Journal of Pharmaceutical Research International

20(3): 1-10, 2017; Article no.JPRI.38553
ISSN: 2456-9119
(Past name: British Journal of Pharmaceutical Research, Past ISSN: 2231-2919, NLM ID: 101631759)

Effect of Different Storage Conditions on the Stability and Efficacy of Some Reconstituted Oral Antibiotic Suspensions Sold in Port Harcourt, Nigeria

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Authors' contributions

This work was carried out in collaboration between both authors. Authors CNS and SEI designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author CNS managed the analyses of the study while author SEI managed the literature searches. Both authors CNS and SEI read and approved the final manuscript.

Article Information

DOI: 10.9734/JPRI/2017/38553
Editor(s):
(1) Wenbin Zeng, School of Pharmaceutical Sciences, Central South University, Hunan, China.
Reviewers:
(1) Sheikh Shahnawaz, India.
(2) R. L. C. Sasidhar, Chebrolu Hanumaiah Institute of Pharmaceutical Sciences, India.
(3) José Francisco Gómez Clavel, National Autonomous University of Mexico, México.
Complete Peer review History: http://www.sciencedomain.org/review-history/22545

Original Research Article

Received 30th November 2017
Accepted 27th December 2017
Published 1st January 2018

ABSTRACT

Antibiotics for pediatric use are commonly available as dry powders for reconstitution into oral suspensions. Once reconstituted, these oral suspensions should be refrigerated to preserve their potency and deliver optimal benefit to the patient. However, for reasons ranging from lack of refrigerator and irregular power supply to lack of information and ignorance, these storage instructions are not always adhered to resulting in varying degrees of degradation of the reconstituted product. In spite of these constraints, Pharmacists have to ensure that patients receive optimum benefit from whatever drug they dispense in the course of rendering pharmaceutical care. This study examined the stability of three frequently prescribed pediatric reconstituted Cefuroxime axetil, Amoxicillin-clavulanate potassium and Azithromycin oral

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suspensions under different storage conditions. The reconstituted suspensions were stored in three different conditions with temperature ranging from 2℃ to 29℃ over a period of 5 days. Samples were assayed periodically using a validated UV/Vis spectroscopic method. Percentage concentrations were within 90 ± 5% up to the fifth day at condition A (refrigerated at 2 - 8℃). Under condition C (submerging in water at room temperature) the suspensions were preserved for at least 3 days. However, under condition B (storing in a cupboard at room temperature), extensive degradation was observed. In both conditions B and C, concentrations had fallen below 80% by the fifth day due to extensive degradation that occurred. Reconstituted oral suspensions should be used within 5 days if refrigerated or within 3 days if submerged in water and stored at room temperature in the absence of better alternatives.

Keywords: Antibiotics; degradation; oral suspension; reconstitution; stability; storage.

1. INTRODUCTION

Antibiotics are types of medications that completely destroy (bactericidal) or inhibit (bacteriostatic) the growth of bacteria [1].

Bacteria are life forms [2]. The US National Library of Medicine defines antibiotics as powerful medicines that fight bacterial infections which are capable of saving lives when used properly. Antibiotics only work against infections due to bacteria and certain parasites. They are ineffective against infections caused by viruses (for example, the common cold or flu), and fungi such as oral or vaginal thrush and fungal infections of the skin [3].

Antibiotics are among the drugs most commonly prescribed by clinicians. Antibiotics for pediatric use are commonly available as dry powders for reconstitution into oral suspensions. Once reconstituted, these oral suspensions should be refrigerated to preserve their potency and deliver optimal benefit to the patient. However, for reasons ranging from lack of refrigerator and irregular power supply to lack of information and ignorance, these storage instructions are not always adhered to resulting in varying degrees of degradation of the reconstituted product.

Stability is defined as the capacity of a drug substance or drug product to remain within the established specifications, to maintain its identity, strength, quality, and purity throughout the retest or shelf life. Stability testing of an active substance or finished product provides information on how the quality of a drug substance or product varies with time. It is influenced by such factors as temperature, humidity and light. Knowledge from stability studies enables understanding of the long-term effect(s) of the environment on drugs. Stability testing also provides information about the degradation mechanisms, potential degradation products, possible degradation pathways as well as interaction between the drug and the excipients in the drug product [4,5].

Antibiotic suspensions were chosen for this work because reconstituted antibiotics usually require refrigeration, a condition that may be difficult to meet in many resource-limited environments. Studies in Iraq and Sudan had also shown that antibiotics were the most commonly encountered drugs stored and consumed by patients in their homes with the beta-lactam antibiotics of penicillin and cephalosporin derivatives constituting the highest percentage of 26.43% and 22% respectively [6,7].

