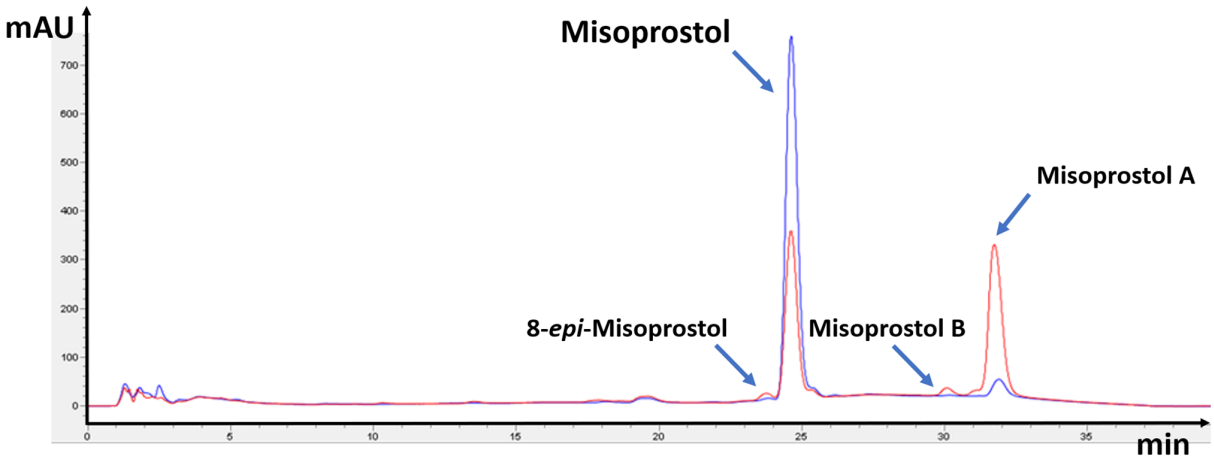

Fig 3. HPLC analysis of misoprostol tablets stored in damaged blisters at 40 °C and 75 % RH for six months (red line). KONTRAC 200 tablets of Fourrts (India), batch E0571 (see Table 1) were used for this experiment. Tablets of the same batch stored in intact blisters at 25 °C and 60 % RH for six months are shown as comparison (blue line). HPLC analysis for related substances was carried out according to Kahsay et al. [35].

4.3.4. Stability at long-term storage conditions

Parallel to the accelerated stability testing at 40 °C and 75 % RH, a control experiment was conducted at 25 °C and 60 % RH, which represents the long-term storage condition for non-refrigerated products in climatic zone II [26, 31]. As shown in Fig 2b and in S1 Table, the assay results of all five investigated batches remained in specifications under these conditions for the six months testing period. Even the sample with the punctured blisters remained in specifications, though it showed the lowest final misoprostol content of all investigated samples (Fig 2b).

Also at 25 °C and 60 % RH, a decline of the misoprostol content over six month was visible (Fig 2b). Assuming that misoprostol degradation followed first-order kinetics [22], i.e. with a linear relationship between the logarithm of the content and time, we calculated from the measurements depicted in Fig 2b the expected misoprostol content at the time point two years after the date of manufacture upon continued storage at 25 °C and 60 % RH. This calculation predicted that four of the investigated batches would show contents between 90.2 and 96.6 % of the declared amount, i.e. would remain in specifications for assay during that time period. In contrast, the batch which had failed assay testing after accelerated stability testing (KONTRAC 200, batch E0571) was predicted to show a content of 87.3 % at the time of its expiry date, which would be out of specifications. Results of such extrapolations need to be

evaluated with care, but still they indicate that the differences in stability observed at 25 °C were consistent with those observed at 40 °C.

4.3.5. Dissolution testing

All collected batches, as well as the sample with the intentionally punctured blisters, were also tested for dissolution of the active pharmaceutical ingredient (API). The International Pharmacopoeia requires that at least 80 % of the declared amount of misoprostol dissolves under the defined conditions (see Methods). As shown in Table 2, all five investigated batches perfectly complied with this requirement, showing the nearly complete dissolution of the API. Storage in intact blisters for six months at 40 °C and 75 % RH resulted only in moderate decreases of the dissolution values (Table 2). These mainly reflected the loss of the API content described above, while the dissolved percentage of the API showed only small changes over six months (Table 2, right columns). All five batches remained within specifications, but the batch of the non-SRA product which had failed assay testing after storage under these conditions showed a final dissolution value of 81.3 %, passing the 80 % threshold of the pharmacopoeia only by a narrow margin.

Table 2. Dissolution testing results of misoprostol tablets stored at two different conditions, 0 and 6 months.

		Dissolution (% of declared content)				Dissolution (% of assay determined at the respective month [see S1 Table])	
Storage condition	**Sample**	**Month 0**	**RSD**	**Month 6**	**RSD**	**Month 0**	**Month 6**
40˚C +/ - 2˚C	Kontrac 200, batch E0571	99.2	0.98%	81.3	2.81%	98.6	94.3
	Kontrac 200, batch D2205	100.4	5.76%	95.1	1.72%	96.3	98.2
	Ace Miso	99.7	2.59%	90.8	1.57%	97.7	96.9
75% RH +/- 5%	Cytotec, batch B16131	104.0	3.03%	92.3	1.86%	101.1	98.3
	Cytotec, batch B17173	99.9	2.82%	91.0	2.26%	97.5	95.8
	Kontrac 200, batch E0571; punctured blister	99.2	0.98%	42.4	3.87%	98.6	87.9
25˚C +/- 2˚C	Kontrac 200, batch E0571	see above		90.6	2.93%	see above	95.6
	Kontrac 200, batch D2205			93.1	2.21%		94.6
	Ace Miso			93.0	2.93%		96.8
60% RH +/- 5%	Cytotec, batch B16131			93.1	1.97%		96.3
	Cytotec, batch B17173			94.6	1.88%		97.9
	Kontrac 200, batch E0571; punctured blister			89.7	2.46%		97.5

RSD: relative standard deviation. RH: relative humidity.

The sample with the punctured blisters clearly failed dissolution specifications after six months at 40 °C and 75 % RH, showing 42.4 % dissolution of the declared API amount. Assay testing had shown a final API content of 48.2 % of the declared amount. Therefore, storage in damaged

blisters at these conditions strongly reduced the API content, and to some extent also reduced the dissolved percentage of the remaining API (Table 2).

After storage at 25 °C and 60 % RH, all tested samples, including the one with the punctured blisters, still complied with the specifications of the pharmacopeia for dissolution of misoprostol (Table 2).

4.4. Discussion

The results of the present study confirm that the limited stability of misoprostol represents a problem for safeguarding the quality of misoprostol tablets especially in countries with hot and humid climates. This problem can be reasonably managed by appropriate packaging and by professional formulation of the tablets. The two investigated batches of the originator product, as well as the WHO-prequalified product, were found to remain within the specifications of the International Pharmacopoeia over six months of accelerated stability testing at 40 °C and 75 % RH, despite the fact that already 11, 16 and 19 months had elapsed since their date of manufacture when they entered stability testing, respectively. The WHO-prequalified product even exceeded its expiry date 2 months before the end of the stability test. Still, it conformed to specifications both in assay and in dissolution at the end of the six-months testing period. This may be seen as a further example that WHO prequalification is a reliable assurance of the good quality of medicines. However, of the two tested batches of a product without WHO prequalification and produced in a country without stringent regulatory authority (SRA), one batch was clearly out of specifications at the end of the accelerated stability testing. The other batch of this manufacturer showed much less degradation during accelerated stability testing. The clearly different results between the two batches may raise doubts about the batch-to-batch consistency in the manufacturing of this product.

In this study, misoprostol tablets were included in the stability test only if they were within specifications at the beginning of the test. One brand of misoprostol tablets collected in Rwanda (packaged in double-sided aluminium blisters; manufactured in a country without SRA; not WHO-prequalified) had to be excluded due to extreme deviations from specifications in the assay. Furthermore, Hall [25], Anyakora et al. [11] and Hagen et al. [12] have reported insufficient misoprostol contents in many misoprostol preparations collected in LMICs. Therefore, quality and/or stability problems are certainly not a rare observation in misoprostol tablets circulating in LMICs.

The observation that several batches investigated in this study remained in specifications during accelerated stability testing does not prove the absence of stability problems. The current ICH/WHO guidelines [26, 31] require that the investigated preparations remain in specifications over the six-months testing period and that the content of the API does not change by more than 5 %. None of the five investigated batches complied with our accelerated stability test criterion. This result has to be interpreted with care, since the products had been

stored for extended periods of time, at unknown conditions, before the stability test was conducted. Therefore, the present results do not prove that the preparations failed their stability requirements at the time when they left the manufacturing company. Nevertheless, the data reported in the present study confirm that the stability of misoprostol tablets is problematic. This is also stated in the assessment report of the WHO prequalification programme for the misoprostol tablets produced by Acme (Table 1): “The product is chemically not very stable; the data show an increase of degradation with time at accelerated and long-term storage conditions, though within justified limits” [38].

In the present study, a decrease of the misoprostol content was observed both at 40°C and 75 % RH and at 25 °C and 60 % RH (Fig 2). An investigation of the storage conditions of misoprostol tablets in health facilities in Malawi had shown mean kinetic temperatures at the different storage sites ranging from 21.4 °C to 31.0 °C [12]. In view of these observations, a reduction of the stated shelf-life of the originator brand (Table 1) from three to two years may be advisable, especially for those batches which are exported to countries with hot and humid climates. Also, the omission of a storage requirement on the packaging of the originator product marketed in Rwanda is surprising and should be corrected by the manufacturer.

Manufacturers are aware of the stability problems of misoprostol. They frequently manufacture tablets with an initial content of more than 100 % of the stated amount to ensure that the misoprostol content of their preparations does not fall below the pharmacopeial limit of 90 % of the declared amount within their shelf-life. This was observed in the present study: the content of all preparations at the beginning of the stability testing exceeded 100 % of the stated amount, despite the time which had elapsed since manufacture. The data reported by Hall [25] showed that out of 215 samples of misoprostol tablets which were investigated at an age between zero and three years after their date of manufacture, approximately 43 % contained between 100–110 % of the stated content, and 5 % even exceeded the pharmacopeial limit of 110 % at the time of analysis. It has to be expected that the prevalence of overdosed preparations would be even higher if all of them had been investigated shortly after their date of manufacture. Also for the preparations investigated in the present study, extrapolation of the data shown in Fig 2b indicates that their misoprostol content at the time of manufacture may have exceeded the pharmacopeial limit of 110 %. However, as mentioned above, results of such extrapolations need to be interpreted with care.

It is encouraging that all brands of misoprostol tablets distributed by wholesalers and government stores in Malawi and Rwanda at the time of sample collection were packaged in double-sided aluminium blisters. This may be a consequence of the report by Hall [25] that plastic-aluminium blisters are grossly inadequate to ensure stability of misoprostol tablets. According to the information listed on the website of the WHO prequalification of medicines programme [39], all three WHO-prequalified brands of misoprostol tablets are packaged in double-sided aluminium blisters for protection of the tablet against moisture, and are produced from 1:100 misoprostol dispersion in HPMC [38].

Both the originator product [40, 41] and at least one generic preparation produced in India [12, 25] are also marketed in screw-cap plastic bottles containing 60 or 100 tablets. While such bottles in principle offer good protection if also a desiccant in sufficient amount is included in the packaging [42], their use in LMICs is not advisable since proper closure of the bottle may not always be ensured and thereby access of humidity may severely affect the quality of the tablets. A preparation packaged in such bottles found in Malawi contained only 48.8% of the misoprostol amount declared on the label 29 months after the date of manufacture [12].

For misoprostol tablets packaged in double-sided aluminium blisters, the intactness of the blister is of principal importance for stability. This was reported by Berard et al. [23] and has been clearly confirmed in the present study. If the blister was damaged, we observed more than 50 % loss of misoprostol within 6 months at 40 °C and 75 % RH. The velocity of the degradation was strongly dependent on the environmental conditions: at 25 °C and 60 % RH, only 8.7 % loss occurred within 6 months. 1.5 % loss was observed in the first month under these conditions. This is somewhat different from the time pattern reported by Berard et al. [23], who had investigated another brand of misoprostol tablets for a period of one month after complete removal from their blister packs. At 25 °C and 60 % RH, these authors reported a loss of misoprostol content of approximately 10 % within the first week, followed (somewhat surprisingly) by a loss of only about 0.5 % per week in the following three weeks.

The results of our study show that unprotected storage of misoprostol tablets for extended periods at high temperature and humidity is extremely detrimental, while unprotected storage for one month at 25 °C and 60 % RH only had a small effect on the misoprostol content. Nevertheless, in view of the instability of misoprostol, storage outside of intact blisters should be avoided, even for short periods.

The key stability problem of misoprostol tablets is the degradation of the API, especially in the absence of proper protection from humidity. In contrast, dissolution of misoprostol was hardly affected during accelerated stability testing in this study, and even unprotected storage did not affect dissolution of the remaining amount of misoprostol very strongly (see Table 2).

Limitations of this study

This study investigated only those brands and batches of misoprostol tablets which were available at government medical stores and pharmaceutical wholesalers in Malawi and Rwanda during the time of sample collection, and may not be representative for other brands or batches. Furthermore, while this approach to sample collection provides a realistic picture of the stability of the products which were distributed in these countries at that time, it does no give a precise picture of the condition of the samples at the time when they left the manufacturing companies. Differences in the results obtained for different brands and batches may be influenced by the different age of samples at time of analysis, and by their different storage condition prior to collection.

Conclusions

The stability of misoprostol tablets is problematic and must be ensured by intact, quality assured double-sided aluminium blisters and by appropriate manufacturing using HPMC as stabilizing agent. Misoprostol tablets of very different quality and stability are on the market, and careful supplier qualification is required in the procurement process. In doubt, procurement should be restricted to WHO-prequalified products, and/or products manufactured under supervision of a stringent regulatory authority. In the prevention and treatment of PPH, misoprostol tablets offer the advantage over oxytocin injections that they do not need parenteral administration. However, the notion that misoprostol tablets present fewer stability problems than oxytocin injections may be misleading, especially in hot and humid climates.

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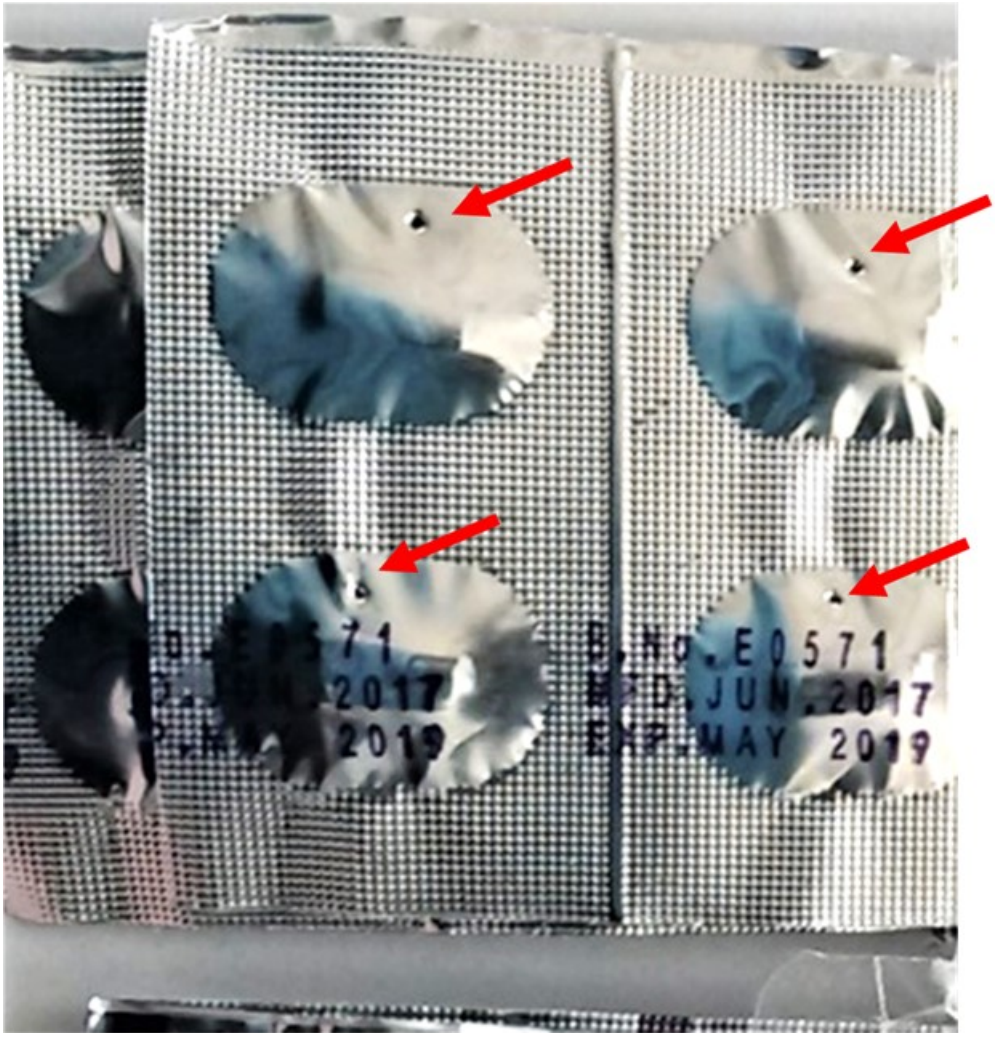
Supporting information

S1 Fig. Intentionally punctured blister of misoprostol tablets (Kontrac 200, batch E0571). Needle punctures are highlighted by arrows.



