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Original Research Article

Physico-chemical properties and antibacterial drug performance of amoxicillin from streets in Bamenda, Cameroon

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ABSTRACT

Background: The advent of microbial drug resistance creates a concern about the quality of anti-infective drugs. The rise in some microbial resistance to therapy has urged us to investigate physico-chemical properties and antibacterial performance of amoxicillin from streets in Bamenda, Cameroon.
Methods: Amoxicillin samples were purchased from street vendors in Bamenda. Cameroon. Visual inspection was done for defects and signs of discoloration, deterioration or other physical disfiguration. Weight uniformity test for each sample of antibiotic tablet was carried out. The time for drug disintegration for each sample of amoxicillin was evaluated. Microbial antibiotic susceptibility tests were done using both the agar disc diffusion and micro well broth dilution methods to check drug performance. Data analysis was done and comparison checked with Student-Newman-Keuls at p<0.05.
Results: The mean weight of amoxicillin drugs ranged from 632.00±11.5 to 748.06±17.9 mg. Tablets from Austria had a significant decrease in weight compared to those from Germany and Nigeria. This was similar with the capsules from India and China. Average disintegration varied significantly from as small as 2.2 to 14.4 minutes. All the amoxicillin samples were active against the tested bacteria with mean zones of inhibitions ranging from 8.33±0.57 to 39.33±0.57 mm. The MICs and MBCs values range from 1 to 64 µg/ml. For the same bacterium the growth inhibitory effects of the various drugs were not all similar.
Conclusions: Some of the amoxicillin samples studied in this paper showed significant differences in their weights and performances (antibacterial activities). Probably the required amount of API was not respected.

Keywords: Antibacterial performance, Amoxicillin, Bamenda-Cameroon, Physico-chemical properties

INTRODUCTION

The advent of microbial drug resistance creates a concern about the quality of anti-infective drugs; especially in areas of endemic diseases.[1] The leading group constitutes antibiotics, due to their high demand globally. The high-volume sales of antibiotics attract manufacturers of "counterfeit" drugs making much profit from these medicines and sometimes at the expense of the population health.[2] It is obvious that use of poor quality drugs can lead to poor treatment outcomes, waste of financial resources due to prolonged illness, increase in the potential of recrudescence, and propagation of drug resistance.[3] It has been observed that very limited scientific investigations have assessed the prevalence of poor quality antibiotics.[4,5] Moreover, in the developing world, antibiotics can be

easily obtained without prescription from pharmacies, drug stores and mobile drug dealers.[6] Amoxicillin is a β-lactam antibiotic with a widespread use in the treatment of bacterial infections. In some parts of the world amoxicillin prescription still being the first choice antibiotic.[7] Although most of the generic drug development are meant to lower public health costs there are still some doubts regarding their qualities and performances compared to branded products.[8] Hence a substantial amount of locally produced amoxicillin among antibiotics do not complying with the U.S. Pharmacopeia (USP). This has been reported in the Middle East and North Africa.[9]

Some microorganisms have been reported to have some resistance to amoxicillin, for example *S. aureus, E. coli*, and *S. pneumonie*.[7,10] Antibiotic resistance is a problem so serious that it threatens the achievements of modern medicine.[1] In the Bamenda community, there are many pharmacies and a great number of drug dealers in streets and local markets. The rise in some microbial resistance to therapy in this area let us to investigate physico-chemical properties and antibacterial performance of amoxicillin from streets in Bamenda, Cameroon.

METHODS

Determination of physico-chemical properties of the various drug samples

Antibiotic drugs

Amoxicillin, commonly used over the counter, antibiotics were purchased from street vendors (five samples each from a different locality) and a reference pharmacy (one sample) in Bamenda, Cameroon.

