Bioequivalence of generic and branded amoxicillin capsules in healthy human volunteers

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Abstract:
CONTEXT: The Medical Council of India urges doctors to prescribe generic drugs as far as possible. The Indian Medical Association had responded earlier saying that it requires guarantees on the quality of generic forms of drugs. Although no published scientific reports are available on the issue of therapeutic inequivalence, unconfirmed clinician accounts and newspaper reports of therapeutic inequivalence exist.
AIM: This study was planned to ascertain whether bioequivalence of branded and generic amoxicillin capsule is comparable.
SETTINGS AND DESIGN: An open-label, randomized, single-dose, two-treatment, two-sequence, two-period crossover oral bioequivalence study was conducted in 12 healthy, adult human subjects under fasting condition.
MATERIALS AND METHODS: Serum samples, collected at 8 time points, were analyzed by a validated ultraviolet spectrophotometer method. Pharmacokinetic (PK) parameters such as area under the curve (AUC) \(_{0–t}\), AUC \(_{0–\infty}\), \(C_{\text{max}}\), and \(T_{\text{max}}\) were determined along with time above minimum inhibitory concentration (MIC).
STATISTICAL ANALYSIS USED: The log-transformed PK parameters (\(C_{\text{max}}\), AUC \(_{0–t}\), AUC \(_{0–\infty}\)) were analyzed using a Two One-Sided Test ANOVA in SAS for each parameter. \(T_{\text{max}}\) and MIC were analyzed by Wilcoxon rank-sum test in GraphPad Prism.
RESULTS: Geometric mean ratio of \(C_{\text{max}}\) fell within bioequivalence criteria. The upper and lower confidence limits of both AUC \(_{0–t}\) and AUC \(_{0–\infty}\) geometric mean ratio fell below bioequivalence criteria. Time above MIC of generic preparation was significantly lower than that of branded version.
CONCLUSIONS: The generic capsule was not bioequivalent to the branded amoxicillin capsule.

Keywords:
Amoxicillin, generic, India, therapeutic equivalency

Introduction

In the market, two types of drugs are available, branded generic considered as Branded drugs and unbranded Generic drugs.

A branded drug as per the US Food and Drug Administration (FDA) refers to the original or innovator product that has undergone and passed the rigorous tests and evaluations involved in developing the product. The US FDA also states that a generic drug is the same as a branded drug with respect to conditions of use, active ingredients, route of administration, dosage form, strength, safety, etc. Generic medicinal products are marketed at low cost following patent expiration of the brand name preparations.

In the Indian drug regulations, the terms “branded drug” or “generic drug” have not been defined. The Drug and Cosmetic

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Act 1940 and Rules 1945 is related to quality and sale/manufacturing license of the medicines in India but does not differentiate between generic and branded drugs.\(^2\)

The term “branded drug” broadly encompasses drug formulations manufactured and sold by a company under a popular brand name which is promoted. It does not correspond to innovator drug as in the US.

Generic drugs are manufactured by a pharmaceutical company under a brand name which is not promoted and so not widely known.

Two important factors regarding the use of generic drugs in India are low availability of competitively priced generics to the consumers\(^3\) and the quality of such drugs. The Central Drugs Standard Control Organization has made stringent rules and regulations in bringing out medicines in public use in India, requiring extensive in vitro and in vivo testing before marketing.\(^4\)

Despite these tests and the economic advantages of generic drugs, there is reluctance among doctors to prescribe and patients to use these agents because of perceived lesser efficacy of these drugs. Quality assurance of medicines, whether generic or innovator, is a feature of all procedures and processes involved throughout the production and supply chain. This quality assurance includes the quality of raw materials, excipients, packaging, stability studies, and bioequivalence.\(^5\)

Bioequivalence is important for all drugs and more so for antimicrobial agents. If drugs are found inequivalent, they would be considered as substandard or counterfeit drugs. According to the WHO, up to 10% of the drugs worldwide may be counterfeits with up to 50% of them being antimicrobial agents. In a recent literature review, out of 163 counterfeit antibiotics detected in the world until 2009, 50% were β-lactams with India leading with highest reported counterfeit/substandard antibacterial agents.\(^6\)

Amoxicillin was chosen for this study as it is a widely used and effective agent to treat community-acquired bacterial infections such as respiratory, urinary, ear, and nose.