In Nigeria due to the epileptic power situation, high level of poverty and illiteracy, directing patients on proper storage of reconstituted antibiotics at home for optimal benefit poses a major challenge for pharmacists. The consequences of delivering suboptimal doses of the antibiotic or toxic bye products of degradation of the drug due to poor storage are only too obvious. This study, therefore, determined to ascertain the effect(s) of in-home storage conditions on the stability of three reconstituted antibiotic suspensions, Cefuroxime axetil, Amoxicillin-clavulanate potassium and Azithromycin with a view to recommending alternative storage conditions and appropriate pharmacists' instructions to the patient when adequate refrigeration is unachievable.

2. METHODS

2.1 Survey of Community Pharmacies in Port Harcourt

This phase of the study was carried out in the Port Harcourt metropolis of Rivers state, Nigeria. Ninety-one (91) community pharmacies were visited and through a single-blind study, information concerning the availability of the

drugs, presence of a pharmacist, how well the patient was advised on the proper method of reconstitution of the antibiotic dry powder and storage of the reconstituted suspension, as well as the willingness to dispense with or without a prescription, were obtained and recorded.

2.2 Simulated in-Home Study

2.2.1 Sample collection

Three (3) antibiotics Cefuroxime axetil (125 mg/5 ml), Amoxicillin-clavulanate potassium (228.5 mg/5 ml), and Azithromycin (200 mg/5 ml) were used for this study. Two (2) brands of each antibiotic were selected. All the drugs were available as dry powders for reconstitution. The samples were purchased from a reputable and registered pharmacy and were within the stated expiry date on the packs as shown in Table 1 at the time of carrying out the study.

2.2.2 Sample preparation and estimation of the antibiotics

Eighteen (18) samples; consisting of six (6) samples of Cefuroxime axetil (3 Zinnat™ and 3 Ziva®), six (6) samples of Amoxicillin-clavulanate potassium (3 samples of Augmentin® and 3 samples of Fleming®), and six (6) samples of Azithromycin (3 samples of Zithromax® and 3 samples of Suitrox®) oral suspensions were freshly reconstituted with distilled water. The reconstituted preparations were distributed into groups (n=6) and subjected to three different simulated conditions that represent the different in-home storage conditions. Samples stored under condition A were refrigerated with fluctuating temperatures between 2-8℃ due to power outages during the period. Samples in condition B were stored inside a cupboard at room temperatures of 25-29℃ and samples stored under condition C were submerged in a bowl filled with water at temperatures of 22-25℃ for a period of 5 days. A thermometer was used to measure the temperatures.

On day 0 (immediately after reconstitution), 5 ml of each sample was measured and transferred into the clean cuvet, and inserted into the UV/Vis spectrophotometer. The equipment was set at the wavelength of maximum absorption appropriate to the antibiotic being assayed at the time. Its absorbance was then determined and recorded.

This procedure was repeated at days 1, 3 and 5 respectively and the absorbance of the different samples stored under the three (3) simulated in-home storage conditions were obtained and recorded.

The concentrations of Cefuroxime axetil, Amoxicillin-clavulanate potassium, and Azithromycin remaining after 1, 3 and 5 days were calculated using the Beer-Lambert equation:

$$A = \varepsilon . c . l$$

Where:

A= Absorbance
Ɛ = Molar absorptivity value or molar extinction coefficient (Wavelength dependent)
C = Concentration of the absorbing specie(s)
l = Optical path length

The values obtained were plotted in a curve and the percentage assay purities were then evaluated.

2.3 Instrumentation and Spectroscopic Conditions

Cefuroxime axetil, Amoxicillin-clavulanate and Azithromycin were assayed using a modified stability ultraviolet (UV)/Visible spectroscopic method. The wavelength of UV detection was set at 292 nm for simultaneous detection of Amoxicillin and Clavulanic acid, 276 nm for detection of Cefuroxime axetil, and 235 nm for Azithromycin. The path length of the cuvet was 10 mm (1 cm) and water was used as the blank at zero calibration for all three antibiotics.

The molar absorptivity values used in the Beer-Lambert law to determine the concentrations were: $2.1x10^{-5}mol^{-1}cm^{-1}$ for Cefuroxime axetil, $1.71x10^{-4}mol^{-1}cm^{-1}$ for Amoxicillin-clavulanate potassium, and $4.3x10^{-4}mol^{-1}cm^{-1}$ for Azithromycin.