Visual inspection

The packages of the various antibiotic drugs were examined for defects and signs of discoloration, deterioration or other physical disfiguration. Dates of manufacture and expiration of each sample were checked and recorded as well as the pharmaceutical form of the drug whether tablet or capsule, powder, ointment or suspension.[11]

Weight uniformity test

The test for uniformity of weight for each sample of antibiotic tablet was carried out as described by British pharmacopoeia.[12] The weights of twenty tablets/capsules were individually measured using a Kern ABT 120-40M sensitive electronic balance. The mean standard weight and standard deviation of each tablet/capsule were then determined.[13]

Disintegration test

For each sample of amoxicillin antibiotic, ten tablets/capsules were individually place in separate beakers containing 5 ml of distilled water all placed in a water bath set at a temperature of 37ºC. The time each tablet took to disintegrate completely was noted and the mean time determined for ten tablets/capsules.[13]

Antibiotic susceptibility test

Preparation of antibiotic stock solution and test solution

First a stock solution of the antibiotic was prepared by dissolving a tablet or powder obtained from each capsule of the drug in 4 ml of sterile water; this gave stock solutions varying in concentrations 125 mg/ml of active ingredient as per drug package. The stock solutions were made in higher concentrations to maintain their qualities when stored at -20ºC. Once taken out, they were not refrozen or reused.[14] With a micropipette, 40 µl of the different antibiotic drugs with concentrations of 125,000 µg/µl were pipetted and dissolved in 5 ml of sterile distilled water to give a test solution with a concentration of 1,000 µg/ml.

Preparation of antibiotic disc

Using Whatmann No. 1 filter paper, discs of 6 mm diameter were cut out using a paper puncher. The discs were then separated out in a large size glass Petri dish and sterilized in a 100 0^C hot air oven for one hour. Then cold and dried paper discs were first dipped in the test solution with a concentration of 1,000 µg/ml and air dried. A 6 mm disc absorbs approximately 10 µl of test solution giving it a drug charge of 10 µg.[15]

Microorganisms

Clinical isolates and standard strains of some bacteria were obtained from Pasteur Centre Research Unit Yaounde-Cameroon. They include five Gram negative (*Salmonella typhi* ATCC6539, *Klebsiella pneumonia* ATCC2513883, *Escherichia coli* ATCC28913, *Providencia stuartii*) and four Gram positive (*Staphylococcus aureus* ATCC29213, *Staphylococcus aureus* ATCC1026, *Streptococcus pneumonia, Enterococcus faecalis*, ATCC29212) bacteria. These microorganisms were maintained on nutrient agar during the test period.

Preparation of microbial suspension and inoculum

A flamed sterile wire loop was used to collect a few colonies from 14 hours grown cultures on nutrient agar plates separately and transferred into a corresponding sterile tube containing 5 ml of 0.9% sterile saline water. The microbial suspensions were the vortexed and their turbidity match with that of 0.5 MacFarland standard. This gave us a microbial suspension with approximately 1.5x10¬8 CFU/ml. A quantity of 466 µl of microbial suspension was then pipetted and diluted with 70 ml of 0.9% saline water giving a final microbial load of 1x106 CFU/ml for agar inoculation.[16,17]

Determination of microbial growth inhibition zones

Agar well diffusion method was used for this assay. Agar plates were prepared using Mueller Hinton Agar (MHA) according to the manufacturer's indications. A volume of 100 μl of the inoculum were pipetted and placed on the surface of each agar plate containing and with the aid of a sterile glass spreader rod, the inoculum suspension was evenly spread on the agar surface by streaking. The surface of the seeded plates were allowed to dry for 30 min before a careful positioning of the prepared antibiotic discs (drug charge of 10 μg) on the solidified agar plates using a sterile forcep. The susceptibility plates were then placed at 37°C in incubator for 18 hours. This procedure was repeated for all the samples of amoxicillin antibiotic drugs. After the incubation period, zones of growth inhibition appeared around the antibiotic containing disc for those bacteria that were susceptible to the tested drug. The diameters of zone of inhibition were measured using a ruler held on the back of the inverted Petri plate. Zones were measured to the nearest whole millimeter in a radial manner and their average determined.[18,19]

Determination of minimum inhibitory concentration (MIC)