This study was planned to ascertain whether bioequivalence of branded and generic amoxicillin capsule is comparable.

**Materials and Methods**

The study was conducted after obtaining approval from the institutional ethics committee at a tertiary care teaching hospital of Maharashtra. The study was registered and is under review by the CTRI with reference number REF/2015/09/009837.

This was an open-label, randomized, single-dose, two-treatment, two-sequence, two-period crossover oral bioequivalence study conducted in 12 healthy, adult human subjects under fasting condition.

Male participants, between 18 and 65 years, were screened, and medical history and examination, electrocardiograph, hematology, biochemistry, serology, urine analysis, and chest X-ray were done within 14 days before start of the study. Participants having a history of major illnesses, habituated to tobacco or alcohol, and a history of hypersensitivity to amoxicillin were excluded from the study.

After being given detailed information about the study, the participants were requested to sign an informed consent. Participants were randomized into two groups of six volunteers each, and each group received the two drug treatments at two different times, with a 1-week washout period.

The drugs were administered orally with 250 ml of water, after overnight fasting of 10 h. Venous blood was collected through indwelling catheters at 0, 0.5, 1, 1.5, 2, 4, 6, and 8 h after dosing. Amoxicillin has a half-life (t\(\text{1/2}\)) of 1–1.5 h, 8 time points were selected at 0, 0.5, and 1 covering absorption phase and 1.5, 2, 4, 6, and 8 h covering elimination phase after dosing.

Using a refrigerated centrifuge, blood samples were spun at 3800 ± 20 rpm for 10 min at 10°C ± 2°C to separate serum. The serum samples were then stored upright in a freezer at −80°C ± 2°C until analysis.

Amoxicillin was assayed in serum by an ultraviolet (UV) spectrophotometric method. The method given by El-sayed et al. was modified and adapted to human serum and validated.\(^7\) The method was validated for sensitivity, specificity, linearity, accuracy, and precision. A volume of 0.5 ml of serum sample was mixed with 4 ml of distilled water, and 0.5 ml of 70% perchloric acid was added to precipitate serum protein. The mixture was shaken for 5 min, and then centrifuged for 40 min at 2500 rpm. The supernatant was collected and read at 235 nm.

Pharmacokinetic (PK) parameters such as area under the curve (AUC\(_0\rightarrow t\)) AUC\(_0\rightarrow \infty\), C\(_{\text{max}}\), and T\(_{\text{max}}\) were determined. Time above minimum inhibitory concentration (MIC) was also determined.

Serum concentrations at different times obtained were tabulated in Microsoft Excel. Pharmacokinetic parameters were derived.

The log-transformed PK parameters (C\(_{\text{max}}\), AUC\(_0\rightarrow t\), AUC\(_0\rightarrow \infty\)) were analyzed using a Two One-Sided Test ANOVA in SAS (Statistical Analysis System, is a
software suite developed by SAS Institute for advanced analytics, multivariate analyses, business intelligence, data management, and predictive analytics) for each parameter. \( T_{\text{max}} \) and \( \text{MIC} \) were analyzed by Wilcoxon rank-sum test in GraphPad Prism. The level of significance was set at \( P < 0.05 \).

## Results

Fourteen participants were enrolled for the study after meeting selection criteria and 12 participants completed the study. The overall characteristics of participants are shown in Table 1. Baseline hematology and clinical biochemistry laboratory parameters were within normal limits for all participants.

Standard solutions of amoxicillin trihydrate in blank serum were extracted and run through different wavelengths in the range of 200–320 nm. The maximum absorption was obtained at 235.0 nm. A calibration curve using six standard concentrations was constructed. Linearity was observed in the range of 1–16 \( \mu \text{g/ml} \). A mean accuracy of 97.4% with >98% precision was observed. Limit of quantification was 0.5 \( \mu \text{g/ml} \) with limit of detection at 0.1 \( \mu \text{g/ml} \).

