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Quality and bioavailability of ampicillin capsules dispensed in a Nigerian semi-urban community

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Summary
Five samples of ampicillin capsules with a label claim of 250mg were purchased from different dispensing points in a small town in Nigeria. The pharmaceutical quality of the products and a sample from a batch produced by a local manufacturer was evaluated and five of the capsule samples were employed in an *in vivo* bioavailability study. Three of the five capsule samples from dispensing points were found to be of lower quality than the officially prescribed standards of pharmaceutical quality. The quality lapses observed were sufficient to bring about determinable differences in biological availability. The results demonstrate that ampicillin capsules of sub-standard chemical quality are being dispensed within the study sources from authorised and unauthorised sources and that this may have biological, clinical and epidemiological consequences.

Résumé
Cinq specimens d'ampicellin en capsule avec un lebelle suppose etre de 250mg avaient été acheté dans differentes pharmacil dans une petite ville du'Nigeria. La qualite pharmaceutique de ces produits et un specimen provenant d'un batch produit locallement etait evalue et les cinq capsules avaient été utilisé pour une etude de bio avalabilité in vitro. Tros de ces cinq capsules des differentes pharmacies etaient de qualité inferieur que la qualite standard prescript par les pharmacies. Le lapse de qualite observe etait suffosante pour apporter des differences significatives dans la disponibilite biologique. Les resultats montrent que les ampicilin en capsule sont de qualité chimque sous standard et sont vendus dans la region on question par des pharmacies officielles et des boutiques et ceci pourrait avoir des consequences biologiques, cliniques et epidemiologiques.

Introduction

Antibiotics are available in Nigeria and other developing countries with or without prescription from diverse sources [1-4]. This has led to a situation in which their misuse is contributing to the selection and spread of resistant bacteria in these countries at a rate that far exceeds that seen in industralised nations where their use is more controlled [2, 3]. The possibility that undesirable consequences of antibiotic use will occur increases when products of poor quality are dispensed without the cognisance of the prescriber. This is because such antibiotic preparations, by promoting sub-inhibitory concentrations in biological fluids, may in addition to permitting therapeutic failure encourage the selection of resistant strains during therapy [5]. In addition, products that are incompletely absorbed provide suitable conditions for selection of resistant strains in the gut and may cause gastrointestinal side effects.

The current trend in biopharmaceutic research is toward newer antibiotics which are relatively uncompromised by the growing spread of acquired antibiotic resistance. These drugs are however too expensive for the majority of Third World residents who depend on cheap broad spectrum older agents for the first line management of infections [6]. Furthermore, the older drugs are produced by a wider range of manufacturers in both developing and developed countries and so there is a greater likelihood of products of inferior bioavailability being dispensed, especially in developing countries where bioavailability tests are not always required before distribution.

In a study designed to evaluate the bioavailability of tetracycline from capsules dispensed in Ile-Ife, a semi-urban town in Nigeria, it was demonstrated that the products sampled were chemically and biopharmaceutically below the standards expected from the products [3]. The poor quality seen with the products suggested that it was likely that quality lapses made some contribution to the high rates of tetracycline resistance prevalent among isolates within the area. Ampicillin, another agent to which resistance is common, is particularly vulnerable to quality lapses because the drug is heat and moisture labile and previous Nigerian workers have recorded the existence of chemically substandard ampicillin capsules on the Nigerian market [7, 8]. Furthermore, ampicillin, which is incompletely absorbed from the gut and has a very short half life is prone to bioavailability problems [9]. Although a few earlier studies have failed to detect bioequivalence in commercial capsule formulations of ampicillin [10-12], a number of such surveys have revealed that some or all test samples produce blood or urine levels significantly below what is seen with the innovator's product or other reference standards [13-15]. Food and other substances, many of which are peculiar to developing countries and have not been exhaustively evaluated, affect the absorption of ampicillin and related drugs. A recent report describing the negative effects of chewing 'Khat', a traditional stimulant on the bioavailability of ampicillin and the related drug, amoxycillin serves to highlight the problem [16].

Materials and methods

Capsule samples

Seventy ampicillin capsules were purchased from each of five different dispensing points in Ile-Ife, a small town in

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South west Nigeria. All samples were dispensed from large containers containing one thousand capsules. Special permission was obtained to purchase from the hospital which does not dispense to non-patients of the institution. No demands for a prescription were made by the dispensers at the other points. Where information was available, the brand name, place of manufacture, batch number and date of expiry were noted. A sample from a batch was also obtained from a local manufacturer in Lagos (Table 1).