Briefly, 100 μl of nutrient broth was first introduced into all the wells of the micro-titre plate. Then 100 μl of each antibiotic solution with a concentration of 256 μg/ml was further added into the first three wells of each column successively to give a final volume of 200 μl in first row of wells. The content of these wells was mixed using a pipette and two-fold serial dilution was performed in a consecutive manner from the first well to the last was and the left over thrown away. This exercise gave antibiotic concentrations of 128, 64, 32, 16, 8, 4, 2 and 1 μl/ml in each series of wells. Now 100 μl of broth containing 2×10^6 CFU/ml was then pipetted and added to all the wells of the plate except the first four in the last column which served as a sterility control. The last four wells in that column which were void of antibiotics were inoculated to serve as a positive control. After inoculation, the final antibiotic test concentrations were 64, 32, 16, 8, 4, 2, 1, and 0.5 μg/ml. All the plates were incubated at 37oC for 18 hours.[20] The MICs of each test sample was detected following addition of 50 μl (0.2 mg/ml) p-iodonitrotetrazolium chloride (INT, Sigma-Aldrich, South Africa) solution into all the wells except for those in column 3, 6, 9 and 11 which are further used for the determination of MBCs. Viable bacteria reduced the clear dye to a pink colour. The MIC corresponded to the lowest well concentration where no colour change was observed, indicating no growth of microorganism. All tests were performed in triplicates.[18,19]

Determination of Minimum Bactericidal Concentrations (MBCs)

Immediately after reading the results of the MIC for the various drugs on the different bacteria, 150 μl of freshly prepared Mueller Hinton broth was pipetted into all the wells of new sterile plates. Then 50 μl of broth test mixtures from MIC test that did not receive INT (wells from columns 3, 6, 9 and 11) was then pipetted and added onto the 150 μl broth in the new plate in a sequential manner from the first well in the series right down to the well indicating the MIC value. This was procedure was done in duplicate for all the antibiotic samples and bacteria strains. The plates were then incubated at 370C for 18 hours. The MBCs of each test sample was detected following addition of 50 μl INT into all the wells. The MBC which is the lowest concentration of an antibiotic required to kill a particular bacterium corresponded to the lowest well concentration where no colour change was observed.[21]

Statistical analysis

Statistical evaluation of the data generated was carried out using Statistical Package for Social Science (SPSS) version 20. Multiple comparisons were done using Student-Newman-Keuls at $p < 0.05$.

RESULTS

Physico-chemical properties of the various amoxicillin samples

Visual inspection and categories of samples collected

All the samples of amoxicillin (amox) collected were perfectly packaged and the visible characteristics such as shape, colour, and any evidence of deterioration or other physical disfiguration normally attributed to poor quality drugs were not observed (Table 1).

From the labels, the approximate shelf-life of all the samples was 3 years except for Amoxicillin1 that was having a shelf-life of 4 years.
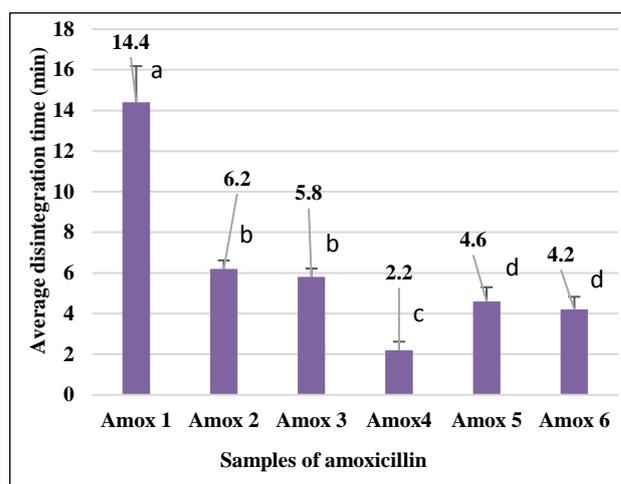
Figure 1: Average disintegration time for the different samples of amoxicillin.

Average weight of antibiotic drug samples

The mean weight of amoxicillin drugs in this study ranged from 632.00±11.5 to 748.06±17.9 mg (Table 2). Within these drug samples, the tablets from Austria had a significant decrease in weight compared to those from Germany and Nigeria. This was similar with the capsules from India and China.