Intentionally punctured blister of misoprostol tablets (Kontrac 200, batch E0571).

S1 Table. Assay testing results of misoprostol tablets stored at two different conditions over 6 months.

		Misoprostol assay (% of declared content)									
Storage condition	**Sample**	**Month 0**	**RSD**	**Month 1**	**RSD**	**Month 2**	**RSD**	**Month 3**	**RSD**	**Month 6**	**RSD**
40°C +/- 2°C **75% RH** +/- 5%	**Kontrac 200, batch E0571**	100.7	0.82 %	99.4	0.42 %	96.2	1.71 %	95.0	1.85 %	86.2	0.15 %
	Kontrac 200, batch D2205	104.3	0.43 %	103.2	0.14 %	99.0	1.22 %	96.8	0.50 %	96.8	0.42 %
	Ace Miso	102.0	0.44 %	101.0	0.58 %	98.4	0.60 %	90.3	2.81 %	93.7	0.85 %
	Cytotec, batch B16131	102.8	0.41 %	102.7	1.56 %	98.1	0.42 %	94.8	2.07 %	93.9	0.72 %
	Cytotec, batch B17173	102.4	1.13 %	102.1	0.20 %	99.0	1.91 %	96.8	1.12 %	94.9	0.34 %
	Kontrac 200, batch E0571; punctured blister	100.7	0.82 %	90.5	0.69 %	77.8	1.75 %	68.6	0.47 %	48.2	0.24 %
25°C +/- 2°C **60% RH** +/- 5%	**Kontrac 200, batch E0571**	see above		99.6	1.28 %	95.2	1.12 %	97.1	1.32 %	94.8	0.20 %
	Kontrac 200, batch D2205			100.3	1.16 %	100.4	1.03 %	98.6	1.09 %	98.4	0.48 %
	Ace Miso			99.0	0.21 %	95.8	0.62 %	97.5	0.93 %	96.0	0.87 %
	Cytotec, batch B16131			100.5	0.37 %	98.6	0.17 %	98.3	0.77 %	96.6	0.62 %
	Cytotec, batch B17173			101.4	0.29 %	97.8	0.39 %	97.2	0.27 %	96.6	0.14 %
	Kontrac 200, batch E0571; punctured blister			99.2	0.49 %	95.5	0.45 %	93.4	2.01 %	92.0	1.04 %

RSD: relative standard deviation. RH: relative humidity.

S2 Table: Intermediate precision of misoprostol assay and dissolution

Intermediate Precision Calibration Curve Assay Misoprostol											
Reference concentration (µg/ml)	Mean AUC (mAU*s) Month 0	*RSD*	Mean AUC (mAU*s) Month 1	*RSD*	Mean AUC (mAU*s) Month 2	*RSD*	Mean AUC (mAU*s) Month 3	*RSD*	Mean AUC (mAU*s) Month 6	*RSD*	**Mean AUC (mAU*s) all months**	**RSD**
25	1706.08	*0.76%*	1669.21	*0.21%*	1582.35	*1.45%*	1597.30	*0.41%*	1656.25	*0.13%*	**1642.24**	**3.14%**
20	1308.22	*0.64%*	1292.34	*0.58%*	1299.18	*0.34%*	1315.76	*0.36%*	1306.89	*0.49%*	**1304.48**	**0.69%**
15	1044.85	*0.50%*	975.97	*0.47%*	975.27	*0.52%*	1006.14	*0.25%*	981.14	*0.60%*	**996.67**	**2.98%**
10	708.98	*0.24%*	651.95	*0.70%*	700.70	*0.75%*	671.94	*1.07%*	645.61	*0.29%*	**675.84**	**4.20%**
5	353.04	*1.06%*	325.19	*0.62%*	377.79	*1.35%*	372.71	*0.53%*	372.04	*0.30%*	**360.15**	**6.02%**

Intermediate Precision Calibration Curve Dissolution Misoprostol											
Reference concentration (µg/ml)	Mean AUC (mAU*s) Month 0	*RSD*	Mean AUC (mAU*s) Month 1	*RSD*	Mean AUC (mAU*s) Month 2	*RSD*	Mean AUC (mAU*s) Month 3	*RSD*	Mean AUC (mAU*s) Month 6	*RSD*	**Mean AUC (mAU*s) all months**	**RSD**
0.6	94.37	*3.16%*	88.62	*1.34%*	92.06	*0.15%*	91.01	*4.57%*	89.74	*1.72%*	**91.52**	**2.61%**
0.4	61.13	*4.35%*	57.88	*1.12%*	60.58	*2.55%*	59.28	*0.97%*	60.26	*1.73%*	**60.13**	**1.46%**
0.32	49.90	*9.32%*	45.88	*1.17%*	48.36	*2.33%*	45.60	*1.77%*	47.13	*2.72%*	**47.43**	**4.34%**
0.24	34.61	*5.22%*	33.66	*3.49%*	34.07	*4.63%*	33.59	*1.40%*	36.97	*0.55%*	**33.98**	**1.37%**
0.1	11.28	*9.05%*	13.40	*5.32%*	12.80	*4.30%*	13.23	*2.08%*	15.01	*4.07%*	**12.68**	**7.61%**

AUC: area under the curve. RSD: relative standard deviation. Mean AUC calculated based on five measurements for reference concentration 20 μg/ml, and three measurements for all other reference concentrations.

Chapter five: Quality of oxytocin and misoprostol in health facilities of Rwanda

Abstract

Sustainable Development Goal 3.1 calls for reducing the maternal mortality ratio to less than 70 per 100,000 live births by 2030. The most important cause of maternal mortality is post-partum haemorrhage (PPH). Oxytocin injections and misoprostol tablets are medicines of first choice for managing PPH in low- and middle-income countries (LMICs). Unfortunately, both substances are chemically unstable, and previous studies have revealed serious quality problems of these medicines in LMICs. The present study is the first report on their quality in Rwanda.

From 40 selected health facilities by simple random (hospitals, health centers, retail pharmacies, and private clinics) in different parts of Rwanda and from six wholesalers and government stores, oxytocin injections and misoprostol tablets were collected. Oxytocin storage temperatures in the health facilities were monitored for six months using temperature data loggers, and found to correctly follow the storage requirements stated by the manufacturers (2-8 °C, or room temperature) with few minor deviations.

Oxytocin injections (57 samples, representing seven batches of four brands) were tested for their oxytocin content and pH value according to the United States Pharmacopeia. Twenty-four samples from three European manufacturers passed all tests. However, all nine samples of one batch of a Chinese manufacturer showed an excessive oxytocin content (range 117.2-121.5% of the declared amount). Another batch of the same manufacturer showed extreme variations in the preservative benzyl alcohol concentration.

According to the International Pharmacopeia, Misoprostol tablets (25 samples, representing ten batches of six brands) were tested for content and dissolution. Fifteen samples passed, but all 10 samples of two brands from India failed with extreme deviations, containing only 42.5-48.7% of the stated amount of misoprostol.

In conclusion, oxytocin quality in Rwanda was better than reported from other African countries. However, two extremely substandard brands of misoprostol tablets were found. The Rwandan authorities reacted quickly and efficiently, and recalled these substandard medicines from the market. For oxytocin and misoprostol, with their well-known quality and stability

problems, procurement should possibly be restricted to medicines that are WHO-prequalified or manufactured in countries with stringent regulatory authorities.

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No data were changed, only minor edit adjustments were made to fit the paper into the Microsoft office word format and accommodate comments from panelists.

5.1. Introduction

In the year 2017, an estimated number of 295,000 women around the world died due to complications of pregnancy and childbirth [1]. The highest maternal mortality ratio is observed in the sub-Saharan region of Africa [1,2]. Post-partum hemorrhage (PPH) is the most important cause of maternal mortality, and 50 % of all cases of PPH worldwide occur in Africa [3]. Rwanda, a low-income country in sub-Saharan Africa [4], has successfully lowered its maternal mortality ratio from 1160 down to 248 per 100,000 live births in the years from 2000 to 2017 [1]. Efforts are made by the government of Rwanda to further reduce the maternal mortality ratio to less than 70 per 100,000 live births by 2030, in accordance with the Sustainable Development Goal (SDG) 3.1 [5,6]. To achieve this target, oxytocic medicines which are used to treat and prevent PPH are of principal importance. Oxytocin injections and misoprostol tablets are among the medicines of first choice for the prevention and treatment of PPH [3,7]. They are included as oxytocics (uterotonics) in the WHO model list of essential medicines [8] and in the Rwanda National List of Essential Medicines for adults [9]. Also, they have been included in the list of 13 life-saving items prepared by the United Nations Commission on Life-Saving Commodities for Women and Children (UNCoLSC) [10], as the only medicines for the management of PPH.

The use of substandard and falsified medicines has been shown to result in serious public health problems [11]. Especially in LMICs, the quality of medicines often fails to meet the pharmacopeial specifications, and this has far-reaching adverse consequences for patients, families, national health systems and the economy [11,12]. The use of substandard oxytocin or misoprostol preparations in the management of PPH may lead to therapeutic failure in the treatment of excessive bleeding, and even to the death of the patient [13,14]. Avoiding such preventable deaths is one of the key measures required to reach SDG 3.1.

Unfortunately, oxytocics are sensitive to environmental conditions. Oxytocin itself is a peptide hormone containing nine amino acids with an intramolecular disulfide bridge (Fig 1) [15]. It is highly sensitive to elevated temperatures and may degrade quickly when inappropriately stored, especially in tropical climates [16,17]. The World Health Organization (WHO) recommends to store all preparations of oxytocin in the refrigerator, i.e. between 2 °C and 8 °C [16]. However, some commercial oxytocin preparations carry recommendations for non-refrigerated storage [18,19]. Oxytocin stability furthermore depends on the pH value, with an optimum stability at pH 4.5 [20]. Both the United States Pharmacopeia (USP) and the

International Pharmacopeia (Ph. Int.) demand that oxytocin injections must have a pH value between 3.0 and 5.0 [19].

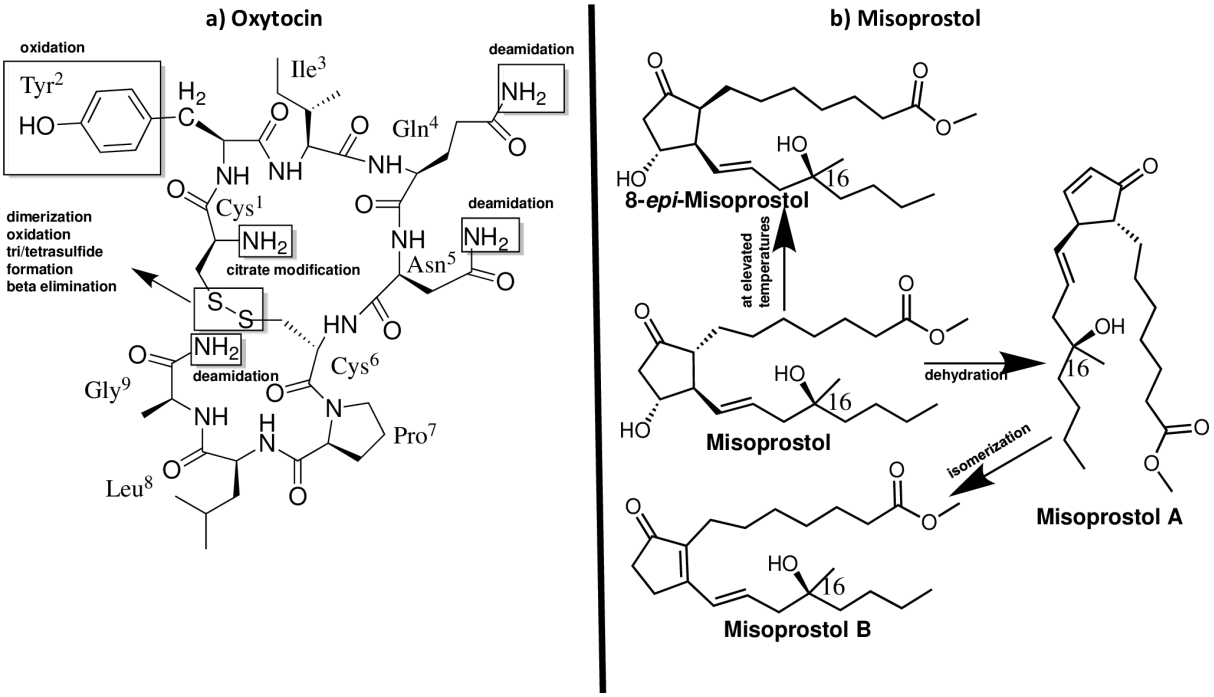

Fig 1. Structures of oxytocin (modified from [21]) and misoprostol, and their typical degradation mechanisms. Commercial misoprostol is a mixture, containing the depicted structure, its epimer at C16, and the enantiomers of both compounds. Likewise, the degradation products of misoprostol contain the corresponding stereoisomers.

A systematic review published by Torloni et al. in 2016 [13] listed eight studies on the quality of oxytocin conducted in LMICs. In a subsequent systematic review published by the same authors in 2020 [14], the number of included oxytocin quality studies had increased to 14. Overall, 39.7 % of the oxytocin samples investigated in all these studies had been reported to fail quality testing. An insufficient content of the active pharmaceutical ingredient (API) represented by far the most frequently found deficiency. The overall percentage of failing samples had been 31.4 % for studies conducted in the time period of 2000-2011 (n = 363 samples), but had increased to 44.4 % for studies conducted from the year 2012 onwards (n = 611 samples) [14]. This indicates that in the last two decades, the quality problems of oxytocin have increased rather than decreased.

The percentage of oxytocin samples reported to fail quality testing varies notably between different studies. Anyakora et al. [22] reported that 74.2 % of the 159 oxytocin samples collected in Nigeria failed quality testing. Similarly, Stanton et al. [23] reported a failure rate of 73.9 % upon investigation of 46 oxytocin samples from Ghana. On the other hand, Hagen et al. [24] showed that only 11 % out of 65 oxytocin samples collected in Malawi failed testing, and only with moderate deviations from the pharmacopeial specifications. Another study conducted in Ethiopia reported a failure rate of only 4 % within 45 oxytocin samples [25]. In all these studies, the failure rate resulted nearly exclusively from an incorrect API amount determined in the samples [14]. Therefore, the different failure rates reported are not due to different parameters being tested in the different studies.

