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# A comparative study on the prevalence of substandard ampicillin/cloxacillin preparations in the Nigerian market: mid 1990's and present

*[1]Obodozie,O.O,[1] Mustapha,K.B, [1]Ebeshi,B.U and [1] Inyang,U.S.

[1]*Department of Medicinal Chemistry and Quality Control*
*National Institute for Pharmaceutical Research and Development,*
*P. M. B. 21, Idu Abuja, Nigeria.*

**Abstract**

A Comparative evaluation of the quality (content of active pharmaceutical ingredients) of different brands of Ampicillin/Cloxacillin products available in Nigerian market, for the period between mid 1990s and 2004 was carried out. The samples consisted of ten, eight, and four different brands of Ampicillin/Cloxacillin capsules, suspension and injections respectively. All the different brands were analyzed using a slightly modified HPLC, U.S.P. method at a wavelength of 225nm. The absolute drug content of the various brands were calculated and found to range from 66.4 to 118.2% for Cloxacillin and 80.0 to 96.5% for Ampicillin in the capsule formulations. The absolute drug content was found to range from 37 to 173.5% for Cloxacillin and 78.3 to 128.7% for Ampicillin in the suspension dosage forms. Also, the percentage content in injection formulation ranged from 80.2 to 118.2% for Cloxacillin and 91.8 to 122.7% for Ampicillin. The prevalence of substandard ampicillin/cloxacillin capsule, suspension, and injection in this study was found to be 40%, 50% and 25% respectively. In comparison, similar study done in our laboratory in the mid 1990's gave the following prevalence of substandard ampicillin/cloxacillin products; 80% capsules, 100% suspension and 40% for injection. The findings in this study indicate that there is almost a 50% drop in the prevalence of substandard ampicillin/cloxacillin preparations in the Nigerian market. The implication of this findings to Nigeria is that the current efforts by the food and drug regulatory authority to wage war against substandard drug is yielding positive results but a lot still needs to be done.

*Keyword: Ampicillin/Cloxacillin,* HPLC, Substandard drugs, Nigeria.

## Introduction

Counterfeiting and faking of Pharmaceutical products is a global phenomenon, and the faking of commercial products is an age long problem [(1)]. The proliferation of substandard and adulterated products has been of great concern to many countries including Nigeria [(2)]. The incidence of substandard products especially, in Nigeria is due to a number of factors. Principal among them are: Ineffectiveness of drug regulatory authorities, poor drug procurement practices; low literacy level and lack of awareness of the existence of substandard drugs; high level of smuggling of pharmaceutical products within the West African sub-region and political instability.

The problem of substandard drugs despite its global status did not receive prompt attention by the WHO as it deserves until recently when the international organization, recommended that all importing countries should protect themselves among other things, by undertaking sampling of products within the distribution chain as an element in quality surveillance [(3)].

Ampiclox® is the innovator product of a combination antibiotic preparation, made up of Ampicillin and Cloxacillin in equal proportion depending on the presentation. It is an

*Correspondence: Dr O. O. Obodozie Email: obyobodozie@yahoo.com

important and first line antibiotic that is widely used in all hospitals and health centers in Nigeria. It is usually available as capsule, injection and suspension. Capsules are usually for adults while suspensions are for children and infants [4].

The development of resistance to commonly used antibiotics is one of major causes of treatment failures in our healthcare delivery system. This is as a result of a number of factors, principal among them is the administration of substandard dose of antibiotics. Administration of sub-dose of an antibiotic may result from misuse of the drug as compliance to dosage regimen is completely ignored. Another crucial factor is the increased incidence of importation of fake and substandard drugs occasioned by free drug markets in Nigeria.

There are numerous brands of ampicillin/cloxacillin capsules and injections in Nigeria. To ascertain the quality of most imported and locally manufactured products, routine quality assurance is conducted by subjecting the drugs to qualitative and quantitative analyses. In order to encourage and facilitate the frequency and ease of conducting routine quality control assessment of these products, it is imperative to develop a cost-effective, simple, reliable, accurate and sensitive method for the quantitative analysis of the active pharmaceutical ingredient.

