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Evaluation of Amoxicillin Content in Commonly Used Multisource Injectable Brands in Veterinary Practice

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G.F. Aondover[1], O.S. Charles[2], A.R.A. Moshood[2], O.O. Oladipo[2] and S.F. Kundu
[1]*Department of Veterinary Pharmacology and Toxicology, Federal University of Agriculture, Makurdi, Nigeria.*
[2]*Department of Veterinary Medicine, University of Ibadan, Nigeria.*

THE AIM is to evaluate amount of amoxicillin in the array of its injectable formulations from multisource marketed and commonly used in veterinary practice in Nigeria. The amount of amoxicillin in each of the 10 brands sampled was analysed using a high-performance liquid chromatography. Thereafter, percentages of the labelled amount of amoxicillin were determined and compared with 90-120% specified in USP and IP. Samples G, H, and J contained 93%, 98%, and 108%, respectively of the labeled amount of amoxicillin, while B, C, and A contained only 39%, 56%, and 58%, respectively. Again, samples I, F, D, and E contained 124%, 135%, 147%, and 413%, respectively of the labelled amount. Thus, only brands G, H, and J passed assay quality test (AQT) since the amount of amoxicillin they contained was within the specified USP and IP range, and are considered pharmaceutically equivalent, consequently, interchangeable for intravenous administrations. Whereas, brands A, B, and C failed AQT because they contained less than the amount of amoxicillin required, so even when used prudently there could be therapeutic failure, bacterial resistance, and public health implications. Similarly, brands D, E, F, and I could cause toxicity and high tissue residues because they contained higher than the required and labelled amount of amoxicillin. About 30% and 40% of the analysed amoxicillin brands contained less and more than the required amount of amoxicillin, respectively. However, 30% contained the amount within the specified range. Consequently, there is high rate of substandard amoxicillin injectable brands for veterinary use in Nigeria hence the need for regular monitoring.

Keywords: Brands, Assay quality test, Substandard drugs, Pharmaceutical equivalence, Nigeria.

Introduction

Amoxicillin is chemically (2s.5r.6r)-6- [(2R)-2-amino-2-(4-hydrowyphenyl)-acetyl] amino-three, 3-dimethyl-7-oxo-four-thia-l-azabicyclo [3.2.0] heptane-2-carboxylic acid with a molecular formula and weight of $C_{16}H_{19}N_3O_5S$ and 365.404g/mol, respectively [1]. It is a semi-synthetic antibiotic derived from a precursor molecule, 6-aminopenicillanic acid [2]. Figure 1 shows the chemical structure of amoxicillin.

Amoxicillin is bactericidal and extended spectrum β-lactam antibiotic used for treating susceptible Gram- positive and Gram- negative bacterial infections in animals and human. It acts by interfering with the cell wall synthesis in bacteria by inhibiting cross-linkage of peptidoglycan molecules, a major cell wall component in Gram-positive and few Gram-negative bacteria [3]. Amoxicillin is listed in a number of Pharmacopoeias such as United States Pharmacopoeia (USP), British Pharmacopoeia (BP), European Pharmacopoeia (EP), and International Pharmacopoeia (IP).
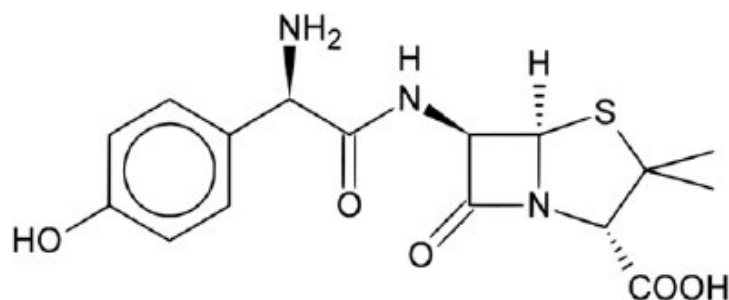
Fig. 1. Chemical structure of amoxicillin.