The mean serum profiles for branded and generic amoxicillin are shown in Figure 2. Table 2 shows the summary statistics of the PK parameters including \( C_{\text{max}} \), \( \text{AUC}_{0–t} \) and \( \text{AUC}_{0–\infty} \).

Table 3 shows that confidence intervals of \( C_{\text{max}} \) geometric mean fall within bioequivalence criteria. The confidence intervals of both \( \text{AUC}_{0–t} \) and \( \text{AUC}_{0–\infty} \) geometric mean fell below bioequivalence criteria. Branded drug had a \( T_{\text{max}} \) of 1.57 h and generic drug had a \( T_{\text{max}} \) of 1.64 h. No statistical difference was observed in \( T_{\text{max}} \) of these drugs. Figure 3 shows that the time above MIC of generic preparation was significantly lower than that of branded version.

## Discussion

There have been many doubts on the efficacy of generic drugs, both by doctors and patients.\(^8\) Various anecdotal reports by doctors also testify that a therapeutic inequivalence is seen when generic drugs are compared to their branded counterparts.\(^9\)

In this study, bioequivalence of the formulations was tested in 12 healthy controls. A sample size of 12 was chosen as it provides a power of 80% at \( \alpha = 0.05 \) in two-way crossover studies, which is sufficient for statistical analysis.\(^10\)

A crossover design was adopted as it is the design of choice for bioequivalence trials. An advantage of this design is that it reduces the between-subject variability, because the comparison of different treatments is done on the same participant. This also results in a smaller sample size. Figure 1 shows the flow of study events.

The main disadvantage of this design is carryover effect which can be overcome by giving an adequate washout period in a single-dose study. A washout period of 7 days was given to the participants in this study, which is more than 10 \( t_{1/2} \) of amoxicillin, whose \( t_{1/2} \) is 1–1.5 h. Carryover effects can be detected in a bioequivalence trial, if the predose samples contain any measurable amount of study drug administered in the previous period. No amoxicillin was obtained in the predose samples of period 2 in the present study.

Bioequivalence is the absence of a significant difference in the rate and extent of absorption of drugs under consideration. Bioequivalence testing considers \( C_{\text{max}} \) as this parameter indicates both the rate and extent of absorption. \( \text{AUC}_{0–t} \) and \( \text{AUC}_{0–\infty} \) determine the extent of drug absorption in the body. AUC is dependent on the rate of absorption and rate of elimination of the drug from the body. \( T_{\text{max}} \) along with \( C_{\text{max}} \) determines the rate of absorption.\(^11\)

The goal of equivalence testing is to test whether the 90% confidence interval of geometric mean \( \text{AUC} \) and \( C_{\text{max}} \) ratio of Test (Generic drug) and Reference (Branded drug) is between the bioequivalence interval of 80% and 125% or 0.80 and 1.25. The basis of this range, 80%–125%, is that differences in systemic drug exposure up to 20% are not clinically significant.\(^12\) Log-transformation of \( C_{\text{max}} \) and AUC parameters is required for calculation of the ratios as it normalizes the distribution of data.

The confidence limits of the geometric mean ratio of \( C_{\text{max}} \) fell into the required range of 0.80–1.25, but the confidence limits of the geometric mean of \( \text{AUC}_{0–t} \) and \( \text{AUC}_{0–\infty} \) lay outside the bioequivalence interval, with the lower limit being <0.8 [Table 3].

Coefficients of variation were <5% for \( \text{AUC}_{0–t} \) [Table 3]. Coefficients of variation can reflect within-subject

### Table 1: Participant demographics and physical examination

| Parameter                  | Mean±SD       | Range       |
|----------------------------|---------------|-------------|
| Age (years)                | 32.86±9.16    | 25–52       |
| Height (cm)                | 178.71±4.94   | 165.10–185.42 |
| Weight (kg)                | 64.43±3.74    | 60–72       |
| Heart rate (bpm)           | 67±3.29       | 64–74       |
| Diastolic blood pressure (mmHg) | 80±1.46  | 78–84       |
| Systolic blood pressure (mmHg) | 124±3.76 | 120–130     |
| Temperature, oral (°C)     | 36.4±0.2      | 35.9–36.9   |

SD=Standard deviation
variability. Since it was <5%, it indicated minimal within-subject variability.