High Performance Liquid Chromatography (HPLC) is a versatile analytical tool for the separation and quantitation of a mixture of closely related substances simply because of its simplicity, high resolving power, accurate quantitative measurement, repetitive and reproducible analysis and automation of analytical procedures and data handling [5].

The food and drug regulatory authority in Nigeria has in recent times, made tremendous efforts to rid the country of substandard products. Its success has largely been measured by the general public and media commendations of its activities. In order to evaluate this effort through an evidence-based assessment of the prevalence of fake and substandard drugs, we undertook a study on the quality assessment of different brands and dosage form preparations of Ampicillin/Cloxacillin presently found in the Nigerian market using a modified HPLC method [6] for the analysis of Cloxacillin. The result was compared to that obtained from earlier work done in our laboratory in the mid 1990s. The objective of the study therefore is to compare the prevalence rates of fake and substandard ampicillin/cloxacillin preparations in Nigeria between two periods of approximately ten year time difference, in a bid to measure the impact of the current sustained efforts by NAFDAC in waging war against substandard products.

## Experimental

*Instrumentation & apparatus*

The HPLC system employed consisted of Agilent 1100 series programmable solvent module Quaternary pump G1311A (Agilent technology, USA), equipped with an auto degasser G1322A, and a variable wavelength detector G1314A. The column used was a reverse phase ultrasphere ODS (C-18), 5µm particle size and 250 x 4.6mm I.D, (Phenomex, USA) equipped with a guard, ultra sonicator (Decon lab Ltd, East susex), Milli-Q (Molsheum, France), Whirlmixer (ultratech), table centrifuge (Sorvall instrument), precision pipettes (Eppendorf), pasteur pipettes and extraction tubes.

*Reagents*
Standard reference compounds of ampicillin and cloxacillin were obtained from Sigma chemical company (St Louis, USA), di-sodium hydrogen orthophosphate (Sigma chemicals, USA), phosphoric acid (BDH Chemicals Ltd., England), HPLC grade acetonitrile (Merck, Darmstadt, Germany), distilled water was further purified by a milli-Q Plus (Millipore, USA).

*Sample collection*
Ten (10) brands of ampicillin & cloxacillin capsules, eight (8) brands of the suspension and four (4) brands of the injections were randomly purchased from health centers, pharmacy stores, markets and patent medicine shops in Abuja, Federal Capital Territory at two different periods i.e. 1994 1996 and 2004. A pre-study survey conducted prior sample collection indicated that brands collected for the different dosage forms were all that was available and circulating in the market as at each study period.

*Analytical Method*
A British Pharmacopeia method for the assay of cloxacillin in drug products using an ultrasphere column rather than that with Li packing column and adjusting flow rate to 1ml/min. Ampicllin and cloxacillin were detected simultaneously at a wavelength of 225nm.

*Method Validation Within-day Runs (Precision)*
Two sets, each set consisting of four centrifuge tubes, were used. Each tube in the first set contained 1ml of blank diluent spiked with the stock solution of the drugs to give a concentration of 0.5µg/ml. The second set also contained 1ml of diluent spiked with stock solution of the drugs to give a concentration of 2µg/ml of each. The samples were whirlmixed before 20L was injected onto the HPLC. Three replicate injections of each were made. The coefficient of variation of each set was computed.

*Day-to-day Runs (Accuracy)*
The procedure above was followed but a sample for each set was analyzed daily for 4 days.

*Limits of detection of analytical method*
Varying concentrations of the reference drug samples up to the minimum non-detectable levels were prepared using the buffer solution whirlmixed before 20L was injected onto the HPLC.

*Preparation of standard stock of Ampicillin and Cloxacillin solutions*
The standard stock solutions of 11mg/ml ampicillin and cloxacillin were prepared using the buffer (0.02M $KH_2PO4$) solution at pH 5.0.

*Calibration curve for Ampicillin/Cloxacillin*
Varying amounts of the stock solutions of Ampicillin and Cloxacillin were added to blank diluent (0.02M $KH_2PO_4$) in extraction tubes to give calibration curves between 0.37 2.2mg/ml for Ampicillin and Cloxacillin. The samples were vortexed in a whirlmixer for

one minute before injecting 20iL unto the HPLC. The peak area was plotted against the concentration of each of the compound injected to obtain calibration curves. The regression analysis was carried out with the aid of a Microsoft Excel 2000.