Misoprostol (Fig 1) is an analog of prostaglandin E1. Commercial preparations contain a mixture of both its epimers at C-16, and their enantiomers [26]. Misoprostol is a viscous oil at room temperature and is extremely unstable in the presence of water [27]. Both the raw material and the finished products have to be carefully protected from humidity [27,28]. In finished pharmaceutical products, misoprostol must be stabilized in form of a 1 % dispersion in hydroxypropyl methylcellulose (HPMC) [29], since in the absence of HPMC misoprostol quickly undergoes dehydration, isomerization and epimerization reactions (Fig 1), resulting in a loss of activity. A study by Hall [28] on 215 misoprostol samples, collected in 15 LMICs, reported an incorrect API content in 45 % of the samples, and a decomposition of misoprostol in those samples which were packaged in plastic-aluminium blisters. Therefore, it has been strongly recommended that misoprostol tablets should be packaged in double-sided aluminium blisters to protect them from moisture [28]. Storage of misoprostol tablets outside the blisters exposes them to moisture and has been shown to quickly decrease the amount of the active ingredient, and also to reduce hardness and increase friability of the tablets [27]. In spite of these well-documented problems, misoprostol quality in LMICs has received much less attention than oxytocin quality. The above-mentioned review published by Torloni et al. [14] lists, besides the study by Hall, only two further studies which investigated the quality of misoprostol tablets: Anyakora et al. [22] reported that 56 (33.7 %) out of 166 misoprostol samples collected in Nigeria failed quality testing due to incorrect API content, but the study did not state the exact amount of API detected in the samples. Hagen et al. [24] reported that 5 (17 %) out of 30 misoprostol samples from Malawi failed pharmacopeial specifications, notably all five with extreme deviations since they contained only 12.7-30.2 % of the declared amount.

So far, no data on the quality of oxytocin injections and misoprostol tablets in Rwanda have been published, although the above-mentioned findings from other LMICs indicate that the presence of substandard preparations is likely. Therefore, in the present study samples of oxytocin injections and misoprostol tablets were collected from randomly selected government, faith-based and private health facilities and drug outlets, as well as from government medical stores and private wholesalers in Rwanda, and were investigated for their quality according to the United States Pharmacopeia (USP) and the International Pharmacopeia (Ph. Int.), respectively. In parallel to this study, an evaluation of the availability and prices of essential medicines in health facilities of Rwanda, also beyond oxytocin and misoprostol, has been carried out, and the results have been published elsewhere [30].

5.2. Methods

5.2.1. Study design and ethical approval

The study protocol and the methods for collection and investigation of the samples were designed following the MEDQUARG guidelines [31] and the WHO Guidelines on the Conduct of Surveys of the Quality of Medicines [32]. Ethical approval to conduct this study was obtained from the College of Medicine and Health Sciences Institutional Review Board (CMHS-IRB) of the University of Rwanda with approval notice No. 026 /CMHS IRB/2018. An authorization to access health facilities and to conduct this study was kindly granted by the Ministry of Health, Rwanda (reference No. 20/1361/DGPHFIS/2018). In fulfilment of the requirements for sample transfer from Rwanda to Germany, a Material Transfer Agreement (MTA) was signed between investigators and the Rwandan Ministry of Health. Consent to import medicine samples for analysis was also obtained from German authorities (Regierungspräsidium Tübingen, Leitstelle Arzneimittelüberwachung).

5.2.2. Selection of sampling sites

Samples of oxytocin and misoprostol were collected in Kigali city and in five districts representing the provinces of Rwanda, i.e. Bugesera district (Eastern Province), Karongi district (Western Province), Musanze district (Northern Province), and Muhanga and Kamonyi districts (Southern Province). Government, faith-based and private facilities were included, i.e. government district hospitals and health centers, faith-based district hospitals and health centers, private retail pharmacies and private clinics/hospitals.

A list of these health facilities in Kigali city and the five selected districts was obtained from the Ministry of Health, comprising 13 district hospitals (government or faith-based), 77 government health centers, 44 faith-based health centers, 36 private clinics, and 234 private retail pharmacies. For each of the five districts and for Kigali city, two hospitals and two health facilities of each of the other categories (government health centers; faith-based health centers; private retail pharmacies; private clinics) were selected (simple random) using the RAND function of Microsoft Excel. However, in each of the two districts, Muhanga and Kamonyi, only a single district hospital existed; these were included in the study. In Musanze district, no district hospital existed, but a government referral hospital and this was included.

Therefore, a total of 57 health facilities and private retail pharmacies were selected. In the course of the study visits, it turned out that one of the selected district hospitals was a

specialized orthopedic hospital and did not stock oxytocin or misoprostol. Also, 11 of the 12 selected private clinics stated that they did not store these medicines, and 5 of the 12 retail pharmacies had none of these two medicines available. Therefore, oxytocin and/or misoprostol could be collected from 40 health facilities and retail pharmacies, and these are listed in S1 Table (Supplementary Information).

In addition to health facilities and retail pharmacies, oxytocics were also collected from the government central medical store (Medical Procurement and Production Division [MPPD]), from two government district pharmacies and three large private wholesalers. Therefore, samples were collected from a total of 46 different facilities. Fig 2 shows the location of the facilities on a map of Rwanda.

In health facilities which stored oxytocics both in their pharmacy stores and in their maternity wards, samples were collected from both sites if these medicines were available there. Thereby in total, samples were collected from 44 storage rooms and 20 maternity wards, as listed in S1 Table (Supporting Information).

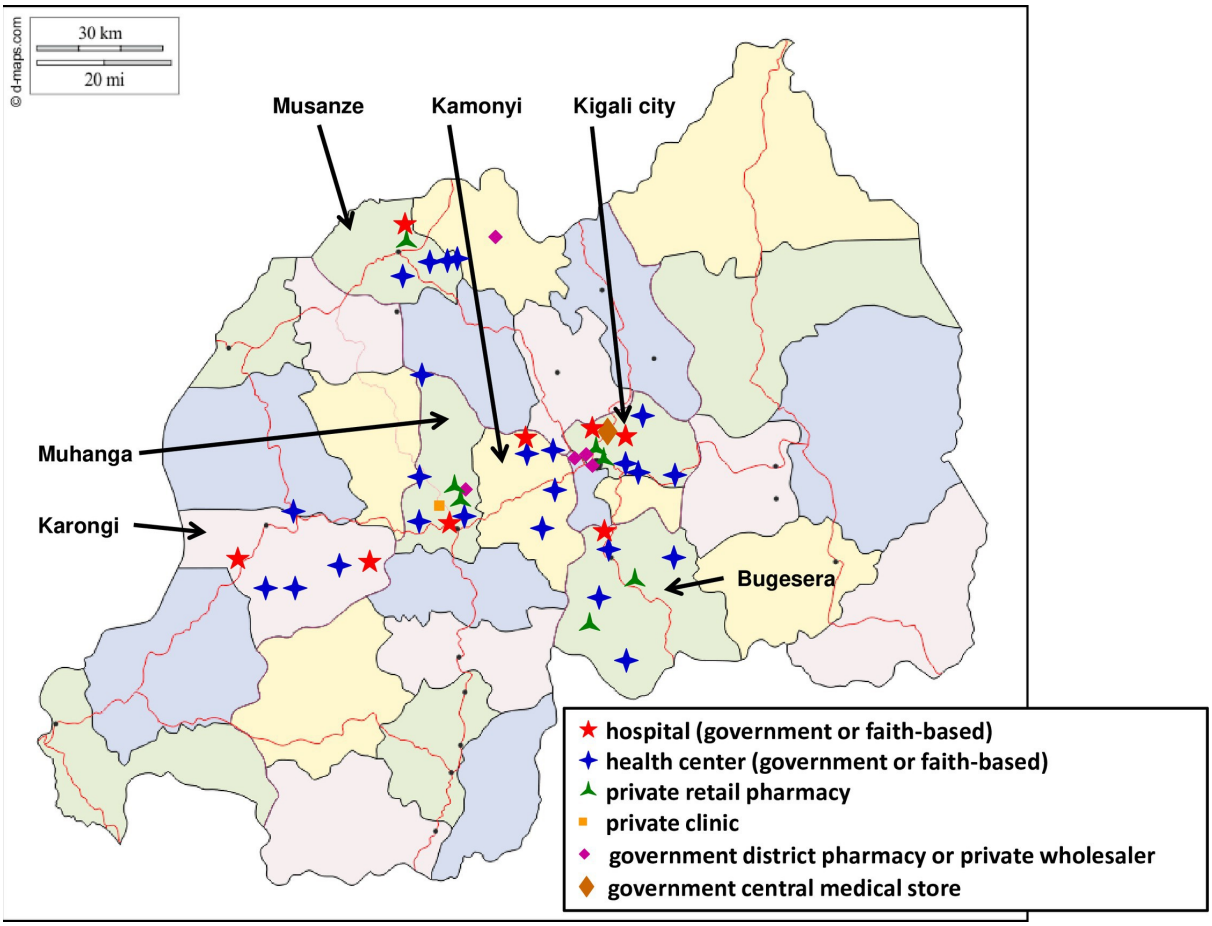

Fig 2. Map of the location of the 46 facilities from which oxytocics were collected. Reprinted (with modifications) from https://d-maps.com/conditions.php?lang=en under a CC BY license, with permission from d-maps.com, original copyright 2007-2020.

5.2.3. Sample collection

Sampling was done by the investigator T.B. in March 2018 for the pilot study in Muhanga district, and in September-October 2018 for the main study in the other districts and Kigali city. At each sampling site, from every available brand and batch, 10 vials of oxytocin and 50 tablets of misoprostol were collected if available. A minimum amount of 5 vials of oxytocin and 10 tablets of misoprostol was collected.

When sampling from government and faith-based health facilities, the collected vials and tablets were replaced with medicines purchased by the investigator from the government central medical store or two government district pharmacies to avoid causing stock-outs in the health facilities the sampling. Samples collected from private facilities (retail pharmacies, private clinics and wholesalers) were paid for in cash. In health facilities and retail pharmacies, samples were collected by an overt approach, i.e. the investigator informed the staff about the purpose of the visit, and the "Sample Site and Drug Purchase Records" shown in S1 Fig (Supporting Information) were filled and signed by the investigator and by the responsible person at the health facility. Samples from private wholesalers were obtained through a local retail pharmacy using a mystery shopper approach, and no "Sample Site and Drug Purchase Records" were prepared for these purchases.

Each sample was immediately labeled with a unique sample code upon sample collection. All samples of oxytocin were transported to a central collection site in Rwanda in a 12 V plug-in refrigerator, and subsequently stored between 2 °C and 8 °C until shipment to Germany. Misoprostol tablets were transported and stored at a temperature not exceeding 25 °C. Samples were hand-carried to Tübingen University, Germany, by investigator T. B. on commercial passenger flights in May 2018 (for the pilot study) and in November 2018 (for the main study). They were placed into appropriate storage conditions at Tübingen University within 24 hours after departure from Rwanda. A temperature data logger was kept with the samples to record the temperature during transport and intermediate storage.

5.2.4. Assessment of oxytocin storage temperatures

Oxytocin storage temperatures were recorded using temperature data loggers (Tempmate M1 by imec Messtechnik GmbH, Heilbronn, Germany). The investigator placed these at the storage sites of oxytocin at the time of sample collection, and they automatically recorded the temperature every 10 minutes. Private wholesalers were contacted by a mystery shopper approach, and therefore no temperature data logger could be placed. The investigator recollected temperature data loggers after six months, and the mean kinetic temperature (MKT) was calculated by the imec Messtechnik software.

5.2.5. Registration status of collected medicines

To enquire the registration status of the medicines collected in Rwanda, the Rwanda Food and Drug Authority (RFDA) was contacted. However, the full registration process of medicines by RFDA had not yet been in effect at the time of sample collection. It could not be established which of the collected preparations had been pre-registered according to the previous procedures.

5.2.6. Packaging examination

The information stated on the packaging and in the package inserts of the samples were examined visually for the presence of irregularities or inconsistencies, such as spelling mistakes, unusual batch numbers, unexpected or modified manufacturing or expiry dates, or signs of repacking. Furthermore, the individual dosage units (oxytocin vials and misoprostol tablets) were inspected for visible deficiencies, like color changes, suspended particles within vials, etc. In addition, for misoprostol tablets the material of the primary packaging was recorded, as this is important for misoprostol stability [28].

5.2.7. HPLC analysis

Oxytocin injections were analyzed for identity, assay and pH value following the respective monograph of the United States Pharmacopoeia 40 (USP 40). The assay was performed using high-performance liquid chromatography (HPLC; Agilent Infinity 1260 II with binary pump, refrigerated autosampler, integrated column compartment and variable wavelength detector; Agilent Technologies, Santa Clara, CA, USA). A Reprospher column 100 C18, 5 µm; 12.5 cm x 4.6 mm (Dr. Maisch GmbH, Ammerbuch, Germany) was used with a column temperature of 21 °C. A linear gradient of mobile phase A (0.1 M aqueous NaH_2PO_4 buffer) and mobile phase B (acetonitrile/water 1:1) was used: 0 min, 30 % B; 10 min: 40 % B; 17.5 min: 65 % B; 20.5 min, 65 % B; 23.5 min, 30 % B; 26 min, 30 % B. Flow rate: 1.5 ml/min. Detection: 220 nm.

Injection volume: 70 µl. The diluent used to prepare standard solutions was prepared by dissolving 500 mg of chlorobutanol in 0.5 ml glacial acetic acid, adding 500 mg of ethyl alcohol, 110 mg of sodium acetate and filling up to 100 ml with bi-distilled water. Analysis was performed for three different vials per sample, each injected twice, yielding six measurements per sample. Oxytocin USP Reference Standard purchased from Merck KGaA, Darmstadt, Germany, was used for comparison. The pH value was measured twice for each vial, testing three vials from each sample of oxytocin, and the average value was calculated.

The concentration of the preservative benzyl alcohol, which was only contained in the oxytocin samples manufactured by Jiangxi Xierkangtai Pharmaceutical Co. Ltd, China, was determined using a method modified from Rego and Nelson [33]. The analysis was carried out using HPLC (Agilent 1200 Series with a diode array detector; Agilent Technologies, Santa Clara, CA, USA), with the same column as described above. The mobile phase was composed of 20 % acetonitrile and 80 % water. Flow rate: 1.5 mL/min. Detection: 254 nm. Injection volume: 50 µl. Samples were diluted 1:30 with bi-distilled water prior to injection, except for sample QOR04 which was injected without dilution. Analysis was performed for at least two different vials per sample, each injected twice. Benzyl alcohol pharmaceutical secondary standard (SIGMA-ALDRICH, St. Louis, MO, USA; Lot LRAC1678; certified purity of 99.98 %) was used as reference material.

Misoprostol tablets were analyzed following the respective monograph of the International Pharmacopoeia 2017 (Ph. Int. 2017) for identity, assay and dissolution testing. The Agilent Infinity 1260 II HPLC instrument described above was used with a stainless-steel column packed with ReproSil-XR 120 C18, 5 µm, 150 mm x 4.6 mm (Dr. Maisch GmbH, Ammerbuch, Germany) and a guard column containing the same material. The column oven was kept at 35.0 °C. A premixed mobile phase composed of acetonitrile and bi-distilled water in a ratio of 45:55 was used, at a flow rate of 1.5 ml/min in isocratic mode. The injection volume was 100 µl for assay and 250 µl for dissolution testing. HPLC vials were kept in a cooled autosampler at 4 °C until sample injection to avoid degradation. Detection was carried out by UV at 200 nm. Sample and standard solutions were freshly prepared using the mobile phase as diluent. For misoprostol assay, two separate determinations were carried out for each sample. For each determination, five tablets were placed into 50 ml of the mobile phase. In the case of three of the collected samples, the number of available tablets was insufficient for this procedure, and in these cases for each of the two determinations one tablet was placed into 10 ml of the mobile

phase. Misoprostol was dissolved from the tablets using an ultrasonic bath, and ice was added to the bath to avoid degradation by heat. The solution was filtered through Rotilabo PTFE 0.20 µm filters (Carl Roth GmbH and Co. KG, Karlsruhe, Germany) and injected into the HPLC, with three injections of each of the two solutions, yielding six measurements per sample. European Pharmacopoeia Reference Standard (batch N° 3.0) from the European Directorate for the Quality of Medicines (EDQM) was used for comparison.