Disintegration time of antibiotic drug samples

The average time each samples of amoxicillin drug took to disintegrate completely in water varied significantly from as small as 2.2 minutes in sample 4 (amox 4), to 14.4 minutes (amox 1) as indicated on Figure 1.

Table 1: Visual inspection features and sources of the various antibiotic drugs sampled.

Sample	Collection point	Country of origin	Expiring date	Shelf-life (years)	Pharmaceutical form	Colour
Amox 1	Licensed pharmacy	Austria	07/2017-07/2021	4	Tablet	Gray
Amox 2	Local market	India	02/2018-01/2021	3	Capsule	Yellow-red
Amox 3	Local market	China	02/2018-01/2021	3	Capsule	White-orange
Amox 4	Local market	Germany	04/2018-04/2021	3	Tablet	White
Amox 5	Local market	China	09/2017-09/2020	3	Capsule	Yellow-red
Amox 6	Local market	Nigeria	01/2018-01/2021	3	Tablet	White

Table 2: Average weights of the various antibiotic drug groups.

Samples (form)	Brand/Country of origin	Average weight±Standard Deviation (mg) N=20	Relative standard deviation (%)
Amox 1 (Tablet)	Amoxycillin/Austria	670.27±2.8[b]	0.4
Amox 2 (Capsule)	Healmoxy/India	684.93±9.7[c]	1.4
Amox 3 (Capsule)	Healmoxy/China	632.00±11.5[a]	1.8
Amox 4 (Tablet)	Amozem/Germany	745.44±8.4[d]	1.1
Amox 5 (Capsule)	Amoxicillin/China	685.58±7.7[c]	1.1
Amox 6 (Tablet)	Amoxicillin/Nigeria	748.06±17.9[d]	2.4

Table 3: Mean diameters (mm) of inhibition zones of the various samples of amoxicillin against test organisms.

Bacteria	Amox 1	Amoxi 2	Amox 3	Amox 4	Amox 5	Amox 6
Staphylococcus aureus ATCC1026	23.00±3.07[a]	14.67±3.02[b]	23.67±1.87[a]	22.00±3.08[a]	15.48±0.76[b]	12.33±2.08[c]
Streptococcus pneumonae	35.67±0.57[a]	37.17±1.67[a]	35.67±1.53[a]	35.33±0.57[a]	34.98±0.65[a]	25.67±0.57[b]
Enterococcus faecalis ATCC29212	39.00±1.00[a]	32.67±0.57[b]	33.00±2.00[b]	32.00±1.00[b]	34.52±0.57[b]	33.00±1.00[b]
Staphylococcus aureus ATCC29213	39.67±0.57[a]	36.33±0.58[b]	32.33±0.33[c]	35.67±0.58[b]	32.18±0.25[c]	29.67±1.53[d]
Salmonella typhi ATCC6539	21.67±1.52[a]	9.33±0.57[d]	17.33±0.58[b]	17.35±0.57[b]	18.53±1.00[b]	12.00±2.65[c]
Klebsiella pneumonae ATCC2513883	24.67±3.33[b]	17.67±2.15[c]	27.68±3.81[a]	20.67±2.51[bc]	20.66±1.82[bc]	19.67±0.57[c]
Escherichia coli ATCC28913	18.67±0.58[a]	10.33±2.08[c]	15.33±1.53[b]	17.67±0.58[a]	14.85±0.57[b]	11.67±1.00[c]
Providencia stuartii	39.33±0.57[a]	38.33±0.57[ab]	35.33±0.57[ab]	33.33±2.51[b]	34.32±0.57[b]	26.67±4.51[c]

Antibacterial activity of amoxicillin samples

Diameter of bacterial growth inhibition

All the amoxicillin samples were active against the tested bacteria with mean zones of inhibitions ranging from 8.33±0.57 to 39.33±0.57 mm (Table 3). Generally, the mean zones of bacterial growth inhibition for the same

bacterium varied significantly for the different amoxicillin samples tested.