## Determination of drug samples

*Analysis of tests samples*

Twenty capsules each of the different brands of Ampicillin/Cloxacillin were weighed to determine their weight variation. An amount equivalent to 11g of the drugs was accurately weighed into a 100ml volumetric flask and dissolved using the buffer solution to give expected stock concentration of 11mg/ml.

Varying amounts of the test samples (11mg/ml) of Ampicillin and Cloxacillin were added to 1ml of blank diluent (0.02M $KH_2PO_4$) in extraction tubes to give concentrations between 0.37 2.2mg/ml for Ampicillin and Cloxacillin. The samples were thereafter subjected to the same treatment as for the standards. The resulting peak areas were recorded and the actual amounts of the drugs were determined from the standard calibration curve.

## Results

Table 1

| | | | Coefficient of Variation (%) | |
|---|---|---|---|---|
| | l | No. of samples (n) | Ampicillin | Cloxacillin |
| Within-day | 0.5 | 4 | 3.7 | 3.2 |
| | 2.0 | 4 | 3.3 | 3.5 |
| Day-to-day | 0.5 | 4 | 3.5 | 2.9 |
| | 2.0 | 4 | 3.7 | 2.1 |

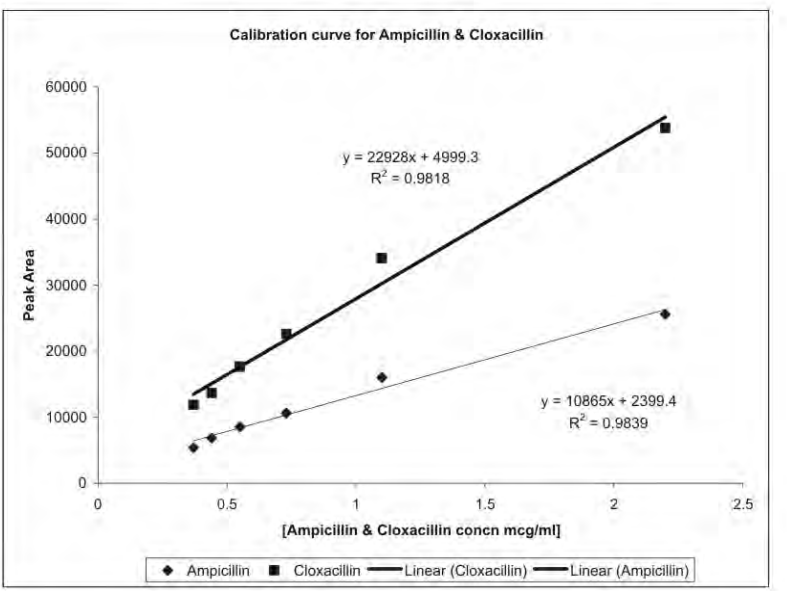


Table 2: **Ampicillin/Cloxacillin capsules at present**

| Code No. | % Content of Ampicillin Found | %Content of Cloxacillin Found | Remark |
|---|---|---|---|
| 1 | 92.7 | 96.4 | passed |
| 2 | 87.9 | 87.2 | Failed (both low) |
| 3 | 92.7 | 118.2 | passed |
| 4 | 93.6 | 110.9 | passed |
| 5 | 92.7 | 113.6 | passed |
| 6 | 96.5 | 112.7 | passed |
| 7 | 80.0 | 103.6 | Failed (amp low) |
| 8 | 86.5 | 66.4 | Failed (both low) |
| 9 | 86.4 | 84.5 | Failed (both low) |
| 10 | 94.6 | 105.5 | passed |

Table 3: **Ampicillin/Cloxacillin capsules mid 1990s**

| Code No. | %Content of Ampicillin | %Content of Cloxacillin | Remark |
|---|---|---|---|
| 1.A54 | 86.99 | 84.94 | Failed (both low) |
| 2.A/25 | 97.02 | 98.37 | Passed |
| 3.A/694 | 85.59 | 56.25 | Failed (both low) |
| 4.A/324 | 103.42 | 43.45 | Failed (clox low) |
| 5.A783 | 92.54 | 61.89 | Failed (clox low) |
| 6.A819 | 98.64 | 107.12 | Passed |
| 7.A753 | 98.50 | 57.21 | Failed (clox low) |
| 8.A768 | 96.44 | 57.21 | Failed (clox low) |
| 9.1509 | 106.76 | 82.78 | Failed (clox low) |
| 10.966 | 177.03 | 49.98 | Failed (amp high, clox low) |