Substandard and counterfeit drugs constitute health challenge not only in the developing countries but also in developed countries with well-established monitoring structures for drug manufacturing, importation, sales, and use [4,5]. Antibiotics account for a large portion of substandard and counterfeit medications in Africa and Asia with the β-lactam antibiotics, notably penicillin and amoxicillin accounting for 50% [6]. Drug quality control is a requisite to ensuring efficacy, safety, and quality of medicines. Use of substandard and/ or counterfeit drugs in animals and man could result in therapeutic failure, toxicity, allergic reactions due to their content, drug resistance, prolonged illness, high cost of treatment, and mortality [7]. Recent reports indicated high rate of adverse drug events following administration of amoxicillin generics, of which there are little explanation for the possible causes [8].

In veterinary medicine, amoxicillin is commonly used in treating susceptible bacterial infections in food and companion animals. Generally, it is included in the list of essential drugs issued by the World Health Organization (WHO). It is also among the most widely counterfeited medicines in the developing countries [9,5]. Little is known about the quality of most antibacterial drugs used in veterinary practice, including injectable amoxicillin dosage form despite being in high demand globally, including Nigeria. Manufacturers of substandard, fake or counterfeit drugs target economically profitable medicines such as antibiotics that have high demand and volume of sales [10]. Nevertheless, poor-quality essential medicines are a substantial and understudied problem in pharmaceutical research [11]. Few studies revealed that inadequate amount of the active pharmaceutical ingredient in relation to the claimed amount as labelled is a major factor in poor quality of pharmaceuticals

[12]. Consequently, this study was conducted to evaluate the actual amount of amoxicillin in the array of injectable dosage form from multisource marketed and commonly used in veterinary practice in Nigeria.

Material and Methods

Preparation of solutions : A method described by Jayakar et al. [1] was slightly modified and adopted for the assay of amoxicillin in the sampled brands as briefly explained below.

Buffer

A 0.2M Potassium dihydrogen phosphate buffer (KH_2PO_4) was prepared by dissolving 27.218g of analytical KH_2PO_4 (E-Merck, India) in1000mL of deionized water. Thereafter, the resulting buffer solution was adjusted to a pH 4.8 using 60% perchloric acid.

Mobile phase

Mobile phase used constituted HPLC grade acetonitrile (Sigma Aldrich, USA) and buffer (5:95; v/v). This was filtered through a 0.45μm membrane filter and degassed under ultrasonic bath prior to use. The mobile phase was pumped through the column at a flow rate of 1ml/min for conditioning.

Amoxicillin reference standard and samples (injectable dosage forms)

A USP amoxicillin (200mg) reference standard (Rockville, MD, USA) was used. The stock solution of the reference standard was reconstituted by dissolving the whole vial of 200mg amoxicillin with 4 ml of deionized water resulting in a concentration of 50mg/ml. Thereafter, 20, 40, 60, 80, and 10μg/ml strengths of working solutions were prepared for development of a calibration curve of the standard. Also, 10 common and frequently used brands of injectable dosage form of amoxicillin in veterinary practice

across Nigeria were purchased from different pharmaceutical and veterinary shops or clinics across the country. Table 1 summaries the basic information on these multisource injectable amoxicillin formulations.

Samples D to J were reconstituted into a stock solution of 100mg/ml by injecting 5 ml of deionized water into each of the vials. Thereafter, 50µg/ml of working solutions were prepared from sample B and stock solutions of samples D to J using the mobile phase. Also, 75µg/ml working solutions were prepared for samples A and C using the mobile phase.

Instrumentation and chromatographic conditions

An Adept Cecil (CE 4200) High Performance Liquid Chromatography (HPLC) system was used for amoxicillin assay from the reference standard and generics injectable dosage form from multisource (test samples). The chromatographic conditions were as follows: flow rate of 1ml/min, wavelength of 223nm, column temperature of 25°C, injection volume of 20µL, and run time of 10 minutes. In addition, a reversed phase column, 120-10C18, 4.6mm x 25cm (Hichrom, Nucleosil) with a guard (Ajo-4282, Phenomenex) was used and the analyte was detected with a UV detector. After each run time, the system was equilibrated for 5 minutes with the mobile phase.