3. RESULTS

3.1 Survey of Community Pharmacies in Port Harcourt

Table 3 below shows the results of the survey conducted. Of the 91 pharmacies visited, 76 had at least one of the three test antibiotics in stock. Out of this number, 71 consented to dispensing without a prescription, while 74 appropriately instructed on reconstitution and storage techniques.

Table 1. The brands, batch numbers, NAFDAC registration numbers, dates of manufacture and expiration of the drugs

Name of drug	Brand name	Batch/Lot number	NAFDAC reg number	Date of manufacture	Expiry date
Cefuroxime axetil	Zinnat™	C769410	04-0463	04/2016	04/2018
	Ziva®	1011	A4-6978	12/2015	11/2017
Amoxicillin-clavulanate potassium	Augmentin®	732588	04-2516	05/2015	05/2017
	Fleming®	160245	04-2396	02/2016	01/2018
Azithromycin	Zithromax®	540500	04-1387	12/2015	12/2017
	Suitrox®	GP0862	A4-3463	09/2016	09/2018

Table 2. Results of the survey conducted in community pharmacies in port Harcourt

Total number of pharmacies visited	91
Number of pharmacies with one or more of the drugs in stock	76
Number of pharmacies without any of the drugs in stock	15
Number of pharmacies that consented to dispensing without prescription	71
Number of pharmacies that declined dispensing without prescription	5
Number of pharmacies that appropriately instructed on storage of the drugs	74

Table 3. The absorbance, concentration and percentage concentration values of Zinnat™

Days	ABSORBANCE ± SEM			CONCENTRATION ± SEM ($x10^{-4}$mol)			% CONCENTRATION ± SEM		
	CA	CB	CC	CA	CB	CC	CA	CB	CC
1	5.82	5.84	5.87	2.77	2.78	2.79	96.89	93.39	95.22
3	5.54	4.99	5.34	2.64	2.38	2.56	95.31	85.61	91.76
5	5.13	3.90	4.72	2.44	1.86	2.25	88.09	66.91	80.65

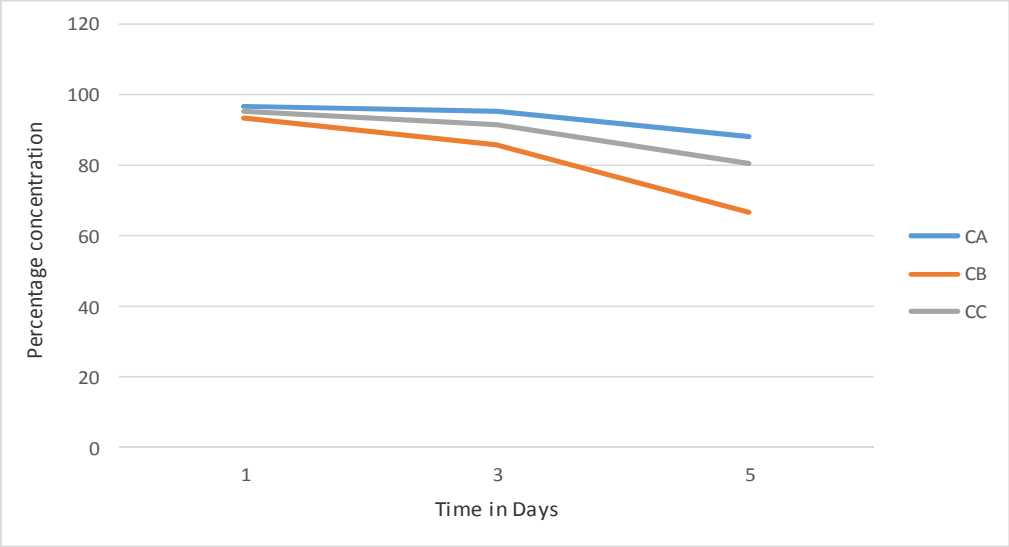

Fig. 1. Showing degradation of Zinnat® over time under different storage conditions

3.2 Simulated in-Home Study

The results from the in-home study show the respective absorbance obtained under the three simulated storage conditions post-reconstitution. These absorbance values were used to determine the concentrations and percentage concentration of the three antibiotics remaining after five days as shown in Tables 3-8.

3.3 Cefuroxime Axetil

The details of absorbance, concentration and percentage concentration of the two brands of cefuroxime axetil and the rate of degradation are presented in Tables three and four and Fig. 1 and 2 respectively.

3.4 Amoxicillin-Clavulanate Potassium

Tables 5 and 6 and Figs 3 and 4 below show the results of the absorbance, concentration and percentage concentration as well as the degradation over time of the two brands of amoxicillin-clavulanate potassium.