Misoprostol tablets were analyzed for related substances according to Ph. Int., using the same HPLC instrumentation and the same column as described above for the assay. The mobile phases consisted of acetonitrile, water and methanol in the ratio 28:69:3 (mobile phase A) and 47:50:3 (mobile phase B), and were used in the gradient mode described in Ph. Int., at a flow rate of 1.5 ml/min and a column temperature of 35 °C. The injection volume was 200 µl. Detection was carried out by UV at 200 nm. Sample solutions were prepared as described in Ph. Int. For comparison, a misoprostol standard solution 20 µg/ml in mobile phase A, and the same standard solution heated for 1 hour at 80 °C, were used. Each of the sample and standard solutions was injected twice. The different degradation products of misoprostol (Fig. 1) were identified as described by Ph.Int., based on their relative retention times compared to misoprostol.

Dissolution testing was conducted according to Ph. Int. using a dissolution tester PT-WS 610 (Pharma Test Apparatebau AG, Hainburg, Germany). Into each of the six dissolution vessels filled with 500 ml of de-ionized water, one tablet was placed. The test was conducted at 37 ± 0.5 °C with a paddle rotational speed of 50 revolutions per minute. Samples were withdrawn after 30 min through an in-line filter, and 250 µl of these solutions were injected into the Infinity 1260 II HPLC system described above.

The system suitability for the method for oxytocin assay was verified according to USP 40, and for the methods for misoprostol assay and dissolution according to Ph. Int.
For the determination of the benzyl alcohol concentration, linearity and precision of the applied method were validated according to the International Council for Harmonization (ICH) guideline Q2(R1) [34]. Relative standard deviation of the measurements (repeatability) was 0.2 %.

Sample analysis was conducted unblinded to packaging. All oxytocin and misoprostol samples were analyzed before reaching their expiry dates.

5.2.8. Mass spectrometric analysis

Gas chromatography-mass spectrometry (GC-MS) was carried out using a Hewlett Packard/Agilent HP6890 GC system coupled with a HP5973 mass selective detector. The injector temperature was 280 °C. An Agilent HP-5ms Ultra Inert (5 %-phenyl)-methylpolysiloxane column 30 m x 0.25 mm with a film thickness 0.25 µm was used. The temperature gradient was 40 to 320 °C with 10 °C/min, followed by 10 min 320 °C isothermal. Helium was used as carrier gas with a flow rate of 1.2 ml/min. Electron impact ionization (EI) was carried out with 70 eV, and a single quadrupole analyzer was used.
HPLC-MS/MS analysis was conducted on a Thermo Scientific UltiMate 3000 HPLC-System coupled with an ESI-TOF Bruker maXis 4G (Bruker Daltonics, Bremen, Germany) in the positive mode.

5.2.9. Definitions for substandard and falsified medicines

Samples were classified as within specification or out of specification (= substandard) based on the criteria of USP 40 for assay and pH value in case of oxytocin injections, and based on the criteria of the Ph. Int. 2017 for assay and dissolution in case of misoprostol tablets. According to these pharmacopoeias, both oxytocin injections and misoprostol tablets must contain not less than 90.0 % and not more than 110.0 % of the declared amount of the active pharmaceutical ingredient (API). For oxytocin vials, the pH value must be between 3.0 and 5.0. For dissolution testing of misoprostol tablets, the amount in solution must not be less than 80 % (Q) of the amount declared on the label.

Following the terminology introduced by earlier studies of WHO, assay results deviating more than 20 % from the declared API content, and dissolution results falling more than 25 % below the pharmacopeial Q value, were considered as extreme deviations [35,36]. Lesser deviations from pharmacopeial specifications were considered as moderate deviations. As per definition of WHO [11], products that deliberately/fraudulently misrepresent their identity, composition or source were considered falsified.

5.2.10. Data analysis

Excel (Microsoft Office Professional Plus 2019) was used to calculate means, medians and percentiles, and relative standard deviations (RSD). Figures of the distribution of the assay test

results for oxytocin injections and misoprostol tablets, and the dissolution test results for misoprostol tablets, were generated using the statistical software JMP 14.2 (SAS GmbH, Heidelberg, Germany).

5.2.11. Information of national authorities and stakeholders

Following the request stated in the permission No 20/1361/DGPHFIS/2018 from the Ministry of Health of Rwanda to conduct the present study, the authors have submitted on December 2, 2018, an alert letter to the Rwandan authorities about two extremely substandard brands of misoprostol tablets found to circulate in Rwanda (see Results section). Furthermore, this manuscript was shared with the Rwandan Food and Drug Authority (RFDA) and with the WHO Rapid Alert System.

5.3. Results

5.3.1. Overview of sampling sites

As shown in Table 1, oxytocics could be collected from 40 of the 57 selected (simple random) health facilities and retail pharmacies, as well as from three government medical stores and three private wholesalers (see Methods section). In health facilities, samples were collected both from medicine storage rooms and from maternity wards if available. Notably, oxytocin injections were available in every visited government and faith-based health facility, i.e. both in hospitals and health centers, consistent with the recommendations of the Rwanda National List of Essential Medicines (REML) [9]. According to the REML, misoprostol tablets are expected to be available as oxytocics in hospitals but not in health centers. Indeed, all eight hospitals but only three of the 24 visited health centers, had misoprostol tablets available in a sufficient amount for sampling.

A total of 12 retail pharmacies had been selected (simple random) and visited, but only seven had misoprostol tablets available, and none stored oxytocin injections. Of the 12 randomly selected private clinics, most stated that they do not offer maternity services, and oxytocin could be collected only from a single private clinic. A detailed list of all sampling sites, with the numbers of oxytocin vials and misoprostol tablets collected at each site, is shown in S1 Table (Supporting Information).

Table 1. Overview of sampling sites for oxytocin injections and misoprostol tablets

Category of health facility	Number of facilities	Sites in facility	Number of sites	Number of oxytocin samples collected	Number of misoprostol samples collected
Government hospitals	3	storage rooms	3	3	4*
		maternity wards	2	2	1
Faith-based hospitals	5	storage rooms	5	5	5
		maternity wards	5	5	2
Government health centers	12	storage rooms	11	11	2
		maternity wards	6	6	0
Faith-based health centers	12	storage rooms	11	11	1
		maternity wards	7	7	0
Private clinics	1	storage rooms	1	1	0
Retail pharmacies	7	storage rooms	7	0	7
Government central medical store	1	storage rooms	1	2*	0
Government district pharmacies	2	storage rooms	2	1	2
Private wholesalers	3	storage rooms	3	3	1
Total	**46**		**64**	**57**	**25**

* Two brands collected in one sampling site.

5.3.2. Overview of collected oxytocin samples

A total of 57 oxytocin samples were collected. All of these were packaged in vials of 1 ml, with a concentration of 10 IU/ml. As shown in Table 2, the 57 samples represented only four different brands and a total of seven different batches. In addition to the facilities listed in Table 1, three further private pharmaceutical wholesalers in Kigali were contacted, as well as the procurement organization for faith-based health facilities, i.e. the Bureau de Formations Médicales Agréés du Rwanda (BUFMAR), but none of these had any other oxytocin brands or batches in stock than those shown in Table 2. Therefore, the preparations listed in Table 2 appear to represent most (or all) oxytocin batches which were in circulation in Rwanda at the time of sample collection.

The most frequently encountered preparation, representing 33 samples, was stated to be manufactured by Jiangxi Xierkangtai Pharmaceutical Co. Ltd, China. On its secondary packaging, the storage requirement was stated as "Store in a cool dry place, away from light", while in the package insert, the storage requirement showed a slightly different wording: "Store in a dark place at room temperature, protect from light."

The three other brands (Table 2) were stated to be manufactured in European countries with stringent regulatory authorities (SRAs) [37], and all of them were labeled for refrigerated storage (i.e. storage at 2-8 °C). The product manufactured by AS Grindeks, Latvia (marketing authorization holder: Peckforton Pharmaceuticals Ltd., UK) was a WHO-prequalified medicine [38,39].

The stated shelf life of three out of the four brands was three years, and four years in case of the preparation by Grindeks. The package inserts of the preparations by Sterop (Belgium), Grindeks (Latvia) and Rotexmedica GmbH Arzneimittelwerk (Germany) stated the excipients, i.e. sodium chloride, different buffering agents, water for injection, and in case of the Sterop preparation also chlorobutanol, a preservative and stabilizing agent [40]. In contrast, no excipients were stated for the product by Jiangxi Xierkangtai.

The preparations by Jiangxi Xierkangtai (China), by Rotexmedica (Germany) and by Grindeks (Latvia) were all found in both government and faith-based facilities. In contrast, the preparation by Sterop (Belgium) was only found in two private pharmaceutical wholesalers at the time of sample collection. No expired samples of oxytocin were found to be in circulation.

Table 2. List of oxytocin brands and batches collected in this study

Brand name and stated manufacturer	WHO Pre-Qualified/ SRA	Stated storage temperature requirement	Batch N°	Manufacture/ Expiry Date	Number of samples
Oxytocin injection Jiangxi Xierkangtai Pharmaceutical Co. Ltd China	-	room temperature [a]	1606573	Jun 16 / Jun 19	24
			1604521	Apr 16 / Apr 19	9
Steroxine 10 IU/1 ml [b] Laboratoires Sterop Belgium	SRA	2-8 °C	160042	Feb 16 / Jan 19	1
			160269	Sep 16 / Aug 19	1
Oxytocin 10 IU/ml AS Grindeks Latvia [c]	WHO-PQ	2-8 °C	37611016	Oct 16 / Oct 20	4
			37711116	Nov 16 / Nov 20	1
Oxytocin 10 Rotexmedica GmbH Arzneimittelwerk Germany	SRA	2-8 °C	70779A	Sep 17 / Sep 20	17

WHO-PQ = WHO-prequalified medicine; SRA = manufactured in a country with stringent regulatory authority

[a] Storage requirement stated on packaging: "Store in a cool dry place, away from light"; storage requirement stated on the package insert: "Store in a dark place at room temperature, protect from light."

[b] Batch 160269 was labeled with the unbranded generic name “Oxytocin 10 IU/1 ml”, all other information was identical as in batch 160042.

[c] Marketing authorization holder: Peckforton Pharmaceuticals Ltd., United Kingdom.

5.3.3. Oxytocin storage conditions

Out of 57 samples of oxytocin, 33 were labeled for room temperature storage, and 24 for refrigerated storage. The actual storage place in the facilities could be inspected at 52 of the 56 sampling sites; it could not be inspected at the pharmaceutical wholesalers which were contacted by a mystery shopper approach. Notably, in all inspected sites the actual storage place (i.e. inside or outside the refrigerator) correctly corresponded to the storage recommendation of the manufacturer.

Temperature data loggers were placed at the storage places of oxytocin and recollected after six months. In five cases, the loggers failed to record data or got lost in the facility, but from 47 oxytocin storage sites temperature recordings were obtained. These comprised 18

refrigerated and 29 non-refrigerated storage places. The results recorded at the individual sites are listed in S1 Table (Supporting Information).

In 13 out of the 18 refrigerated oxytocin storage sites, the recorded mean kinetic temperature (MKT) ranged from 4.3 °C to 7.3 °C, compliant with the manufacturers' storage requirement of 2-8 °C. In one site, the recorded storage temperature was too low (MKT = -1.3 °C). At this site, except for short temperature spikes (possibly due to opening of the refrigerator), the recorded temperature was constantly around -2 °C. Though this indicates an incorrect temperature setting of the refrigerator, it is unlikely to have caused freezing of the preparation, due to the presence of excipients in the oxytocin vials. In three sites, the recorded MKTs in the refrigerators were slightly too high (8.5 °C, 9.1 °C and 10.5 °C, respectively). And in one further site (a maternity ward of a faith-based hospital), oxytocin was not stored in a refrigerator but in a cool box, reportedly only for immediate use and for not more than 24 hours. Indeed, the temperature data logger at that site recorded alternating periods of cold temperature and room temperature. The MKT in this cool box over the entire recording period resulted as 15.8 °C, but the storage temperature at the times of oxytocin storage may well have been correct.

In most of the other storage sites, the temperatures were largely constant over the entire recording period. Only in one case larger fluctuations (between +15 °C and -2 °C) of longer duration were observed. Five sites showed few very brief periods of temperatures above 8 °C which may have been due to occasional power failures, as also mentioned by the staff of these facilities.

At the 29 non-refrigerated oxytocin storage sites, the median value of the recorded MKTs was 23.5 °C (range 19.8-26.3 °C), reflecting the temperate climate of Rwanda, most of which is situated at an altitude of approximately 1500 m.

5.3.4. Packaging examination and visual inspection of oxytocin samples

Packaging examination revealed no irregularities except for a few minor mistakes in the use of upper- and lower-case letters on the packaging and in the package inserts of the product stated to be manufactured by Jiangxi Xierkangtai (China). However, one sampling site (private clinic) stored the vials of oxytocin not in their original secondary packaging but in a box labeled as dexamethasone. Visual inspection showed no deficiencies like color changes or suspended particles.

5.3.5. Chemical analysis of oxytocin samples

The presence of oxytocin could be confirmed for all samples, therefore neither packaging examination nor chemical analysis indicated the presence of any falsified oxytocin samples. Also the pH value important for oxytocin stability, was within USP specifications (3.0-5.0) for all samples (S2 Table, Supporting Information). Fig 3 shows the distribution of the assay results for the investigated brands and batches. Notably, none of the 57 samples showed an insufficient content of oxytocin.

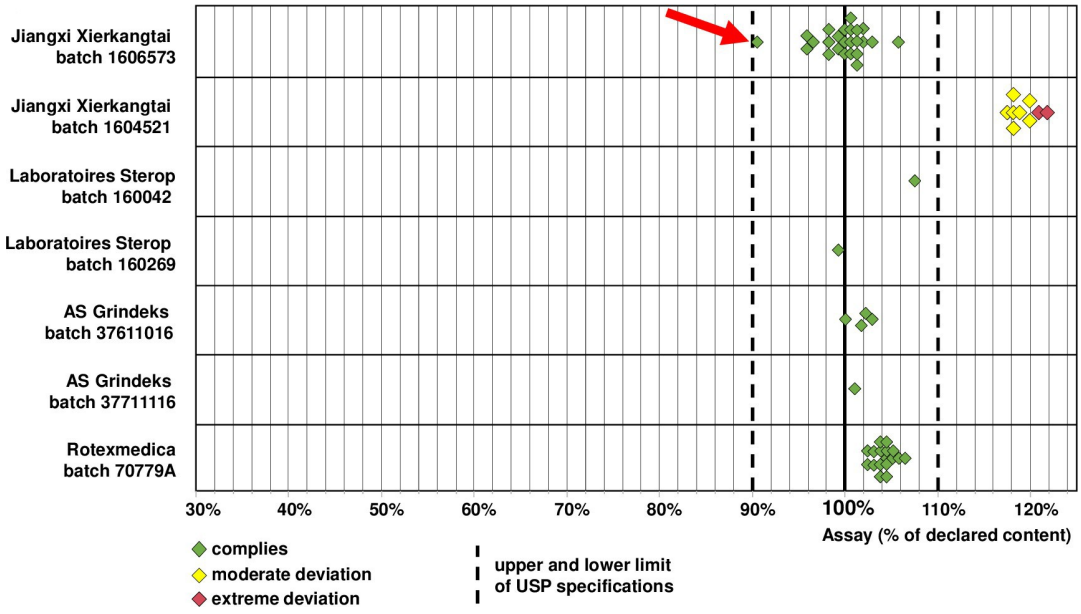

Fig 3. Content of oxytocin determined in each of the investigated samples. The arrow marks a sample with deviating content of the active pharmaceutical ingredient oxytocin and of the preservative benzyl alcohol (see main text and Fig 4).

The 17 samples of Rotexmedica (Germany) all belonged to one single batch and showed a median content of 103.8 % of the declared amount. These samples had been collected at different points of the supply chain (government central medical store; hospitals; government and faith-based health centers), but showed a very uniform API content (range 102.0-105.9 % of the declared amount). Likewise, the samples of AS Grindeks (Latvia) showed a very uniform content (range 99.9-102.8 % of the declared amount). The two samples of Sterop (Belgium), belonging to different batches, contained 99.6 and 107.8 % of the declared amount.