Assay of amoxicillin in reference standard and brands of injectable dosage form

Five different concentrations of amoxicillin (20, 40, 60, 80, and 100µg/ml) earlier prepared for linearity studies were analysed using HPLC with chromatographic conditions described above and corresponding peak responses in areas (mA) were measured. Subsequently, a calibration plot between known concentrations and their corresponding peak areas was constructed to obtain the coefficient of relationship to ascertain linearity, and the regression equation for calculation of the concentrations of amoxicillin in the test samples. Results for the content analysis were expressed as the percentage of the amount of amoxicillin dose as stated on the packaging that was actually quantified. This percentage was compared with the USP and IP which stipulate that the quantified amoxicillin from the injectable commercial preparation should not be outside 90-120% of stated amount on the product label [13].

Result

The study revealed that 60% (6/10) of the sampled brands of injectable dosage form of amoxicillin from multisource commonly used in veterinary practice in Nigeria are imported, and none of the generic injectable dosage form was manufactured in any African country, including Nigeria (Table 1).

Typical chromatograms of amoxicillin in the reference standard and brand of injectable dosage form are presented in Fig. 2. There was no interference or overlapping of the typical amoxicillin peak either with the diluents or excipients. Also, amoxicillin was eluted at about 6 minutes (retention time) from both the reference standard and the commercial injectable dosage form (samples).

TABLE 1. Commonly used multisource injectable dosage form of amoxicillin in some veterinary clinics in Nigeria.

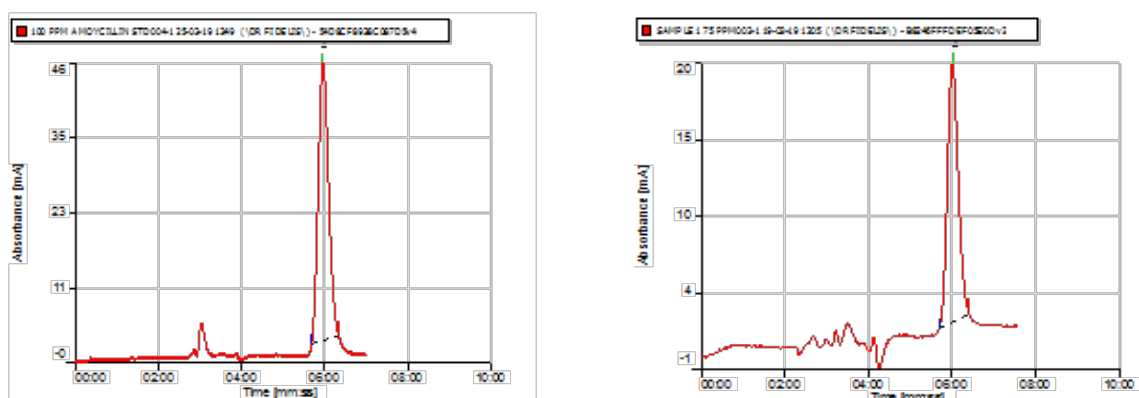| Brand ID	Labelled content			Expiry date
	Amoxicillin	Colistin	Gentamicin	
A	150mg/ml	-	40mg/ml	05/12/19
B	100mg/ml	85mg/ml	-	03/2021
C	150mg/ml	-	-	12/2019
D	500mg/vial	-	-	10/2020
E	500mg/vial	-	-	08/2020
F	500mg/vial	-	-	10/2020
G	500mg/vial	-	-	06/2019
H	500mg/vial	-	-	01/2020
I	500mg/vial	-	-	01/2020
J	500mg/vial	-	-	07/2019