Table 4. The absorbance, concentration and percentage concentration values of ziva®

Days	ABSORBANCE ± SEM			CONCENTRATION ± SEM ($x10^{-4}$mol)			% CONCENTRATION ± SEM		
	CA	CB	CC	CA	CB	CC	CA	CB	CC
1	6.00	6.00	5.99	2.86	2.86	2.79	99.02	95.46	97.16
3	5.86	5.01	5.30	2.79	2.39	2.52	97.55	83.57	88.42
5	5.32	3.92	4.10	2.53	1.87	1.95	88.46	65.38	68.42

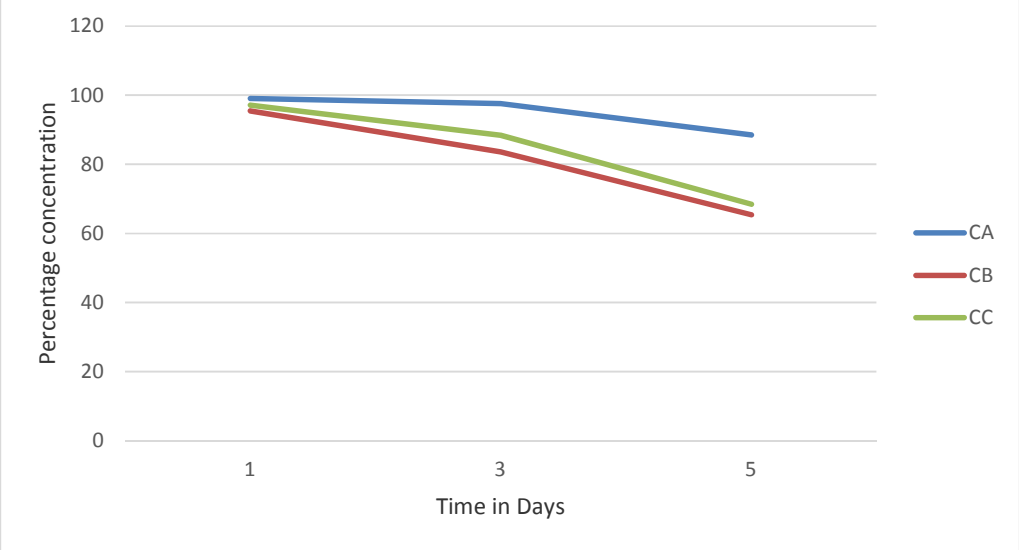

Fig. 2. Showing degradation of Ziva ® brand of cefuroxime over time under different storage

Table 5. The absorbance, concentration and percentage concentration values of augmentin®

Days	ABSORBANCE ± SEM			CONCENTRATION ± SEM ($x10^{-4}$mol)			% CONCENTRATION ± SEM		
	CA	CB	CC	CA	CB	CC	CA	CB	CC
1	6.02	6.00	6.32	3.52	3.51	3.69	96.11	90.33	93.71
3	5.51	4.87	5.25	3.22	2.85	3.07	91.48	81.20	83.20
5	5.00	3.83	4.60	2.93	2.24	2.69	83.24	63.82	72.90

Table 6. The absorbance, concentration and percentage concentration values of fleming®

Days	ABSORBANCE ± SEM			CONCENTRATION ± SEM ($x10^{-4}$mol)			% CONCENTRATION ± SEM		
	CA	CB	CC	CA	CB	CC	CA	CB	CC
1	5.65	5.26	5.29	3.31	3.08	3.10	94.48	89.44	92.17
3	5.00	4.01	4.52	2.92	2.35	2.64	88.21	76.30	85.16
5	4.54	3.14	4.05	2.65	1.83	2.37	80.06	59.42	76.45