In sharp contrast, all nine samples of batch no. 1604521, stated to be manufactured by Jiangxi Xierkangtai (China) were found to deviate from USP specifications (90-110 % of the declared

amount) by containing too high amounts of oxytocin (median 118.0 %). For two of these samples, the obtained assay result exceeded the declared content by more than 20 %, representing an extreme deviation following the definition used in previous studies of WHO [35,36].

In the other batch by Jiangxi Xierkangtai (batch no. 1606573), 23 of the 24 samples ranged in their content from 95.6 to 105.5 % of the declared amount, well inside the content range specified by USP. However, for one sample of this batch, collected from a private wholesaler (sample no. QOR04; collected from facility no. 44 in S2 Table, Supplementary Information), the oxytocin content determined for three investigated vials was 89.7 %, 90.4 % and 91.0 % of the declared amount, respectively, therefore on the borderline of USP specifications.

Fig 3 shows that the oxytocin content varied between different brands and batches. The results of the chemical analysis of each oxytocin sample, and the age of the samples at the time of analysis, are shown in S2 Table (Supporting Information). In case of Jiangxi Xierkangtai batch no. 1606573, samples which were collected during the pilot study in March 2018 were 24 months old at the time of analysis. These did not show a higher content than those samples collected in the main study in September and October 2018 and were 30 months old at the time of analysis. This, and the other data in in S2 Table, provide no evidence that differences in the age of the samples were important for the observed differences in oxytocin content.

Unexpectedly, the HPLC assay of the samples stating Jiangxi Xierkangtai as manufacturer showed a very large peak of an unknown substance eluting approximately two minutes earlier than oxytocin (Figure 4). Neither the packaging nor the package insert gave any information on the identity of this substance. An enquiry was sent to the three e-mail addresses given on the website of the stated manufacturer, but remained unanswered. Therefore, the identity of the unknown substance was investigated by mass spectrometry. GC-MS analysis showed the following mass and fragmentation: m/z 108 (M^+; 92 %), 107 (67 %), 91 (17 %), 79 (100 %), 77 (63 %), 65 (7 %), 51 (12 %), 39 (8 %). Comparison to the database of the National Institute of Standards and Technologies, Gaithersburg, MD, USA (NIST; http://webbook.nist.gov) showed that these data were identical to those of benzyl alcohol, a commonly used preservative in parenteral pharmaceutical preparations [41]. Subsequently, the identity of the unknown substance was confirmed by both GC-MS and HPLC-MS investigation in comparison to authentic benzyl alcohol, showing identical retention times and mass spectrometric

fragmentations. The concentration of benzyl alcohol was determined as 0.9% in nearly all samples.

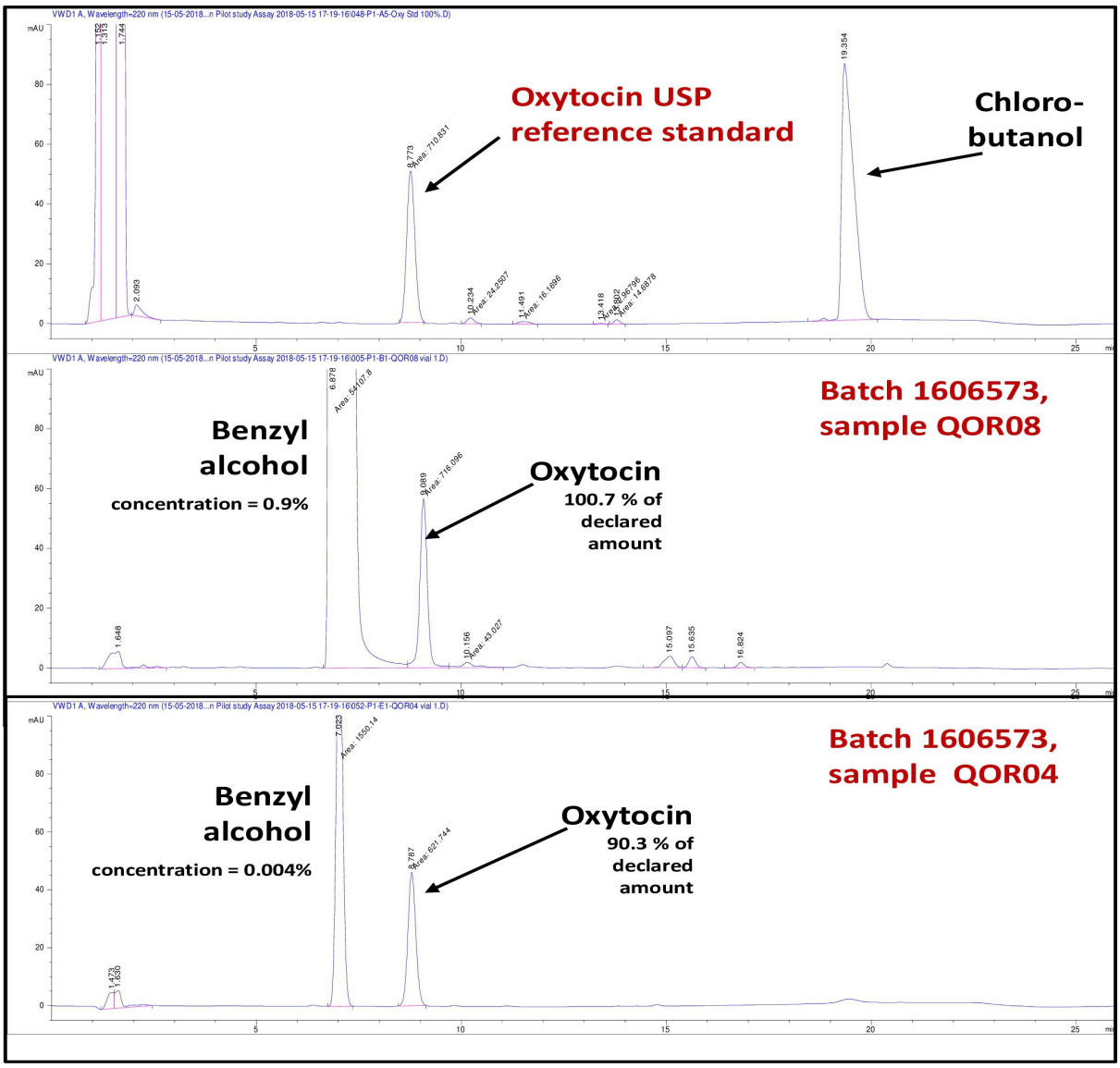

Fig 4. HPLC detection of the undeclared preservative benzyl alcohol, present in different concentrations in oxytocin samples of the same batch, stated to be manufactured by Jiangxi Xierkangtai Pharmaceuticals Co. Ltd (China).

However, the oxytocin sample no. QOR04 (which had already been noticed to contain the lowest oxytocin amount of all investigated samples, see above) was found to contain only 0.004 % benzyl alcohol, showing identical concentrations in all vials. This sample had been collected from a private wholesaler in Kigali. It is highlighted in Fig 3 by an arrow, and in S2 Table by bold print. Its batch number as well as primary and secondary packaging and package insert

were identical to those from the 23 other samples which stated Jiangxi Xierkangtai as manufacturer (S2 Fig, Supporting Information).

Within all other investigated samples by Jiangxi Xierkangtai only a single vial was found with a deviating concentration of benzyl alcohol. It belonged to a sample (no. QOR75) which had been collected in a government district hospital (listed as facility no. 2 in S1 Table). That vial showed a concentration of 0.018 % benzyl alcohol and an oxytocin content of 88.3 % of the declared amount. The other two investigated vials of the same sample QOR075 carried the same batch number and had the same appearance but showed a concentration of 0.9 % benzyl alcohol and oxytocin contents of 99.7 and 100.2 % of the declared amount, respectively.

5.3.6. Overview of collected misoprostol samples

Twenty-five samples of misoprostol tablets were collected. All of them had a stated content of 200 µg per tablet. These samples represented six brands and a total of ten batches (Table 3). As mentioned above for oxytocin, also three further wholesalers as well as the faith-based procurement agency BUFMAR were contacted but had no additional brands or batches of misoprostol tablets in stock. Therefore, the preparations listed in Table 3 appear to represent most (or all) of the batches of misoprostol tablets in circulation in Rwanda at the time of sample collection.

The most frequently encountered preparation, representing ten of the 25 collected samples, was the originator product Cytotec®. For two of the three collected batches, the marketing authorization holder was Pfizer Holding (France), and for the third batch it was Continental Pharma Inc., Belgium (see Table 3). For all three batches, the stated manufacturer of the collected samples was Piramal Healthcare, based in the United Kingdom and therefore in a country with a stringent regulatory authority (SRA). Three further samples were WHO-prequalified medicines [38,39] and had been produced by Acme Formulation Pvt Ltd, India, or by China Resources Zizhu Pharmaceutical Co, Ltd, China, respectively. The remaining 12 samples represented three generic preparations which were manufactured in India (Table 3), a country without an SRA, and were not WHO-prequalified.

Storage requirements were stated on the packaging and/or in the packaging insert of most misoprostol samples, but the indicated temperatures varied (Table 3): two samples were labeled for storage at 15-25 °C, seven samples at 20-25 °C, and eight samples at ≤ 30 °C. For the

remaining eight samples, representing two of the three batches of the originator product Cytotec®, the package insert surprisingly stated: “No special storage requirements.”

To protect it from degradation, misoprostol is very unstable and must be formulated as a 1 % dispersion in hydroxypropyl methylcellulose (HPMC; hypromellose) [29]. For the originator and the WHO-prequalified product by Acme (India), precisely this formulation was stated in the package insert. Zizhu Pharmaceuticals, Co (China), listed hypromellose as an excipient for its product. The Indian company Synokem Pharmaceuticals Ltd (Mizo®) mentioned that misoprostol was contained as a “1 % dispersion”, but failed to mention what it was dispersed in. And notably, for the products by the Indian companies Corona Remedies Pvt., Ltd and Maxtar Bio-Genics, no information on excipients was given, and it remained unclear whether or not HPMC was contained therein.

The shelf life of four of the brands was given as two years, while for C-stol® (Corona Remedies, India) the stated shelf life was three years. For the originator medicine Cytotec® (Piramal Health Care, UK), only an expiry date but no manufacturing date was given on the packaging, but internet databases stated a shelf life of three years for this brand (see Table 3).

Table 3. List of misoprostol brands and batches collected in this study

Brand name and stated manufacturer	WHO Pre-qualified/ SRA	Stated storage requirements	Batch N°	Manufacture/ Expiry Date	Number of samples
Cytotec® 200 µg Piramal Healthcare UK Limited United Kingdom	SRA	No special storage requirements [c]	B15445 [a]	Dec 16 [d] / Nov 19	2
			B17173 [a]	Jul 17 [d] / Jun 20	6
		Store at room temperature (15-25 °C)	B18097 [b]	Nov 17 [d] / Oct 20	2
Ace Miso® Acme Formulation Pvt. Ltd. India	WHO-PQ	Do not store above 30 °C, protect from light	ACE160963	Sep 16 / Aug 18	1
MIZO® SYNOKEM Pharmaceuticals LTD India	-	Store at a temperature not exceeding 30 °C at a dry place	E6SGFT010	Jun 16 / May 18	1
			E6SGLT004	Dec 16 / Nov 18	1
Misoprostol 200 mcg Tablets China Resources, ZIZHU Pharmaceuticals Co Ltd China	WHO-PQ	Store at a temperature not exceeding 30 °C	45180301	Feb 18 / Feb 20	2
C-stol® CORONA Remedies Pvt Ltd India	-	Store below 30 °C. Protect from light and moisture	ERW-005	Mar 18 / Feb 21	3
Cynomax® MAXTAR BIO-GENICS India	-	Store at 20 to 25 °C in a dry area	M8TAB1801	May 18/ Apr 20	4
			MTYX-1604	Aug 16 / Jul 18	3

WHO-PQ = WHO-prequalified medicine; SRA = manufactured in a country with stringent regulatory authority

[a] Marketing authorization holder: Pfizer Holding, France.

[b] Marketing authorization holder: Continental Pharma Inc., Belgium.

[c] Package insert: "Tenir hors de la vue et de la portée des enfants. Pas de précaution particulière de conservation". I.e.: "Keep out of sight and reach of children. No special storage requirements."

[d] Manufacturing date not stated on packaging. Shelf-life listed according to information from the websites www.hpra.ie and www.medicines.org.uk/emc.

5.3.7. Misoprostol blister materials and storage conditions

While elevated storage temperatures primarily cause oxytocin degradation, misoprostol degradation is especially caused by exposure to moisture [27,28]. Therefore, misoprostol tablets must be packaged in double-sided aluminium blisters, not in conventional plastic-aluminium blisters. Indeed, all collected samples were correctly packaged in double-sided aluminium blisters.

In the present study, storage temperatures were systematically recorded for oxytocin but not for misoprostol. However, the storage temperatures recorded at the 29 non-refrigerated oxytocin storage sites and in four of the investigated retail pharmacies (S1 Table, Supporting Information) may present a good approximation for the misoprostol storage temperatures in health facilities and drug outlets of Rwanda. As mentioned above, the recorded mean kinetic temperatures (MKTs) were moderate, and only in five out of 33 sites they slightly exceed 25 °C (with calculated MKTs between 25.1 and 26.3 °C). From one of these sites (MKT 25.4 °C), a misoprostol sample with a stated storage requirement of 20-25 °C was collected (S1 Table, Supporting Information), i.e. the MKT exceeded the demanded storage temperature by 0.4 °C. Therefore, no serious violations of the recommendations for packaging and storage temperatures of misoprostol tablets were observed in the present study.

5.3.8. Chemical analysis of misoprostol samples

The presence of misoprostol was confirmed in all samples, but assay and dissolution testing revealed dramatic shortcomings in two of the six investigated brands. As shown in Fig 5, the assay results were within Ph. Int. specification (90-110 % of the declared amount) for all samples of the originator product, of the two WHO-prequalified brands and of the product by Synokem Pharmaceuticals, India. In sharp contrast, all three samples of C-stol® and all seven samples of Cynomax® showed less than 50 % of the declared content, i.e. failed assay testing with extreme deviations. Therefore, an additional HPLC analysis was carried out for these samples, using the method of Ph. Int. for detection of related substances. This clearly showed large amounts of the typical degradation products of misoprostol (S3 Fig, Supporting Information), suggesting that the low content of misoprostol in these two preparations was due to degradation of the API. The two investigated batches of Cynomax® had different ages at the time of analysis (7 and 22 months since the date of manufacture; S3 Table, Supporting Information) and showed different API contents (mean 44.4% and 47.8 % of the declared content). However, contrary to expectations the higher content was recorded for the older sample, indicating that the difference in content may not have been due to different age but due to differences in manufacture; different transport and storage conditions represent another possible reason.

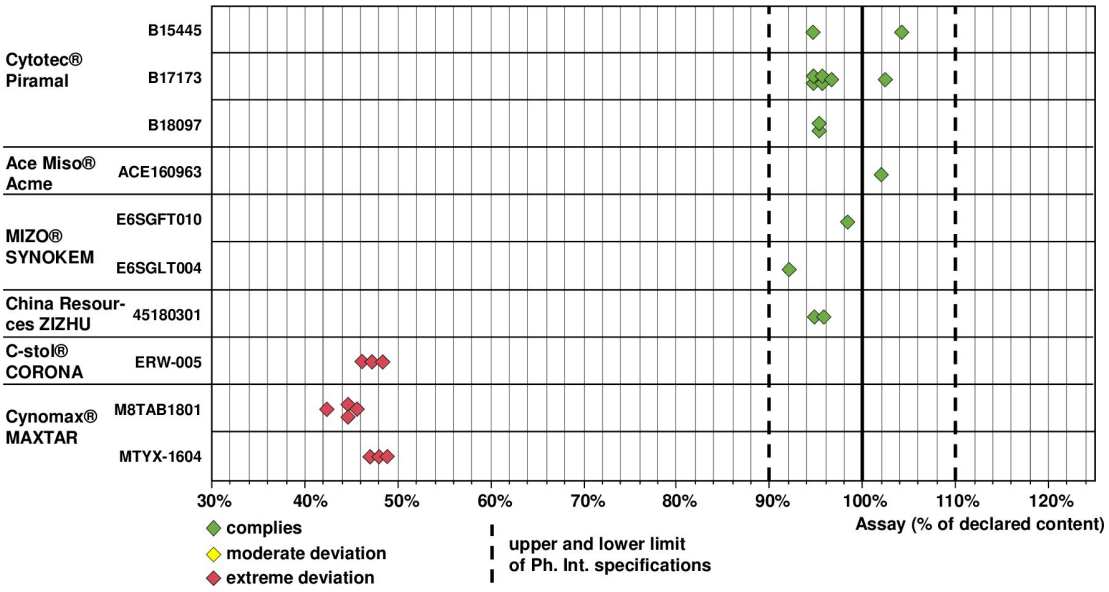

Fig 5. Content of misoprostol determined in each of the investigated samples.