AUC\textsubscript{0-\infty} mean values, as shown in Table 2, for reference drug and test drug did not vary much from AUC\textsubscript{0-t} mean values of the respective drugs. Since AUC\textsubscript{0-\infty} indicates area under curve till complete elimination of the drug, this could be because amoxicillin has a short t\textsubscript{1/2} of around 1–1.5 h, and since serum concentrations up to 8 h were included, the drug was almost completely eliminated from the body as shown in Figure 2.
Bioequivalence can be claimed only when the 90% confidence intervals of geometric mean ratios of both $C_{\text{max}}$ and AUC fall within the range of 0.80–1.25. Since the confidence interval of AUC does not fall into this range, the generic formulation cannot be considered as bioequivalent to the branded amoxicillin product.

The mean time point at which maximum serum concentration ($T_{\text{max}}$) was achieved for both drugs did not vary much. Results of $C_{\text{max}}$ and $T_{\text{max}}$ show that rate of absorption of both drugs was similar to each other.

On extensive literature search, few studies comparing generic amoxicillin to branded with varying results were found. In Italy, two generic drugs were compared with the innovator drug and it was found that the generic drugs were bioinequivalent for $C_{\text{max}}$ on the basis of single-dose study. Another study testing generic amoxicillin in Bangladesh found it to be bioequivalent to its branded counterpart. Equivalence was seen in a study comparing the bioequivalence of amoxicillin 500 mg capsules in Brazil and Malaysia.

Amoxicillin is a β-lactam antibiotic and its antibacterial effect is time dependent. To be therapeutically effective, the optimal antibacterial action is required, and the duration for which the drug is above MIC is important. Hence, this parameter was also evaluated, though it is not required for bioequivalence. MIC of amoxicillin is 6.5 μg/ml.

The generic drug remained above this concentration for 48.38 (±9.12) min, whereas for branded drug, it was 56.75 (±5.07) min. When compared to branded drug, the generic drug showed a significant difference ($P < 0.05$) in the duration of concentration of drug over MIC. The difference seen in these results can play a part in reduced efficacy of generic drug as the concentration below the MIC is subtherapeutic and will not inhibit the microorganisms it is directed against.

In the present study, $C_{\text{max}}$ of both the preparations was equivalent. However, $\text{AUC}_{0-t}$, $\text{AUC}_{0-\infty}$, and time above MIC of generic preparation were significantly less than that of the branded one. Due to this inequivalence, the generic drug under consideration would have subtherapeutic efficacy.

An extension to this study was done to ascertain the reason for biological inequivalence, which could be either due to chemical or pharmaceutical factors. Chemical equivalence of these capsules was studied, and the results showed them to be chemically equivalent.

Pharmaceutical factors also affect the bioavailability of drugs. Various factors such as excipients, additives, lubricants, and also pressure required to compress the mixture into capsule form can affect pharmacological equivalence. Further studies are required to study the pharmaceutical equivalence of these drugs in detail.

The use of such inequivalent drugs can result in adverse clinical outcomes such as lack of effect and treatment failure, risk of development of bacterial resistance, toxicity, or side effects, all of which contribute to the burden of disease and consequently to excess mortality and morbidity. Therefore, a strong regulatory mandate is required to reduce the entry of such drugs in the market.

**Conclusion**

In the present study, comparison of generic and branded amoxicillin capsules showed generic capsules’ bio-inequivalent to branded capsule. Duration above MIC of generic drug was also significantly lower than that of branded capsule. This implies that the generic drug could have lower therapeutic efficacy than that of branded drug.
Limitations
The method used for chemical assay and biological assay is noncompendial and done on UV spectrophotometer. High-performance liquid chromatography or liquid chromatography-mass spectrometry (MS)/MS methods are more sensitive and specific and could have been used.

More time points in the elimination phase would have provided more accurate results.

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Conflicts of interest
There are no conflicts of interest.

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