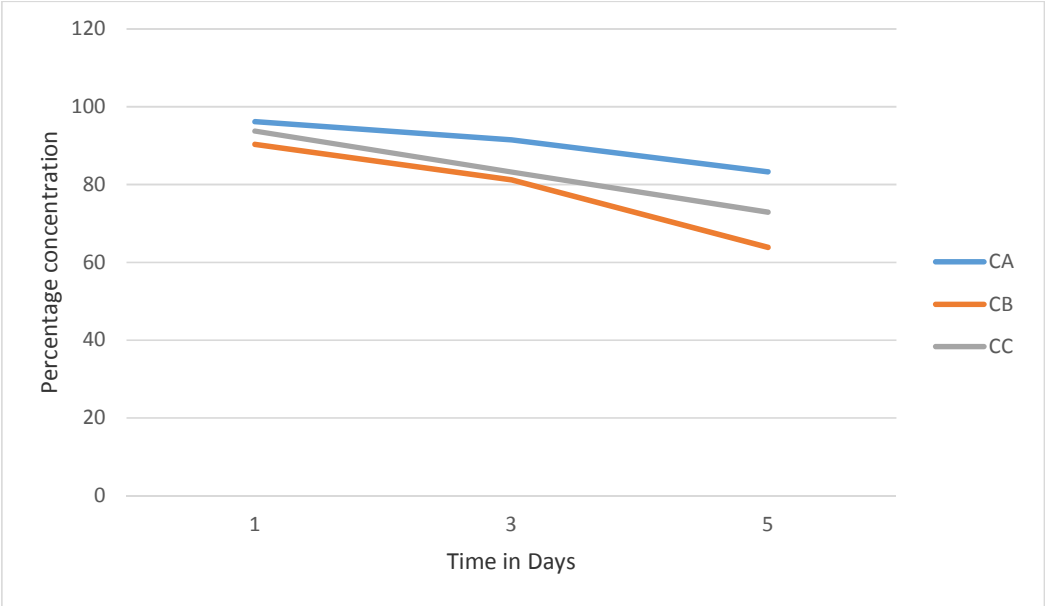

Fig. 3. Showing degradation of Augmentin over time under different storage conditions

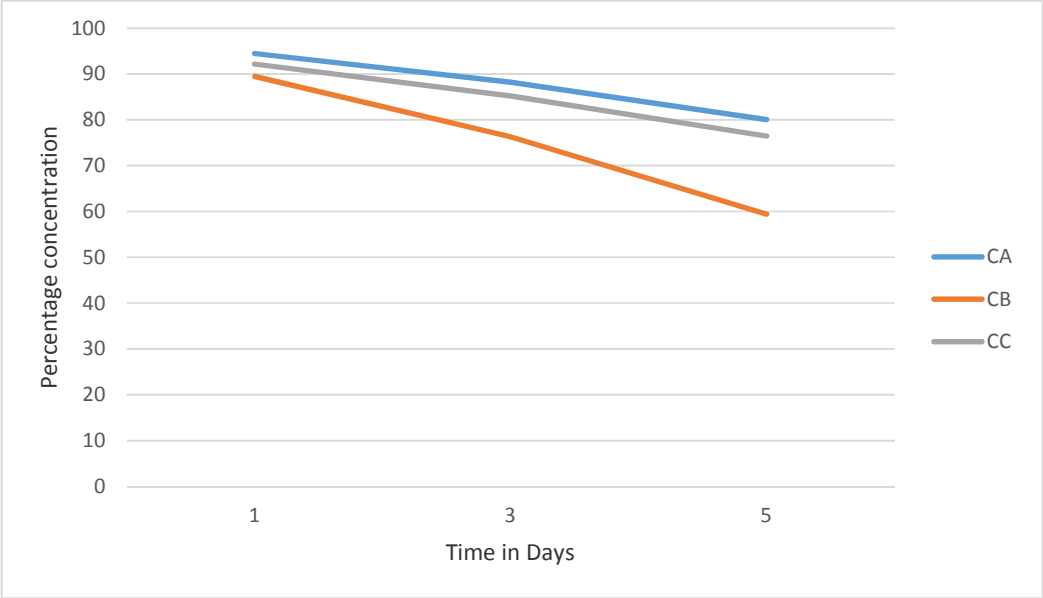

Fig. 4. Showing degradation of Flemming® brand of Amoxicillin clavulanate potasium over time under different storage conditions

Table 7. Results for the absorbance, concentration and percentage concentration values of Zithromax®

Days	ABSORBANCE ± SEM			CONCENTRATION ± SEM ($x10^{-4}$mol)			% CONCENTRATION ± SEM		
	CA	CB	CC	CA	CB	CC	CA	CB	CC
1	4.95	5.02	4.98	1.15	1.17	1.16	98.89	90.61	92.00
3	4.63	4.00	4.24	1.08	0.93	0.99	93.91	79.50	85.34
5	4.00	3.38	3.67	0.93	0.79	0.85	80.87	67.52	73.28