Fig. 2. Chromatogram of amoxicillin in the reference standard (left) and a brand of injectable dosage form (right).
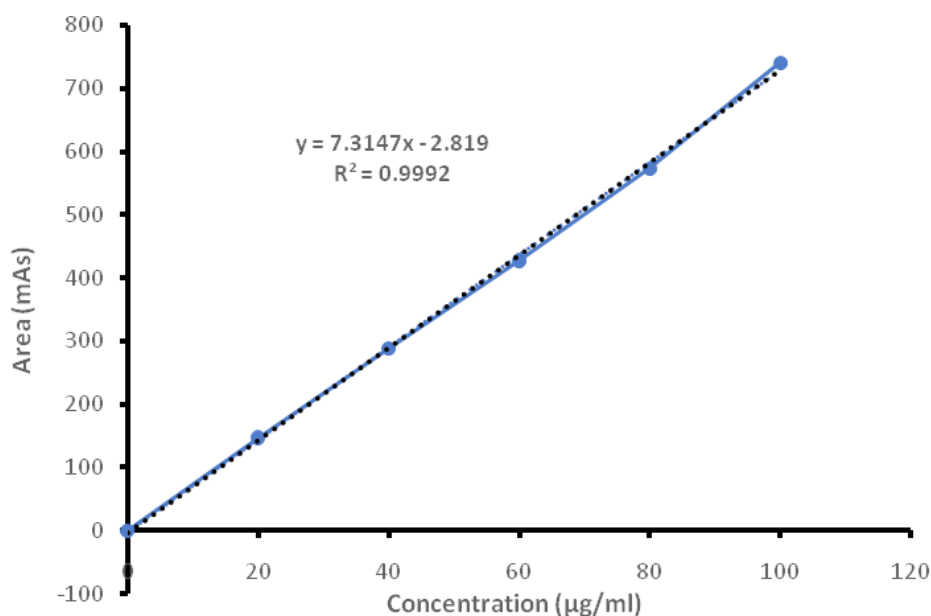

$$y = 7.3147x - 2.819$$
$$R^2 = 0.9992$$

Fig. 3. Calibration plot for the reference standard of amoxicillin.

Calibration plot obtained by the least square regression analysis of amoxicillin concentrations of 20, 40, 60, 80 and 100μg/ml and their corresponding peak areas (responses) showed a strong positive linear relationship with a correlation coefficient (R^2) of 0.9992 (Fig. 3).

The high coefficient of correlation between the peak area and concentration demonstrated a good linearity and suitability of the method over a wide range of concentration of amoxicillin. Assay of amoxicillin in the brands of injectable dosage form were performed using the regression equation (y=7.3147x-2.819) obtained from Fig. 3. Also, the limits of detection (LOD) and quantification (LOQ) were calculated to be

2.10μg/ml and 6.70μg/ml, respectively.

The evaluated amount of amoxicillin in each of the multisource brand as compared to the various manufacturers' labelled claims are presented in Table 2.

Results showed that only three (G, H, and J) of the evaluated brands of injectable dosage form of amoxicillin complied with the compendia required range (90-120% of the labelled amount) for amoxicillin as specified in the USP and IP. Brands I, F, D, and E exceeded the specified maximal limit of 600mg/vial (120% of the labelled amount of 500mg/vial) by 20, 72, 135, and 1465mg of amoxicillin per vial, respectively.

TABLE 2. Assay of amoxicillin in some injectable brands commonly used in veterinary practice in Nigeria.

Brand ID	Labelled amount	Amount found	% of the labelled amount found	*USP/IP Specification (90-120 % of claim)
A	150mg/ml	87mg/ml	58	145-180mg/ml
B	100mg/ml	39mg/ml	39	90-120mg/ml
C	150mg/ml	84mg/ml	56	145-180mg/ml
D	500mg/vial	735mg/vial	147	450-600mg/vial
E	500mg/vial	2065mg/vial	413	450-600mg/vial
F	500mg/vial	675mg/vial	135	450-600mg/vial
G	500mg/vial	465mg/vial	93*	450-600mg/vial*
H	500mg/vial	490mg/vial	98*	450-600mg/vial*
I	500mg/vial	620mg/vial	124	450-600mg/vial
J	500mg/vial	540mg/vial	108*	450-600mg/vial*

Index: *within USP and IP pharmaceutical limit for assay of amoxicillin injectable (90-120% of the labelled amount) [13]

Conversely, brands A and C contained amount of amoxicillin less than the specified lower limit of 145mg/ml (90% of the labelled amount of 150mg/ml) by 58mg/ml (145-87mg) and 61mg/ml (145-84mg) of amoxicillin, respectively. Again, brand B also contained 51mg (90-39mg) of amoxicillin per ml less than the specified lower limit of 90mg/ml (90% of the labelled 100mg/ml).