MICs/MBCs (µg/ml) of amoxicillin samples

The MICs and MBCs of the studied amoxicillin samples against bacterial growth (Table 4) showed that values range from 1 to 64 µg/ml. For the same bacterium the

growth inhibitory effects of the various drugs were not all similar.

Table 4: Minimal inhibitory concentration (MBC)/minimum bactericidal concentration (MBC) of test substances (µg/ml).

Bacteria	Amox 1	Amoxi 2	Amox 3	Amox 4	Amox 5	Amox 6
Staphylococcus aureus ATCC1026	4/16	8/16	32/>64	4/16	4/16	32/>64
Streptococcus pneumonae	8/16	8/32	16/32	8/16	8/32	16/32
Enterococcus faecalis ATCC29212	4/>64	8/>64	8/>64	4/>64	8/>64	8/>64
Staphylococcus aureus ATCC29213	4/32	8/32	8/32	4/16	4/16	8/32
Salmonella typhi ATCC6539	32/64	>64/>64	32/>64	64/>64	32/64	64/>64
Klebsiella pneumonae ATCC2513883	16/32	>64/>64	64/>64	64/>64	32/64	64/>64
Escherichia coli ATCC28913	32/64	>64/>64	64/>64	64/>64	32/64	64/>64
Providencia stuartii	1/16	2/16	2/16	2/16	1/16	2/16

DISCUSSION

From the average weights of twenty tablets or capsules of the different amoxicillin drugs it was observed that tablets from different brands (Amoxycillin/Austria, Amozem/Germany, and Amoxicillin/Nigeria) had significantly different weights. Since the manufacturers were different but having the same rate of active pharmaceutical ingredient (API) 500 mg, then excipients could account for these differences.

Surprisingly, the capsules from the same brand (manufacturer) i.e. Healmoxy from both India and China though rated 500 mg presented a great significant difference in their weights. If these drugs are all genuine and produced by the same manufacturer then the discrepancy in their weights should not be significantly wide. This could be from the excipient, API or both. The dose uniformity and active ingredient homogeneity in drugs/capsules relate directly to the necessary approval requirements of the average weight assay. Once a known weight difference is distinguished it is impossible to assure the active ingredient content of all capsules.[22,23]

The drug dissolution assay performed on the studied amoxicillin samples showed that they were readily soluble in water, taking at most 14 min to dissolve. It is worth mentioning that Amox 1 was bought from a licensed pharmacy while the other samples were obtained from the streets. According to the US Pharmacopoeia the dissolution time for a good drug should be less than or equal to 30 min.[24] The Brazilian Pharmacopoeia establishes the tolerance limit as not less than 80% of the declared released amount into the dissolution medium in 90 minutes.[25] Judging from this dissolution time assay, one could be tempted to say all these drugs are of quality.

Generally, the amoxicillin sample from the pharmacy (amox1) was more active in inhibiting the growth of the studied bacteria. Be it tablet or capsule what is important is the amount of API (amoxicillin). All the drugs as per their manufacturers were rated 500 mg. It was expected that for the same bacterium their activity against bacterial growth should be similar. However, in many cases it was not so. One could think of a reduction in the quantity of PI in some Amoxicillin samples (brands) could account for a decrease in drug performance (in inhibiting bacterial growth). If poor quality medicines contain less than the intended API, pathogens can become exposed to drug concentrations in the 'mutation selection window'-high enough to exert a selection pressure but too low to kill all of the pathogens.[26] Insufficient amount of API may have as consequence the potential to promote antimicrobial resistance (AMR) and treatment failure, now recognized as a major threat to global public health.[27,28] Studies have shown that substandard antibiotics as well as other essential medicines could be due to either poor manufacturing practices or poor storage and transport conditions or both.[27] Although it was not determined how long the imported samples have been stored under tropical climatic conditions, most of the street medicines under poor conservation methods could have a decrease in performance.

CONCLUSION

The Amoxicillin samples studied in this paper showed significant differences in their weights and performances (antibacterial activities). Samples that showed weak antibacterial activity certainly did not contain the required amount of API. The sources and uses of anti-infective agents should be strictly monitored by appropriate personnel.

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