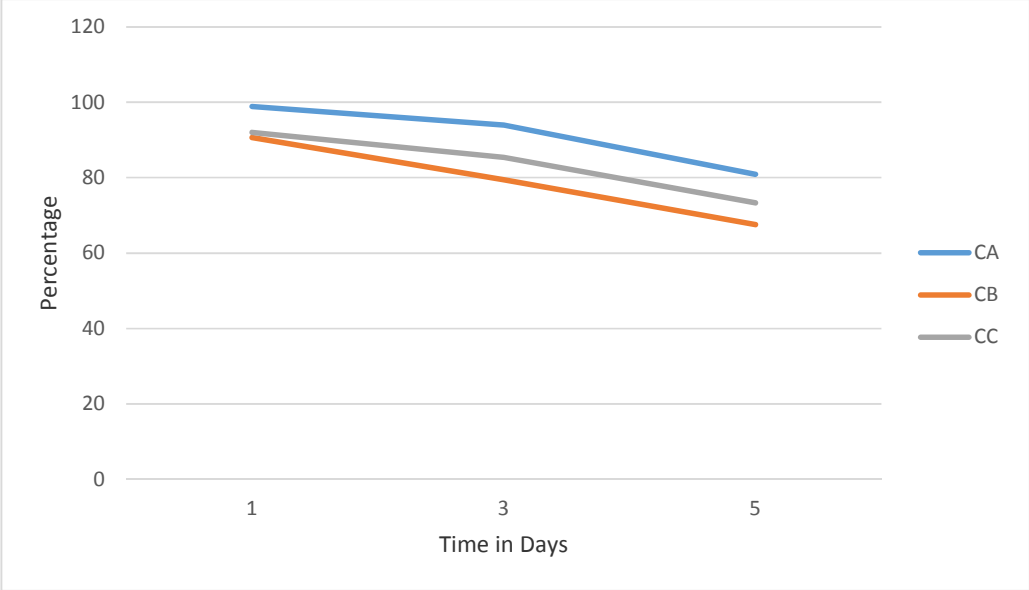

Fig. 5. Showing degradation of Azithromycin over time under different storage conditions

Table 8. The absorbance, concentration and percentage concentration values of Suitrox®

Days	ABSORBANCE ± SEM			CONCENTRATION ± SEM ($x10^{-4}$mol)			% CONCENTRATION ± SEM		
	CA	CB	CC	CA	CB	CC	CA	CB	CC
1	6.00	6.00	6.00	1.40	1.40	1.40	98.33	87.98	90.10
3	5.62	4.56	5.00	1.31	1.06	1.16	93.57	75.71	82.86
5	5.10	3.63	3.97	1.19	0.84	0.92	85.00	60.00	65.71

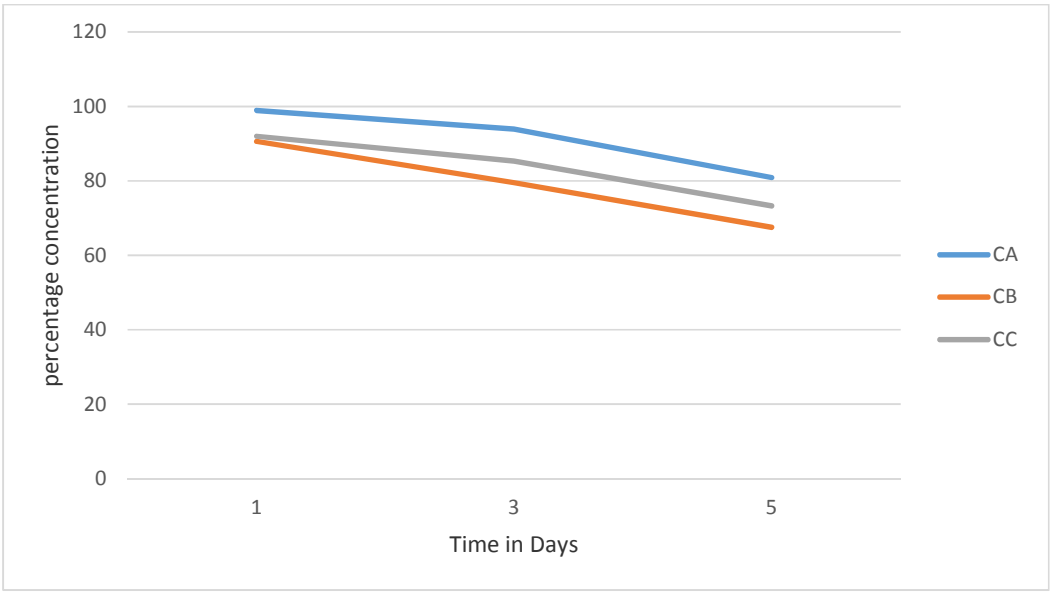

Fig. 6. Showing degradation of Suitrox ® brand of Azithromycin over time under different storage conditions