Table 4: **Ampicillin/Cloxacillin Injections at present**

| Code No. | % Content of Ampicillin found | % Content of Cloxacillin found | Remark |
|---|---|---|---|
| 1 | 91.8 | 118.2 | passed |
| 2 | 122.7 | 96.4 | passed |
| 3 | 109.9 | 80.2 | Failed (clox low) |
| 4 | 114.5 | 100.9 | passed |

Table 5: **Ampicillin/Cloxacillin Injections mid 1990s**

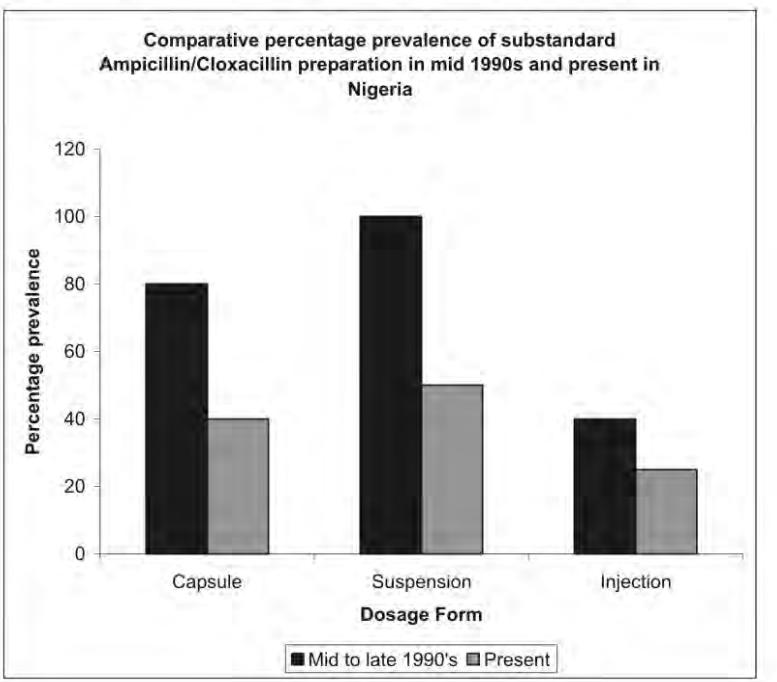
| Code No. | % Content of Ampicillin | %Content of Cloxacillin | Remark |
|---|---|---|---|
| 1.I/628 | 107.82 | 95.30 | Passed |
| 2.I/420 | 89.96 | 78.16 | Failed (clox low) |
| 3.I/1008 | 118.59 | 108.41 | Passed |
| 4.I/422 | 101.76 | 97.00 | Passed |
| 5.I/001 | 98.55 | 125.30 | Failed (clox high) |

Table 6: **Ampicillin/Cloxacillin Suspension at present**

| Code No. | % Content of Ampicillin Found | % Content of Cloxacillin Found | Remark |
|---|---|---|---|
| 1 | 128.7 | 173.5 | Failed (both high) |
| 2 | 122.7 | 162.8 | Failed (both high) |
| 3 | 108.7 | 109.4 | Passed |
| 4 | 119.3 | 113.5 | Passed |
| 5 | 154.6 | 169 | Failed (both high) |
| 6 | 112.2 | 106 | Passed |
| 7 | 104.4 | 115 | Passed |
| 8 | 78.3 | 37 | Failed (both low) |

Table 7: **Ampicillin/Cloxacillin Suspension mid 1990s**

| Code No. | % Content of Ampicillin | %Content of Cloxacillin | Remark |
|---|---|---|---|
| 1.S/1509 | 106.76 | 82.79 | Failed (clox low) |
| 2.S/966 | 154.92 | 55.12 | Failed (amp high, clox low) |
| 3.S/820 | 176.81 | 100.32 | Failed (amp high) |



Results of Precision studies for Ampicillin and Cloxacillin

## Discussion

The HPLC method used for the analysis gave a good resolution of Ampicillin and Cloxacillin. The HPLC method was reproducible with coefficient of variation (which is a measure of precision) for ampicillin and cloxacillin, being less than 5% in the buffer solution (Table 2). The method, therefore, exhibits good precision and sensitivity. The analytical method employed in this study is one of the few methods that can resolve both ampicillin and cloxacillin in samples.