Discussion

Amoxicillin and other β-lactams have saved many human and animal lives since their discovery in the 1930s. However, the use of poor-quality drugs can lead to poor treatment outcomes, waste of financial resources by prolonging illnesses, increase the potential of recrudescence, and propagate the development of drug resistance [14]. Determination of the content and actual amount (potency) of antibacterial agent in its formulation is indispensable to quality evaluation of antibacterial pharmaceuticals for the reason that it is one of the important predictors of the amount of active ingredient administered that reaches and interacts with the infection site and consequently the infectious bacterial organism [15,16].

Out of the 10 brands of injectable amoxicillin evaluated, only 3 (G, H, and J) contained between 90 and 120% of the manufacturer's labelled amount, therefore conforming with the USP and IP specification [13]. This implies that only 30% (brands G, H, and J) of the commonly used brands of amoxicillin injectable dosage form from multisource evaluated passed the assay quality test and are pharmaceutically equivalent. Thus, for intravenous administration, brands G, H, and J can be substituted with one another without compromising the desired therapeutic outcome. Substandard drugs are produced by legitimate manufacturers, but do not fulfill the manufacturing quality standards or do not contain the correct amount of active ingredient(s). In addition, drugs that have passed their expiration date or that have deteriorated due to improper distribution and/or storage conditions (i.e. degraded medicines) may also be considered substandard, even if they were originally genuine and of good quality [17]. Consequently, our study revealed that 70% (7) of the evaluated brands of amoxicillin injectable form were substandard because they contained the equivalent of less than 90% (n=3) or more than 120% (n=4) of the labeled amount of amoxicillin [13]. The high prevalence of poor-quality injectable amoxicillin formulation from multisource observed in our study corroborates previous studies conducted in Saudi Arabia, Ghana, and Brazil where a larger percentage of the analyzed array of formulations of amoxicillin with or without clavulanic acid failed to meet the pharmacopoeia requirement for good quality pharmaceuticals [18,7,19]. The observed poor quality could be due to poor manufacturing standards, poor packaging, transportation, storage conditions, the nature of the active ingredient, or the distribution system [7,20]. The poor quality of these pharmaceuticals cannot be attributed to expiration of the products evaluated because none of them expired as at the time the assays were conducted. Among other equally important factors, low quality antibiotics is incriminated in the development of antibiotic resistance [21].

Treatment of bacteria infected animals with amoxicillin injectable brands A, B, and C will result to underexposure of the incriminated bacterial organisms to the antibiotic even when the indicated dose is administered, because the content of amoxicillin in these pharmaceuticals were found to be well below their manufacturers' claim as indicated on their respective labels. In addition, the evaluated amount of amoxicillin in these products were well below the lower limit of the range specified in the USP and IP. These sub therapeutic injectable dosage form of amoxicillin from multisource were all imported into Nigeria and indicated for animal use exclusively. The suboptimal dosing of antibiotics, including these brands of amoxicillin have a high propensity of causing the development of bacterial resistant strains, consequently adverse public health implications [22]. Conversely, brands D, E, F, and I contained amoxicillin in excess of the maximum permissible amount of 600mg/vial for a 500mg/vial product. Particularly, the assay revealed that the amount of amoxicillin in brand E was 3.4-fold above the specified maximum limit of 600mg/vial for a vial labelled to have contain 500mg of amoxicillin (120%). The implication is that even when the indicated dose of this product is administered to an animal, it will still amount to overdose and consequently toxicity, and high meat residue in food animals even when slaughtered after the withdrawal period. These could explain the high level of adverse drug events earlier reported in dogs treated with amoxicillin with or without clavulanate [8].

Conclusions

To reasonable extent, this study has demonstrated that there is a high rate of substandard brands of amoxicillin injectable dosage form marketed and used in veterinary practice in Nigeria. This could explain the therapeutic failures, increasing emergence of bacterial resistance from previously susceptible bacterial organisms, and toxicities associated with the use of a good number of amoxicillin brands and other antibacterial agents. Thus, regular adequate quality monitoring of drugs, particularly antibacterial agents will go a long way in improving the health of animals and human, as well as reducing the cost of treatments.

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