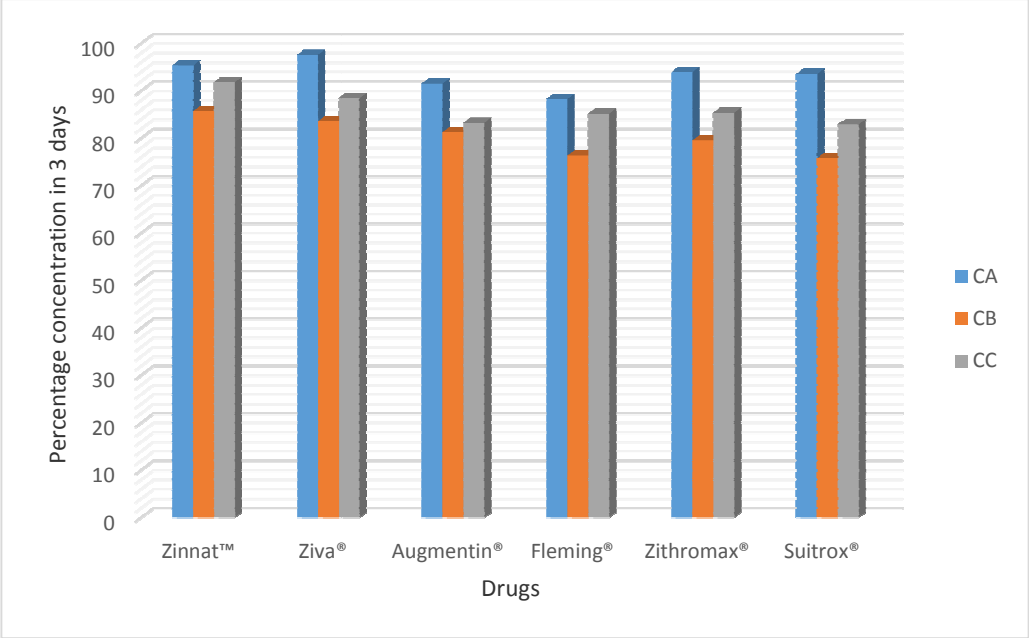

Fig. 7. showing percentage concentration of all the experimental drugs on day 3

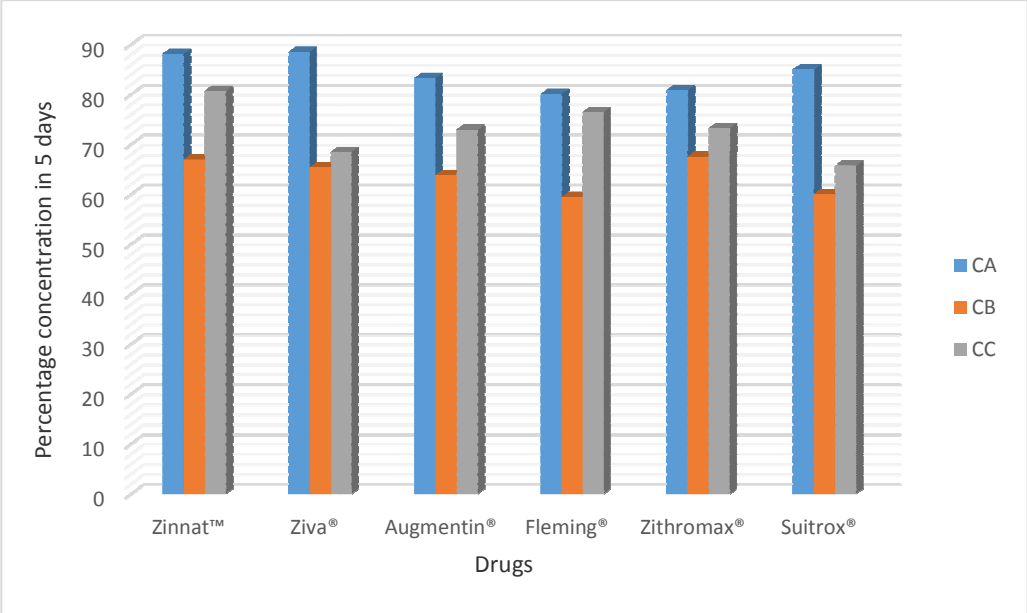

Fig. 8. showing percentage concentration of all the experimental drugs on day 5

KEY: CA= Condition A; Refrigerated with fluctuating power (2-8℃)
CB= Condition B; Stored in a cupboard at room temperature (25 -29℃)
CC= Condition C; Submerged in water at room temperature (22 -25℃)

3.5 Azithromycin

The details of the results for the two brands of azithromycin are as shown in Tables 7 and 8 and Figs. 5 and 6 respectively.