All misoprostol samples were also tested for dissolution of the API. The International Pharmacopoeia demands that from misoprostol tablets at least 80 % of the declared API amount must be released in 30 min under the defined conditions. As shown in Fig 6, all samples which had passed assay testing also passed dissolution testing. However, the samples of C-stol® and Cynomax®, which had been shown already in assay testing to contain less than 50 % of the declared API amount, obviously failed dissolution testing with extreme deviations from USP specifications. From the C-stol® samples, approximately one quarter of the contained amount of the API did not dissolve, proving shortcomings in dissolution in addition to the extreme non-compliance in the assay. The results of the chemical analysis of each misoprostol sample, and the age of the samples at the time of analysis, are shown in S3 Table (Supporting Information).

The two extremely non-compliant brands had been collected both from government and from faith-based health facilities, and from one retail pharmacy.

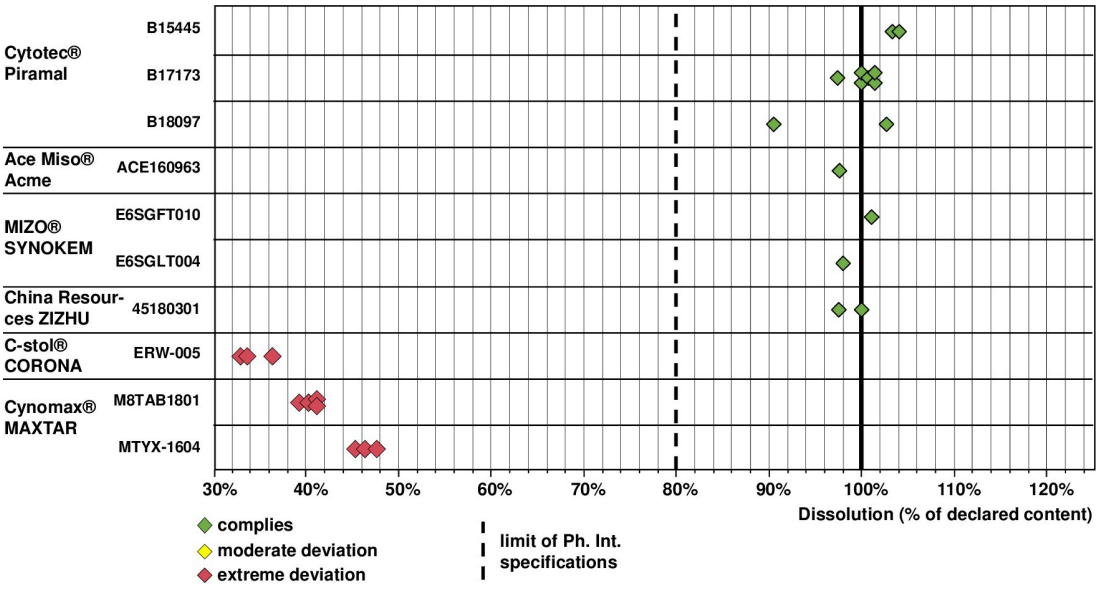

Fig 6. Dissolution of misoprostol determined in each of the investigated samples.

5.3.9. Product recall in Rwanda

The Rwanda Food and Drug Authority (RFDA) was alerted by the authors of this study about the two extremely substandard brands of misoprostol tablets by e-mail on December 2, 2018. RFDA invited the authors to a meeting at RFDA which took place on December 4, 2018. On December 7, 2018 RFDA issued an alert (Ref 079/Rwanda FDA/2018) requesting all suppliers and retailers to stop the distribution of these products and to put them in quarantine; all public and private hospitals, health centers, clinics and retail pharmacies were instructed to stop dispensing these products until investigations on the quality issues by Rwanda FDA were completed. Subsequently, RFDA issued a formal recall (Ref 0108/Rwanda FDA/2019 of 13 February 2019), stating that RFDA's investigations had revealed the indicted batches of misoprostol tablets to be substandard, and instructing all wholesalers, retailers, district pharmacies, public and private health facilities that the indicted batches should be returned to the supplier for suitable disposal.

5.4. Discussion

The present study showed excellent (100 %) availability of oxytocin injections in the investigated government and faith-based health facilities. Also misoprostol tablets were available as oxytocics in all investigated hospitals, as foreseen by the Rwanda National List of Essential Medicines [9]. This proves remarkable success of the health authorities of Rwanda in assuring the availability of these life-saving commodities in hospitals and health centers.

Oxytocin storage inside or outside the refrigerator was found to exactly follow the manufacturers' instructions. For samples labeled for storage at 2-8 °C, the correct storage temperature was maintained well in most of the sites, with only few and probably inconsequential deviations. Again, this proves success of the health authorities of Rwanda, and stands in positive contrast to reports on oxytocin storage conditions in many other LMICs [19,24,25,42-45]. A direct consequence of this success may be the fact that not a single sample of oxytocin was found which had an insufficient content of the API, again in contrast to reports from many other countries [22,24,25]. Of course, it cannot be excluded that knowledge of being monitored in this study may have influenced the behavior of health facility staff.

The number of different brands of oxytocin and misoprostol circulating in Rwanda at the time of sample collection was remarkably small. This may be related to the small market size of Rwanda: many manufacturers and international distributors may not see sufficient economic incentive to engage in medicine sales in this country. Possibly, this can limit the ability of public and private stakeholders to select good-quality, affordable medicines in their procurement.

Out of ten brands collected in total (4 of oxytocin injections, 6 of misoprostol tablets), three were prequalified by WHO [38,39], and three more were manufactured in countries with stringent regulatory authorities (SRAs) [37]. Together, these six brands represented 45 % of the samples collected in this study, and notably all these samples passed the quality tests in this study with good results, indicating that prequalification by WHO, as well as production in a country with an SRA, is a reliable predictor of good quality of medicines.

In stark contrast, three out of the four brands which were neither WHO-prequalified nor produced in a country with an SRA showed serious quality deficiencies. Most alarmingly, every investigated sample of the misoprostol products Cynomax® (stated manufacturer Maxtar

Bio-Genics, India) and C-stol® (stated manufacturer Corona Remedies, India) failed assay and dissolution testing with extreme deviations from the pharmacopeial specifications, likely to result in clinical inefficacy. Large amounts of misoprostol degradation products were detected in these two brands, indicating that the API had degraded to a large extent. Notably, the package inserts of both preparations did not mention whether or not HPMC (hypromellose) had been used in the formulation of these tablets, which is essential for misoprostol stability [29]. Also in Malawi, extremely substandard misoprostol brands, however from different stated manufacturers, have been found [24].

The quality deficiencies observed for the oxytocin injection stating Jiangxi Xierkangtai (China) as manufacturer were not as extreme as those observed for the above-mentioned misoprostol brands, but still alarming. All six samples of one of the two investigated batches of that oxytocin brand exceeded the content limit specified by USP, two of the samples even contained more that 120 % of the stated amount. In pharmaceuticals with unstable APIs, a content slightly higher than 100 % of the stated amount is often intentionally included to ensure that the content is still above the lower pharmacopeial limit towards the end of the shelf life. However, the pharmacopeias define an upper limit to avoid overdosing of the therapy, and this limit was clearly exceeded in the batch in question.

The oxytocin injections stating Jiangxi Xierkangtai as manufacturer were found to contain benzyl alcohol in a concentration of 0.9 %. This compound in this concentration is perfectly acceptable as a preservative in parenteral preparations [39,46]. However, in most countries regulations demand that the presence of such excipients must be declared in the package insert [47], but for the product in question no excipients were declared at all.

The most worrying observation regarding this product, however, was the detection of vials with a two-hundred-fold lower benzyl alcohol concentration, carrying the same batch number as the vials with 0.9 % of that preservative. While this may not cause direct harm to a patient treated with that product, it indicates gross violations of good manufacturing practice, raising strong doubts also about other quality aspects of this product. This kind of problem has not been reported in previous studies of oxytocin quality. However, this problem may easily escape detection in a medicine quality study as it has been visible only in a small number of the investigated vials.

The oxytocin injections by Jiangxi Xierkangtai were labeled for non-refrigerated storage, as also is the case for oxytocin from many other manufacturers [19,24,42-44]. Obviously, the use of oxytocin products which do not have to be stored in a refrigerator appears to be an attractive option, especially in LMICs. However, recent studies have shown that oxytocin products labeled for non-refrigerated storage may not have any better stability than products labeled for refrigerated storage, and on the contrary may even be less stable [40,44]. Therefore, international stakeholders including WHO have issued the recommendation "Buy quality oxytocin, keep it cool" and recommended that all oxytocin products should be stored at 2-8 °C [16,48], irrespective of the manufacturer's storage recommendation. Health authorities in Rwanda and elsewhere may consider this recommendation. A heat-stable formulation of the oxytocin analogue carbetocin has recently been added to the WHO Essential Medicines List, and in future it may become a further treatment option for post-partum hemorrhage for use in facilities where storage at 2-8 °C is problematic.

Several previous studies have reported problems of oxytocin quality in LMICs, but the present study shows for Rwanda a decidedly different situation than reported from other countries. The present data suggest that the Rwandan authorities have successfully assured the availability and (in most cases) the appropriate storage of oxytocin according to manufacturers' instructions. The detected problems of oxytocin and especially misoprostol quality must be addressed not so much by improved storage and transportation conditions of the medicines, but by improvements of the supplier qualification in medicine procurement. It may be considered whether for oxytocin and misoprostol, with their well-known problems of quality and stability, procurement should be restricted to WHO-prequalified medicines and to medicines manufactured in countries with stringent regulatory authorities. And as a simple "rule of thumb", the results of this study suggest that medicines for which the excipients are not stated in the package insert should be regarded as doubtful in quality.

So far, misoprostol quality has received much less attention in scientific studies than oxytocin quality [14]. However, the results of this study, of the study of Hall [28] and of a recent study from Malawi [24], show quality problems of different brands of misoprostol which are even much more serious than those reported for oxytocin, with API contents below (or far below) 50 % of the stated amount, and this problem needs attention in future studies.

Rwanda has only recently established its national drug regulatory agency, i.e. the Rwanda Food and Drug Authority (RFDA), and this will certainly contribute to increased patient safety. The extremely quick and efficient action which RFDA took on the reported substandard misoprostol brands holds good promise for the future development of medicine quality in this country.

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Supporting information captions

S1 Table. List of included health facilities, number oxytocin vial and misoprostol tablets collected, and recorded oxytocin storage conditions.

Facility No	Type of facility	District	Sampling site (maternity ward or storage room)	Number of collected misoprostol tablets	Misoprostol storage conditions stated on label	Number of collected oxytocin vials	Oxytocin storage conditions stated on label	Oxytocin storage site	Oxytocin storage shelf: mean kinetic temperature measured over 6 months (°C)	Refrigerator: mean kinetic temperature measured over 6 months (°C)
1	Government district hospital	Kigali city	maternity ward	50	20-25°C	10	2-8°C	refrigerator	-	**9.1**
			storage room	50; 50[e]	<30°C	10	room temperature [f]	shelf	25.3	-
2			storage room	50	<30°C	10	room temperature	shelf	24.2	-
3	Government referral hospital	Musanze	maternity ward	0	-	10	2-8°C	refrigerator	-	**8.5**
			storage room	60	<30°C	10	2-8°C	refrigerator	-	6.5
4	Faith-based district hospital	Bugesera	maternity ward	0	-	6	room temperature	shelf	26.3	-
			storage room	20	20-25°C	10	room temperature	shelf	23.4	-
5		Kamonyi	maternity ward	0	-	10	2-8°C	refrigerator	-	5.4
			storage room	51	<30°C	10	2-8°C	refrigerator	-	6.1
6		Karongi	maternity ward	0	-	10	2-8°C	cool box [a]	-	**15.8[a]**
			storage room	50	15°-25°C	10	2-8°C	refrigerator	-	not det.
7			maternity ward	10	15°-25°C	10	2-8°C	refrigerator	-	6.7
			storage room	50	<30°C	10	2-8°C	refrigerator	-	7.1
8		Muhanga	maternity ward	51	<30°C	10	room temperature	shelf	22.2	-
			storage room	50	20-25°C	10	room temperature	shelf	21.5	-
9	Government health center	Bugesera	maternity ward	0	-	5	room temperature	shelf	23.7	-
			storage room	13	20-25°C	10	room temperature	shelf	24.8	-
10			maternity ward	0	-	10	room temperature	shelf	26.0	-
			storage room	51	<30°C	10	room temperature	shelf	25.4	-
11		Kamonyi	maternity ward	0	-	8	room temperature	shelf	23.5	-
			storage room	0	-	10	room temperature	shelf	24.4	-
12			maternity ward	0	-	10	room temperature	shelf	not det.	-
			storage room	0	-	10	room temperature	shelf	25.0	-
13		Karongi	storage room	0	-	10	2-8°C	refrigerator	-	5.8
14			storage room	0	-	10	2-8°C	refrigerator	-	4.5
15		Kigali city	storage room	0	-	10	room temperature	shelf	23.7	-
16			maternity ward	0	-	10	room temperature	shelf	24.2	-
17		Muhanga	maternity ward	0	-	9	room temperature	shelf	22.3	-
			storage room	0	-	10	room temperature	shelf	21.4	-
18			storage room	0	-	10	room temperature	shelf	21.4	-
19		Musanze	storage room	0	-	10	2-8°C	refrigerator	-	7.3
20			storage room	0	-	10	2-8°C	refrigerator	-	5.7
21	Faith-based health center	Bugesera	maternity ward	0	-	10	room temperature	shelf	23.8	-
			storage room	0	-	10	room temperature	shelf	not det.	-
22			maternity ward	0	-	10	room temperature	shelf	25.1	-
			storage room	30	20-25°C	10	room temperature	shelf	**25.4 [g]**	-
23		Kamonyi	storage room	0	-	10	room temperature	shelf	22.7	-
24			maternity ward	0	-	10	room temperature	shelf	22.2	-
			storage room	0	-	10	room temperature	shelf	22.1	-
25		Karongi	storage room	0	-	10	2-8°C	refrigerator	-	4.3
26			maternity ward	0	-	10	2-8°C	refrigerator	-	**-1.3**
27		Kigali city	storage room	0	-	6	2-8°C	refrigerator	-	6.5
28			storage room	0	-	10	2-8°C	refrigerator	-	**10.5**
29		Muhanga	maternity ward	0	-	9	room temperature	shelf	21.0	-
			storage room	0	-	10	room temperature	shelf	21.9	-
30			maternity ward	0	-	10	room temperature	shelf	22.7	-
			storage room	0	-	10	room temperature	shelf	22.4	-
31		Musanze	maternity ward	0	-	10	2-8°C	refrigerator	-	not det.
			storage room	0	-	10	2-8°C	refrigerator	-	5.1
32			storage room	0	-	10	2-8°C	refrigerator	-	4.7
33	Private clinic	Muhanga	storage room	0	-	10	room temperature	shelf	not det.	-
34	Retail pharmacy	Bugesera	storage room	25	no requirements	0	-	n.a.	24.1 [c]	-
35			storage room	29	no requirements	0	-	n.a.	24.0 [c]	-
36		Kigali city	storage room	50	no requirements	0	-	n.a.	24.2 [c]	-
37			storage room	28	no requirements	0	-	n.a.	23.9 [c]	-
38		Muhanga	storage room	50	no requirements	0	-	n.a.	-	-
39			storage room	48	20-25°C	0	-	n.a.	-	-
40		Musanze	storage room	50	no requirements	0	-	n.a.	-	-
41	Government central medical store	Kigali city	storage room	0	-	100;200[d,e]	2-8°C	refrigerator	-	not det.
42	Government district pharmacy	Burera	storage room	250[d]	<30°C	0	-	n.a.	-	-
43		Muhanga	storage room	200[d]	20-25°C	160[d]	room temperature	shelf	19.8	-
44	Private wholesaler	Kigali city	storage room	0	-	100[d]	room temperature	not det. [b]	-	-
45			storage room	0	-	100[d]	2-8°C	not det. [b]	-	-
46			storage room	252[d]	no requirements	100[d]	2-8°C	not det. [b]	-	-

Storage conditions which deviate from manufacturers' requirements are given in **bold, underlined** letters.
n.a. = not applicable
not det. = not determined (temperature logger not placed, lost after placement, or no data recorded).