The limit of detection for ampicillin and cloxacillin was 10ng/ml, which renders this method suitable for detecting these entities in matrices where they may be present in low levels, for example, biological fluids. The calibration curve for ampicillin was linear with correlation coefficient greater than 0.98 (Fig. 1). The samples were subjected to weight variation test and all passed the test.

Six brands out of the ten brands of Ampicillin/Cloxacillin capsules conformed to the official requirements as stated in the USP. The USP specifies 90 to 120% of the stated amount for the absolute drug content of ampicillin/cloxacillin (USP, 1990). The average content is between 166mg to 295.5mg, as compared to two brands out of ten brands of ampicillin/cloxacillin capsules that passed the test in the earlier study of mid 1990s (Tables 2 & 3). This indicates that the prevalence of substandard ampicillin/cloxacillin in mid 1990s was 80%, compared to the current level of 40% for capsule dosage form.

Three out of four brands of Ampicillin/Cloxacillin injections had absolute drug content in conformity to the stated amount which differs from the result of the earlier study which showed that three out of five brands passed the content uniformity test (Tables 4 & 5). Therefore, the current prevalence of substandard injectable preparation of the drug is 25%, compared to the 40% obtained in our previous studies.

Furthermore, four of the eight brands of ampicillin/cloxacillin suspension in the present study had absolute content of the drug within the specified range, whereas none out of three brands had absolute drug content up to the specified range (tables 6 & 7).

The prevalence of substandard ampicillin/cloxacillin products under investigation varies from one dosage form to the other. Whereas the suspensions dosage form dropped from a 100% prevalence in 1994-1996 to 50% in 2004, the injection dosage form dropped to 25% from 45%. It was also observed that most failure in the suspension dosage form in the 2004 study resulted from higher drug content (overages) of ampicillin/cloxacillin than that stipulated in the official compendium, unlike the mid 1990s study where the substandard products were as a result of low drug content of both ampicillin/cloxacillin. This may be as result of the manufacturers or importers trying to beat the regulatory authority at all cost incase of deterioration of products on storage. The implication is that this particular dosage form is used by infants and children, thereby putting the life of this vulnerable group into high risk of toxicity occasioned by consumption of higher amounts of the drugs from overages or treatment failure and possible drug resistance in cases of sub-optimal drug content.

It can also be seen in figure 2 that the prevalence of substandard ampicillin/cloxacillin preparation was highest in the suspension and lowest in the injectable dosage form in the order, suspension > capsule > injection. This tends to suggest that the dosage form most

frequently prescribed and commonly used with high volume sales was most subjected to adulteration and faking, supporting the suggestion sales volume and high consumption rates are factors that increase the vulnerability of drug products to faking and counterfeiting. Therefore it is still evident that economic reason is one of driving factors for the menace and high prevalence of substandard drug products in Nigeria.

The high level of the prevalence of substandard ampicillin/cloxacillin preparation as observed in this study could be due to the chaotic nature of the nation's drug distribution chain where quacks and non-professional hold sway, import liberalization, harsh and badly battered economy, existent weak regulatory activities and corruption.

One limitation of the study however is the small sample size involved in the suspension dosage form of mid 1990s. However, the similarity in the trend and magnitude of fall observed with the other two dosage forms tends to support the data obtained with this sample size.

## Conclusion

There is a significant drop on the prevalence of substandard ampicillin/cloxacillin products marketed in Nigeria at present as compared to the mid 1990s. This may have resulted from the current sustained war by NAFDAC to rid the country of fake and adulterated drug products. Concerted efforts being made by the government to empower and strengthen the regulatory authority to contain this menace in other to save the lives of Nigerians is yielding desired changes although a lot still has to be done to further bring down the prevalence to insignificant proportions.

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