4. DISCUSSION

The survey conducted on community pharmacies investigated how well pharmacists advice their patients on proper reconstitution and storage

techniques and their readiness to dispense antibiotics without valid prescriptions. The results are as shown in Table 2. Ninety-one pharmacies were visited out of which 84% had the drugs in stock while 81% instructed the patient appropriately on proper reconstitution and storage techniques. These results show that at least one of Cefuroxime axetil, Amoxicillin-clavulanate potassium, and Azithromycin oral suspensions were readily available in pharmacies within the Port Harcourt metropolis. It also showed that majority of the pharmacists encountered possessed the required knowledge on reconstitution and storage of the antibiotics.

In the simulated in-home study, it was observed that not all three storage conditions maintained the stability of the reconstituted suspensions over the test period (5 days). The suspensions were judged to be stable if the components maintained at least 90 ± 5% of the label concentrations. During the test period, condition A (refrigerated with fluctuating power between 2-8℃) showed stability for all three antibiotics for the first three days and with the exception of Amoxicillin-clavulanate potassium, the other two antibiotics also showed stability until the fifth day. Amoxicillin-clavulanate potassium showed a small degree of degradation on the fifth day under this condition. This might be due to human or instrumentation error.

Storage under condition B (kept in a cupboard at a temperature of 25-29℃) on the other hand, showed the least level of stability. Degradation was observed on the third day in the three antibiotics. Analysis on the fifth day showed extensive degradation.

Condition C (submerged in water at room temperature of 22-25℃) however, was able to preserve the reconstituted suspensions for three days and degradation was observed on the fifth day.

The result of this study is similar to the work done by [8], which showed that amoxicillin suspension stored in a refrigerator between 2℃ and 8℃ for 7 days showed the lowest level of degradation.

These results are also in line with the work done [9], which showed that reconstituted amoxicillin-clavulanic acid oral suspension is stable for at least 5 days when stored at 5-8℃ in a /refrigerator with fluctuating power. [10] studied the stability of reconstituted cefadroxil and cefixime suspensions at 5℃ and at 25℃ respectively. It was observed that the administration period for the reconstituted suspensions was shown to be 6 days at room temperature and, 8 days if the product was kept in the refrigerator.

A striking finding in this study was the number of pharmacies willing to dispense these drugs without valid prescriptions. Even though antibiotics are usually ''Prescription Only Medicines'', 79% of the pharmacies visited consented to dispensing without a prescription. This may be attributed to the fact that many pharmacists do not spend enough time in their pharmacies as it was observed that a good number of the pharmacies that consented to dispensing without prescription were those that had no pharmacist present at the time of carrying out this study. This practice of indiscriminate dispensing and easy access to prescription drugs coupled with inadequacy of appropriate storage conditions can only worsen the already complex problem of antibiotic resistance. The pharmacist has a huge role to play in mitigating the challenge of antibiotic resistance by ensuring good antibiotic stewardship and being available to counsel and guide patients with respect to proper drug storage and use.

5. CONCLUSION

From the results obtained, it can be deduced that Cefuroxime axetil, Amoxicillin-clavulanate potassium, and Azithromycin powders for reconstitution are readily accessible to the populace in Port Harcourt, Rivers state and the patients are appropriately advised on reconstitution and storage techniques. It can also be concluded that reconstituted Cefuroxime axetil, Amoxicillin-clavulanate potassium and Azithromycin oral suspensions are stable for at least 5 days when stored in a refrigerator with temperature range of 2 to 8℃. When stored submerged in water at room temperature, all three antibiotics were stable for at least 3 days but when stored in a cupboard at room temperature, the antibiotics showed degradation on the third day. The implication of this is that in the absence of adequate storage conditions, patients taking these reconstituted drugs only receive sub-optimal doses after the third day. This practice can only enhance the emergence of antibiotic resistant pathogen. It is therefore important that clinicians take into consideration access to appropriate storage facilities when prescribing antibiotics**.** In the light of the

foregoing, it is recommended that pharmacists should advise the patient to store the reconstituted suspension in the refrigerator at the appropriate temperature to preserve the potency of the drug. Where this is not practicable as in resource limited settings, the patient should be advised to store the drug in a bowl of water in a cool place. Alternatively, as much as possible, antibiotics that are formulated in ready to use single sachets that can be reconstituted and administered when needed should be prescribed.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/22545