[a] Oxytocin was stored in this maternity ward in a cool box, reportedly only ordered for immediate use.

[b] From private wholesalers, samples were purchased through a retail pharmacy using a mystery shopper approach. Therefore, storage conditions were not determined, and temperatures data loggers were not placed.

[c] In a few pharmacies, temperature loggers were placed although they did not stock oxytocin.

[d] Higher number of tablets/vials purchased as replacement samples and for additional stability testing.

[e] Two brands collected in one sampling site.

[f] Storage requirement stated on packaging: "Store in a cool dry place, away from light". Storage requirement stated on the package insert: "Store in a dark place at room temperature, protect from light."

[g] Exceeds storage temperature of misoprostol recommended by the manufacturer of the respective sample.

S2 Table. Results of chemical analysis of all oxytocin samples

Brand name and stated manu-facturer	Stated storage tempera-ture require-ment	Batch N°	Manu-facture/ Expiry Date	Faci-lity No.	Facility type	Site in facility	Mean assay (% of declared content)	RSD assay	Mean pH value	RSD pH value	Age of samples (months) [a]
Oxytocin injection; Jiangxi Xier-kangtai Pharma-ceutical Co. Ltd; China	Room tempe-rature	1606573	Jun 16/ Jun 19	2	gov. hospital	stor.	96.1% [b]	6.62% [c]	4.14	0.98%	30
				4	faith-b. hospital	mat.	102.7%	1.56%	4.03	0.48%	30
						stor.	105.5%	2.22%	4.00	0.68%	30
				8	faith-b. hospital	mat.	99.2%	0.68%	4.48	2.17	24
						stor.	99.9%	1.31%	4.40	0.96	24
				9	gov. HC	mat.	101.3%	0.55%	4.05	0.89%	30
						stor.	101.1%	1.66%	4.05	0.76%	30
				11	gov. HC	mat.	100.5%	1.73%	4.05	0.34%	30
						stor.	100.7%	1.73%	4.05	0.33%	30
				12	gov. HC	mat.	100.4%	2.29%	4.08	0.62%	30
						stor.	99.8%	1.29%	4.07	0.73%	30
				17	gov. HC	mat.	95.8%	1.71%	4.56	3.27	24
						stor.	97.9%	0.52%	4.50	1.33	24
				18	gov. HC	stor.	99.0%	0.88%	4.44	0.97	24
				23	faith-b. HC	stor.	99.1%	1.05%	4.12	0.18%	30
				24	faith-b. HC	mat.	100.4%	1.99%	4.06	0.48%	30
						stor.	95.6%	4.94%	4.19	4.38%	30
				29	faith-b. HC	mat.	98.2%	0.75%	4.46	0.73	24
						stor.	98.0%	0.79%	4.48	0.84	24
				30	faith-b. HC	mat.	99.5%	1.32%	4.38	1.18	24
						stor.	99.7%	1.18%	4.39	0.77	24
				33	private clinic	stor.	100.7%	0.96%	4.41	0.94	24
				43	distr. pharm.	stor.	101.5%	0.45%	4.06	0.32%	23
				44	**wholesaler**	**stor.**	**90.3% [b]**	**0.72%**	**4.08**	**0.84%**	**23**
		1604521	Apr 16/ Apr 19	1	gov. hospital	stor.	119.3%	2.09%	3.85	0.13%	32
				10	gov. HC	mat.	118.0%	1.98%	3.83	0.48%	32
						stor.	117.2%	0.52%	3.82	0.51%	32
				15	gov. HC	stor.	119.9%	3.25%	3.84	0.30%	32
				16	gov. HC	mat.	120.6%	2.24%	3.82	0.11%	32
				21	faith-b. HC	stor.	117.6%	1.02%	3.84	0.44%	32
						stor.	121.5%	1.61%	3.82	0.48%	32
				22	faith-b. HC	mat.	117.3%	1.38%	3.80	0.26%	32
						stor.	117.8%	2.31%	3.78	0.47%	32
Steroxine 10 IU/1 ml [d]; Labora-toires Sterop; Belgium	2-8°C	160042	Feb 16/ Jan 19	46	wholesaler	stor.	107.8%	0.62%	3.79	0.22%	27
		160269	Sep 16/ Aug 19	45	wholesaler	stor.	99.6%	1.11%	3.98	0.57%	20
Oxytocin 10 IU/ml; AS GRINDEKS, Latvia [e]	2-8°C	37611016	Oct 16/ Oct 20	1	gov. hospital	mat.	102.8%	1.63%	4.11	0.13%	26
				5	faith-b. hospital	mat.	102.1%	1.99%	4.09	0.15%	26
						stor.	101.9%	3.29%	4.10	0.22%	26
				20	gov. HC	stor.	99.9%	0.74%	4.11	0.10%	26
		37711116	Nov 16/ Nov 20	41	CMS	stor.	100.9%	0.82%	4.09	0.64%	18
Oxytocin 10; Rotex-medica GmbH Arznei-mittelwerk; Germany	2-8°C	70779A	Sep 17/ Sep 20	3	gov. hospital	mat.	104.1%	2.96%	4.06	0.39%	15
						stor.	102.0%	0.26%	4.02	0.20%	15
				6	faith-b. hospital	mat.	103.7%	2.09%	4.09	0.40%	15
						stor.	103.1%	1.53%	4.06	0.35%	15
				7	faith-b. hospital	mat.	103.9%	1.92%	4.02	0.10%	15
						stor.	103.8%	1.84%	4.03	0.10%	15
				13	gov. HC	stor.	103.8%	1.67%	4.02	0.19%	15
				14	gov. HC	stor.	104.2%	1.93%	4.05	0.30%	15
				19	gov. HC	stor.	102.6%	0.44%	4.00	0.10%	15
				25	faith-b. HC	stor.	103.6%	1.76%	4.02	0.24%	15
				26	faith-b. HC	mat.	103.9%	1.67%	4.01	0.19%	15
				27	faith-b. HC	stor.	105.9%	3.96%	3.97	0.19%	15
				28	faith-b. HC	stor.	105.2%	2.37%	3.99	0.21%	15
				31	faith-b. HC	mat.	103.4%	0.86%	4.00	0.14%	15
						stor.	105.4%	2.44%	3.99	0.19%	15
				32	faith-b. HC	stor.	104.6%	2.51%	4.01	0.20%	15
				41	CMS	stor.	102.4%	0.89%	3.99	0.47%	8

RSD = relative standard deviation; gov. = government; faith-b. = faith-based; HC = health center; CMS = government central medical store; stor. = storage room; mat. = maternity ward. The sample found to contain only 0.004 % benzyl alcohol is shown in **bold print**.

[a] Age of sample at time of analysis.
[b] Deviating concentration of the preservative benzyl alcohol observed, see main text.
[c] See text for explanation of the high standard deviation observed for this specific sample.
[d] Batch 160269 was labeled with the unbranded generic name "Oxytocin 10 IU/1 ml", all other information was identical as in batch 160042.
[e] Marketing authorization holder: Peckforton Pharmaceuticals Ltd., United Kingdom

S3 Table. Results of chemical analysis of all misoprostol samples

Brand name and stated manufacturer	Stated storage requirements	Batch N°	Manu-facture/ Expiry Date	Faci-lity no.	Facility type	Site in faci-lity	Mean assay (% of declared content)	RSD assay	Mean dissolution (% of declared content)	RSD disso-lution	Age of samples (months) at time of analysis
Cytotec® 200 µg, Piramal Healthcare UK Limited; United Kingdom [a]	No special storage requirements [b]	B15445 [a]	Dec 16[c] / Nov 19	38	retail pharm.	stor.	104.2%	0.13%	104.2%	0.61%	18
				40	retail pharm.	stor.	94.7%	0.27%	103.5%	2.79%	24
		B17173 [a]	Jul 17[c] / Jun 20	1	gov. hospital	stor.	95.9%	0.19%	100.1%	1.45%	17
				34	retail pharm.	stor.	94.8%	0.29%	101.2%	2.19%	17
				35	retail pharm.	stor.	94.6%	0.35%	100.1%	2.81%	17
				36	retail pharm.	stor.	94.8%	0.58%	100.8%	1.59%	17
				37	retail pharm.	stor.	95.6%	0.48%	101.5%	3.05%	17
				46	wholesaler	stor.	102.4%	1.13%	97.5%	2.82%	10
	Store at room temperature (15-25 °C)	B18097 [b]	Nov 17[c] / Oct 20	6	faith-b. hospital	stor.	95.1%	0.56%	90.6%	4.49%	13
				7	faith-b. hospital	mat.	95.5%	3.80%	102.8%	2.69%	13
Ace Miso®, Acme Formulation Pvt. Ltd.; India	Do not store above 30°C, protect from light	ACE160963	Sep 16/ Aug 18	42	gov. district pharm.	stor.	102.0%	0.44%	97.7%	2.59%	20
MIZO®, SYNOKEM Pharmaceuticals LTD; India	Store at a temperature not exceeding 30°C at a dry place	E6SGFT010	Jun 16/ May 18	8	faith-b. hospital	mat.	98.4%	0.02%	101.2%	3.51%	24
		E6SGLT004	Dec 16/ Nov 18	10	gov. HC	stor.	92.1%	1.62%	98.1%	3.18%	24
China resources, ZIZHU Pharmaceuticals Co Ltd; China	Store at a tempera-ture not exceeding 30 °C	45180301	Feb 18/ Feb 20	1	gov. hospital	stor.	95.8%	0.20%	97.7%	2.31%	10
				3	gov. hospital	stor.	94.8%	0.16%	100.1%	2.64%	10
C-stol®, CORONA Remedies Pvt Ltd; India	Store below 30°C. Protect from light and moisture	ERW-005	Mar 18/ Feb 21	2	gov. hospital	stor.	46.8%	1.33%	32.9%	15.61%	9
				5	faith-b. hospital	stor.	46.2%	0.14%	33.6%	6.82%	9
				7	faith-b. hospital	stor.	48.5%	2.96%	36.4%	4.53%	9
Cynomax®, MAXTAR BIO-GENICS; India	Store at 20° to 25°C in a dry area	M8TAB1801	May 18/ Apr 20	1	gov. hospital	mat.	42.5%	0.51%	39.8%	1.35%	7
				4	faith-b. hospital	stor.	44.6%	0.95%	39.3%	4.15%	7
				9	gov. HC	stor.	45.6%	1.07%	41.1%	3.53%	7
				22	faith-b. HC	stor.	44.9%	0.27%	41.2%	2.37%	7
		MTYX-1604	Aug 16/ Jul 18	8	faith-b. hospital	stor.	48.6%	0.06%	46.4%	3.36%	22
				39	retail pharm.	stor.	47.1%	0.04%	45.3%	1.57%	22
				43	gov. district pharm.	stor.	47.6%	0.24%	47.6%	4.49%	21

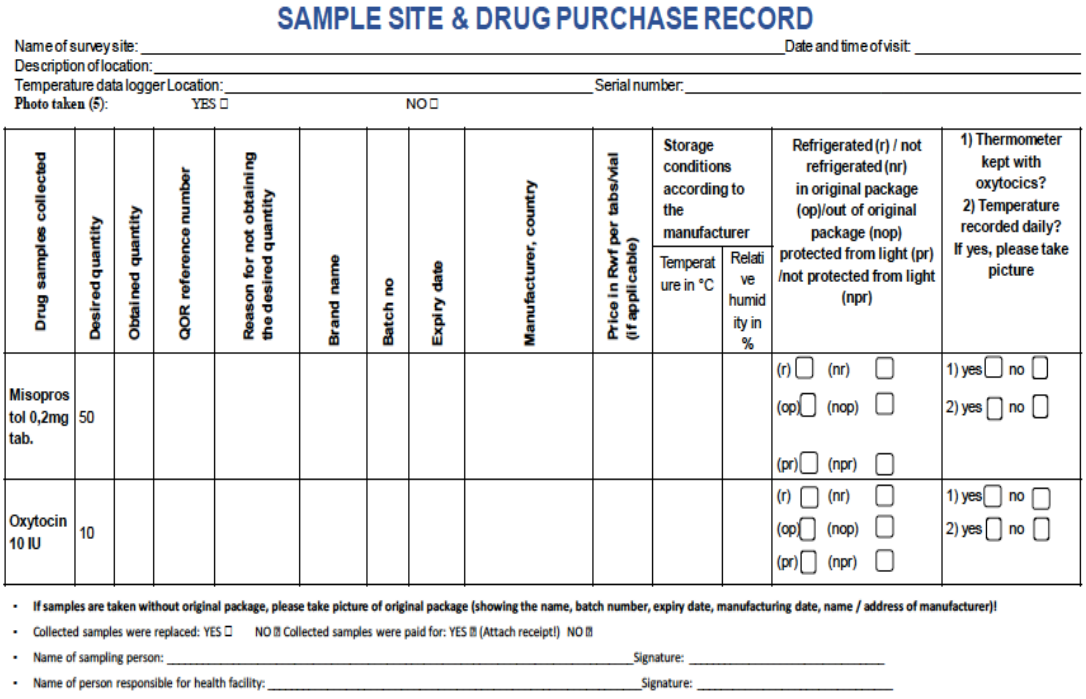
RSD = relative standard deviation
gov. = government
faith-b. = faith-based
HC = health center
pharm. = pharmacy
stor. = storage room
mat. = maternity ward.

[a] Marketing authorization holder: Pfizer Holding, France.

[b] Marketing authorization holder: Continental Pharma Inc., Belgium.

[c] Package insert: "Tenir hors de la vue et de la portée des enfants. Pas de précaution particulière de conservation". I.e.: "Keep out of sight and reach of children. No special storage requirements."

[d] Manufacturing date not stated on packaging. Shelf-life listed according to information from the websites www.hpra.ie and www.medicines.org.uk/emc.



SAMPLE SITE & DRUG PURCHASE RECORD

Name of survey site: _____ Date and time of visit: _____

Description of location: _____

Temperature data logger Location: _____ Serial number: _____

Photo taken (5): YES ☐ NO ☐

Drug samples collected	Desired quantity	Obtained quantity	QOR reference number	Reason for not obtaining the desired quantity	Brand name	Batch no	Expiry date	Manufacturer, country	Price in Rwf per tabs/vial (if applicable)	Storage conditions according to the manufacturer		Refrigerated (r) / not refrigerated (nr) in original package (op)/out of original package (nop) protected from light (pr) /not protected from light (npr)	1) Thermometer kept with oxytocics? 2) Temperature recorded daily? If yes, please take picture
										Temperature in °C	Relative humidity in %		
Misoprostol 0,2mg tab.	50											(r) ☐ (nr) ☐ (op) ☐ (nop) ☐ (pr) ☐ (npr) ☐	1) yes ☐ no ☐ 2) yes ☐ no ☐
Oxytocin 10 IU	10											(r) ☐ (nr) ☐ (op) ☐ (nop) ☐ (pr) ☐ (npr) ☐	1) yes ☐ no ☐ 2) yes ☐ no ☐

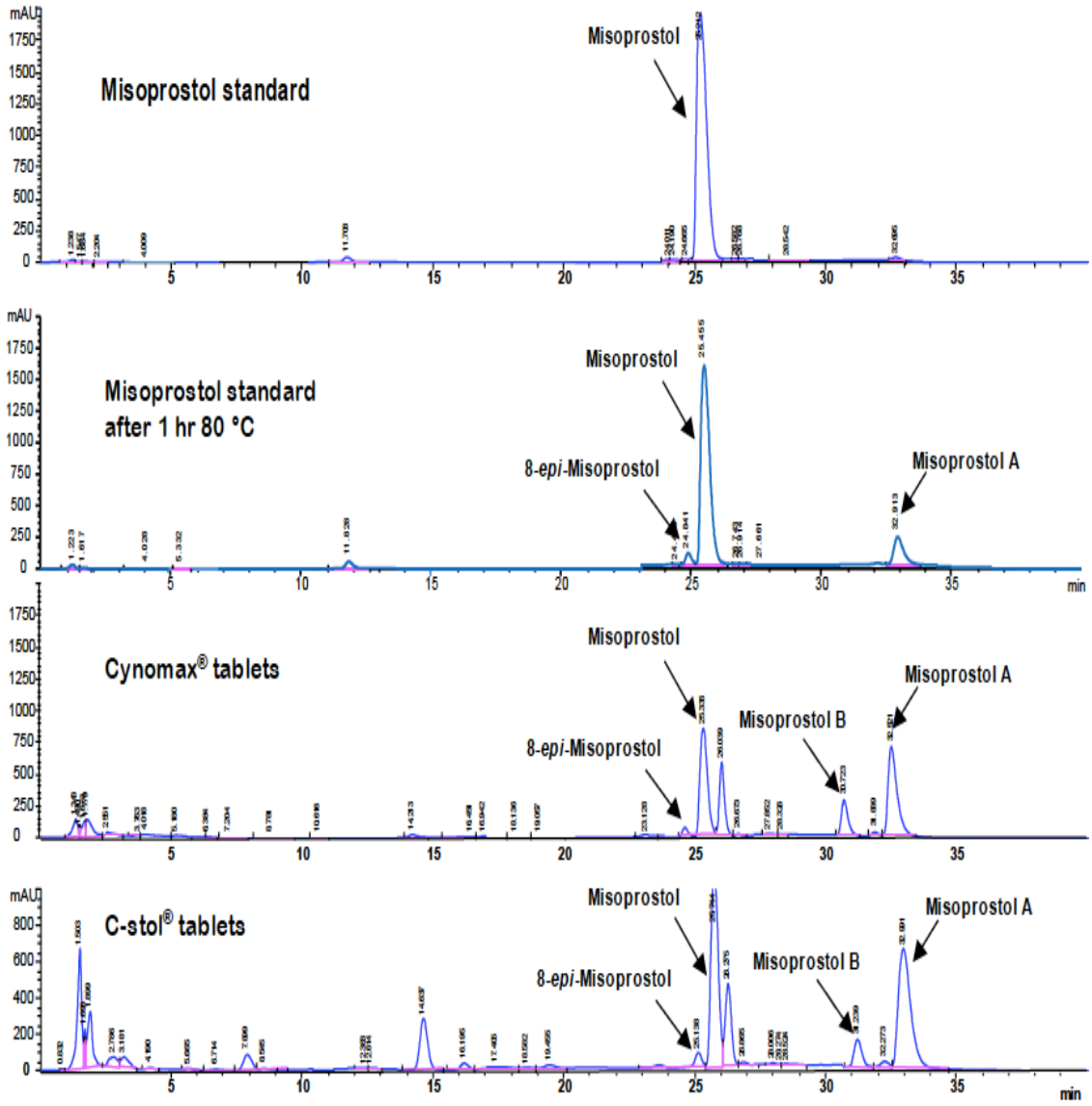
- **If samples are taken without original package, please take picture of original package (showing the name, batch number, expiry date, manufacturing date, name / address of manufacturer)!**
- Collected samples were replaced: YES ☐ NO ☒ Collected samples were paid for: YES ☒ (Attach receipt!) NO ☒
- Name of sampling person: _____ Signature: _____
- Name of person responsible for health facility: _____ Signature: _____

S1 Fig. Sample site AND drug purchase record

S2 Fig. Photos of two samples of oxytocin injections, carrying the same batch number but containing different concentrations of benzyl alcohol.

Top: Sample No. QOR04, containing 0.004% Benzyl alcohol.

Bottom: Sample No. QOR05, containing 0.9% Benzyl alcohol.



S3 Fig. HPLC analysis for related substances in two misoprostol samples.

Cynomax® tablets (batch M8TAB1801) and C-Stol® tablets (batch ERW-005) were investigated. See Methods section for experimental details, and Results section for details of the investigated brands and batches.

Chapter six: General discussion, conclusions and recommendations

6.1. Discussion of findings

Results presented in the four published articles complete each other, and provide the context of the efforts engaged by the Rwandan government to reduce the number of maternal deaths in Rwanda.

Accessibility to oxytocin as the first choice in the management of PPH, was less than but close to 80% in the public and the faith-based sectors, the minimum percent of availability set by the WHO. In addition, even though the patient prices for oxytocin in terms of MPR were more than twice the procurement price, oxytocin was found affordable both in the faith-based and the public sectors. After a six-months accelerated stability study at 25°C and 30 °C, oxytocin brands collected from wholesales of Rwanda were found stable. The survey of the quality of oxytocin samples collected from selected health facilities of Rwanda revealed minor deviations to the stated content in oxytocin.

These results explain the remarkable reduction of maternal deaths in Rwanda, from 2000 to 2017. Rwanda is one of the few countries that have achieved the UN's target set, reducing the Maternal Mortality Ratio (MMR) from 1160 maternal deaths per 100,000 live births in 2000 down to 248 maternal deaths per 100,000 live births in 2017 (1).

Misoprostol, the second choice in the management of PPH, was found accessible in the public and faith-based sectors, but not affordable in the private sector. Misoprostol was available in both the faith-based and the public sectors and poorly available in the private sector. Misoprostol brands found in public and faith-based health facilities were patients' free medicines, but the patients' prices for the same brands of misoprostol in the private sector were more than three times the procurement price. The six months accelerated stability study of misoprostol brands collected from wholesales of Rwanda showed good stability, with a content greater than 90% of the stated content in misoprostol. But, the investigation on the quality of misoprostol brands being used in health facilities of Rwanda, revealed extreme deviations to the pharmacopeial requirements. All samples from two brands, Cynomax and C-Stol, constituting 40% (10/25) of total samples of misoprostol, showed a content of less than 50% of the stated content in misoprostol.

The brands of oxytocin and misoprostol which are WHO-prequalified or manufactured in countries with SRA did not show any quality problems, either during the stability studies or the investigation of the quality of oxytocin and misoprostol brands collected from the Rwandan health facilities. But all brands found with problems of quality, were neither WHO-prequalified nor manufactured in countries with SRA. This shows the importance of the recommendations formulated by the UNCoLSC in 2010 (10). All procurement agencies should prioritize quality-certified and affordable brands.

6.2. Conclusion

The main objective of this PhD thesis was to analyze the treatments of PPH through the measurement of the accessibility of the Rwandan population to oxytocin and misoprostol, and investigation on the quality and stability of oxytocin and misoprostol used in health facilities of Rwanda. The results presented in this thesis on the accessibility to oxytocin and misoprostol as the first and second choices in the treatment of PPH; on the stability of oxytocin and misoprostol; on the quality of oxytocin and misoprostol brands collected from the Rwandan health facilities; all together, show a good work of the Rwandan procurement agencies in ensuring affordability of essential medicines including oxytocin and misoprostol in the government and faith-based sectors.

However, availability of essential medicines including oxytocin and misoprostol was found less than the minimum target set by WHO, and essential medicines were found less affordable in the private sector than in the government and faith-based sectors; this may make essential medicines inaccessible. Also, laboratory analyses were performed in Germany due to the problem of electricity, which is often cut off and unstable to make possible smooth run of the HPLC equipment. Brands of oxytocin and misoprostol collected from the Rwandan health facilities were found stable, but one brand of oxytocin and two brands of misoprostol were found containing inappropriate amounts of the active pharmaceutical ingredients. This quality issue put the Rwandan population at high risks, since oxytocin and misoprostol are classified as life-saving medicines and used as the first and the second choices respectively, in the prevention and management of PPH, which is the leading cause of maternal deaths.

More efforts are still required in ensuring a friendly working environment in the Rwandan laboratories. Prices regulation in the private sector and improvement of the supply chain performance would contribute to improving accessibility of the Rwandan population to quality-

certified oxytocin and misoprostol, in order to move forwards to achieving the goal of less than 70 maternal deaths per 100,000 live births by 2030 in Rwanda.

6.3. Recommendations

To ensure the right and straight move towards achieving the SDG 3.1 of the UN by 2030, the following recommendations need to be considered:

- ⇒ From now Rwandan medicines procurement agencies have to select brands of oxytocin and misoprostol which are either WHO-prequalified or manufactured in countries SRA.
- ⇒ All procurement agencies and health facilities have to continuously ensure that all oxytocin preparations are always refrigerated at a temperature ranging from 2°C to 8°C, and all misoprostol preparations have to be always protected from moisture.
- ⇒ The government of Rwanda should promote and support local analytical laboratories, to strengthen the quality control and quality assurance of medicines in the country.
- ⇒ The Rwandan government also has to maintain efforts in ensuring accessibility of the population to affordable oxytocin and misoprostol, by regulating prices for medicines, not only in the public and faith-based sectors, but also in the private sector.
- ⇒ More research to update data about the accessibility to essential medicines and the quality of medicines, not only oxytocin and misoprostol, is needed for decision-making. The University of Rwanda should improve the working environment to enable laboratory analyses using the premises and equipment available at the university.

Appendices

Appendix 1: Authorization of research by the Ministry of Health

REPUBLIC OF RWANDA

Kigali, 05 MARS 2018
N°20/1361/DGPHFIS/2018

MINISTRY OF HEALTH
P.O .BOX:84 KIGALI
www.moh.gov.rw

BIZIMANA Thomas
Principal Investigator

Re:Authorization of research

Reference is made to the copy of your letter dated 25January 2018 submitted on 19 February 2018 and requesting authorization to conduct a research entitled:"**A survey on quality misoprostol and oxytocin as well as prices, affordability and availability of oxytocics and essential medines in Rwanda**";

Based on Rwandan Health Sector Research Policy and approval from the Institutional Review Board of University of Rwanda, College of Medicine and College sciences, with Ref No: 026/CMHS IRB/2018 dated February 12, 2018;

I am pleased to inform you that the Ministry of Health has granted authorization to conduct this research and to collect data according to the approved protocol.

You are requested to share the results with the Ministry of Health and to provide the final report and datasets to the Ministry.

Sincerely,

MINISTERE DE LA SANTE

Dr. Diane GASHUMBA
Minister of Health

Appendix 2: Ethical clearance

UNIVERSITY OF RWANDA

COLLEGE OF MEDICINE AND HEALTH SCIENCES

CMHS INSTITUTIONAL REVIEW BOARD (IRB)

Kigali, 12th /02/2018

BIZIMANA Thomas
School of Medicine and Pharmacy, CMHS, UR

Approval Notice: No 026 /CMHS IRB/2018

Your Project Title ***"A Survey On Quality Of Misoprostol And Oxytocin As Well As Prices, Affordability And Availability Of Oxytocics & Essential Medicines In Rwanda"*** has been evaluated by CMHS Institutional Review Board.

		Involved in the decision		
		Yes	No (Reason)	
Name of Members	**Institute**		**Absent**	**Withdrawn from the proceeding**
Prof Kato J. Njunwa	UR-CMHS		X	
Prof Jean Bosco Gahutu	UR-CMHS	X		
Dr Brenda Asiimwe-Kateera	UR-CMHS	X		
Prof Ntaganira Joseph	UR-CMHS	X		
Dr Tumusiime K. David	UR-CMHS	X		
Dr Kayonga N. Egide	UR-CMHS	X		
Mr Kanyoni Maurice	UR-CMHS	X		
Prof Munyanshongore Cyprien	UR-CMHS		X	
Mrs Ruzindana Landrine	Kicukiro district		X	
Dr Gishoma Darius	UR-CMHS			X
Dr Donatilla Mukamana	UR-CMHS			X
Prof Kyamanywa Patrick	UR-CMHS		X	
Prof Condo Umutesi Jeannine	UR-CMHS		X	
Dr Nyirazinyoye Laetitia	UR-CMHS	X		
Dr Nkeramihigo Emmanuel	UR-CMHS		X	
Sr Maliboli Marie Josee	CHUK	X		
Dr Mudenge Charles	Centre Psycho-Social	X		

After reviewing your protocol during the IRB meeting of where quorum was met and revisions made on the advice of the CMHS IRB submitted on 12th February 2018, **Approval has been granted to your study.**

Please note that approval of the protocol and consent form is valid for **12 months**.

You are responsible for fulfilling the following requirements:

1. Changes, amendments, and addenda to the protocol or consent form must be submitted to the committee for review and approval, prior to activation of the changes.
2. Only approved consent forms are to be used in the enrolment of participants.
3. All consent forms signed by subjects should be retained on file. The IRB may conduct audits of all study records, and consent documentation may be part of such audits.
4. A continuing review application must be submitted to the IRB in a timely fashion and before expiry of this approval
5. Failure to submit a continuing review application will result in termination of the study
6. Notify the IRB committee once the study is finished

Sincerely,

Date of Approval: The 12th February 2018

Expiration date: The 12th February 2019

Professor Kato J. NJUNWA
Chairperson Institutional Review Board,
College of Medicine and Health Sciences, UR

Prof. J. Njunwa
Vice Chair

Cc:
- Principal College of Medicine and Health Sciences, UR
- University Director of Research and Postgraduate Studies, UR

About the author

Thomas Bizimana was born on May 21, 1972 in Rwanda. He is married and has five children. Thomas Bizimana studied Pharmacy from 2001 to 2006 and graduated in 2007 awarded a bachelor's degree of pharmacy from the "Université Nationale du Rwanda (UNR)". He started teaching at the "Université Nationale du Rwanda" in 2008 as a tutorial assistant, in the department of Pharmacy. In 2009, he started his master's studies in pharmaceutical sciences, Quality assurance/ Quality control at the National University of Rwanda (NUR). In 2011, he went in Dar-Es-Salam, Tanzania, for a six-months research at Muhimbili University of Health and Allied Sciences (MUHAS), working on a project entitled "HPTLC method development and validation for simultaneous determination of Tenofovir and Lamivudine in pharmaceutical formulations" as his master's thesis. He started his PhD studies at the University of Rwanda (UR) in 2017. In 2018, he went in Germany, at Tübingen University, and passed 12 months working on his PhD research project, in the department of Pharmaceutical Biology. Since 2008, he taught the courses of Supply chain management for health commodities, Drug analysis, Health Technologies Assessment and Rational medicines use at the NUR and UR. The following are his authored or co-authored journal articles:

1. Bizimana T, Kayumba PC, Védaste K, Egide K, Kaale E. Development of high performance thin layer chromatography for simultaneous analysis of lamivudine and tenofovir disoproxil fumarate. Rwanda J. 2016;3(1):6.
2. Hagen N, Bizimana T, Kayumba PC, Khuluza F, Heide L. Stability of oxytocin preparations in Malawi and Rwanda: Stabilizing effect of chlorobutanol. Am J Trop Med Hyg. 2020;103(5):2129–41
3. Bizimana T, Kayumba PC, Heide L (2020) Prices, availability and affordability of medicines in Rwanda. PLoS ONE 15(8): e0236411. https://doi.org/10.1371/journal.pone.0236411
4. Hagen N, Bizimana T, Kayumba PC, Khuluza F, Heide L (2020) Stability of misoprostol tablets collected in Malawi and Rwanda: Importance of intact primary packaging. PLoS ONE 15(9): e0238628. https://doi.org/10.1371/journal.pone.0238628
5. Bizimana T, Hagen N, Gnegel G, Kayumba PC, Heide L (2021) Quality of oxytocin and misoprostol in health facilities of Rwanda. PLoS ONE 16(1): e0245054. https://doi.org/10.1371/journal.pone.0245054
6. Bizimana, T.; Kagisha, V.; Nyandwi, J.B.; Nyirimigabo, A.K.; Muganga, R.; Mukanyangezi, M.F.; Kayitare, E. Investigation of the Quality of the 12 Most-Used Antibiotics Available in Retail Private Pharmacies in Rwanda. Antibiotics 2022, 11, 329. https://doi.org/10.3390/antibiotics11030329