Table 1: Capsule samples employed in the study

Sample code	Source	Place of Manufacture	Months to expiration (at time of study)
A1	Indigenous manufacturer	Lagos, Nigeria	32
A2	Roadside stall I	*	*
A3	Community pharmacy	Nigeria	30
A4	Roadside stall II	India	*
A5	Patent medicine stall	Germany	24
A6	Hospital pharmacy	Germany	24

* *Information not available from source*

Weight uniformity test

The method described in the British Pharmacopoeia [17] was used. Twenty intact capsules were randomly selected from each sample and weighed individually. The difference between the weight of 20 intact capsules and their empty shells was recorded as the capsule content weight. For each sample, the deviation of the weight of the contents of each capsule from the mean capsule weight was computed.

Antibiotic assay

Capsule samples were assayed by a paper-disc diffusion microbiological assay employing *Staphylococcus aureus* NCTC 6571 as test organism. Commercially available standard ampicillin discs (PDM, AB Biodisk, Sweden) were used and the test medium was PDM antibiotic susceptibility testing medium (AB Biodisk, Sweden).

Dissolution tests

The British Pharmacopoeia dissolution apparatus I (for tablets and capsules [17] was used, operating at 100 revolutions per minute. The dissolution medium was 0.1M HCl and the test was conducted at 37C. Five mL samples were withdrawn from the dissolution medium after 5, 10, 15, 20, 30, 45 and 60 minutes, each time being replaced with 5mL of 0.1M HCl. The samples were filtered through Whatmans no 1 filter paper and their absorbance was measured at 268nm. The concentration of ampicillin in each sample was calculated from a Beer-Lambert plot prepared from standard solutions of ampicillin in 0.1M HCl. For each capsule sample, five capsules were tested separately and corrections were made for the absorbance of shell constituents.

Bioavailability study

Eight healthy male student volunteers aged 22-29 years and weighing between 62 and 70 kg took part in the study. They all gave written informed consent at the start of the study. The subjects who had no history of renal, hepatic or cardiac disease were subjected to physical examination by a physician and were deemed to be healthy. Furthermore, none had shown sensitivity to beta-lactam antibiotics and they had not ingested any antimicrobials in the month preceding the study. They were all instructed to abstain from all medications and alcohol during the study.

The five test capsule samples and a reference solution (aqueous ampicillin sodium solution containing the equivalent of 250mg base) were administered to the eight subjects as a single blind, randomized crossover design [18]. The subjects fasted overnight before and three hours after drug administration. Each subject received one capsule with 60mL of water (or 20mL of solution) and 3 hours after dosing, the subjects ate breakfast consisting of 300g of white bread, 5g of margarine, one hard boiled egg and one cup of black tea with sugar. Subjects remained ambulatory for at least 10 hours after dosing. An interval of 48 hours was given after urine collection before the next dosing.

There is documented evidence for the correlation between blood and urinary data for ampicillin in man [19] and therefore, the latter, which can be obtained non-invasively was employed in the generation of biopharmaceutic parameters. Urine was collected just before and then 2, 4, 6, 8, 12 and up to 24 hours after drug administration. The volume and pH of each sample were measured immediately. Above 5 mL aliquots were sterilized by passing through bacteria-proof membrane filters and immediately frozen in sterile capped glass test tubes until analyzed within the space of 2 weeks. The ampicillin content of urine specimens was determined by the paper-disc diffusion microbiological assay used to analyse the capsule samples.

The cumulative excretion of ampicllin by the subjects in 24 hours was determined. Relative bioavailability was determined by comparing the mean cumulative amounts of antibiotic excreted in urine after each of the capsule samples with what was obtained after administration of the solution. The percentage unabsorbed at different time points was calculated from urinary data by the method of Nelson (1960) [20]. The absorption rate constants were computed by linear regression analysis, being the semilogarithm plot of the percentage unabsorbed versus time. Statistical analysis of data was carried out by Analysis of variance and the Wilcoxon's signed rank test at 95% confidence intervals [21].

Results

One of the capsule samples tested did not comply with the BP requirements for uniformity of weight. The range of capsule fill weight for this sample was 321.3 to 434.2mg. All the other capsule samples fulfilled this Pharmacopoeial requirement, although samples A2 and A3 did not meet the minimum assay requirement which requires the range to fall between 92.5 and 107.5 of label content and both capsule samples had mean capsule contents below the minimum

permissible amount [17]. As evident from Table 2, the other samples, even though the mean capsule content was within the stipulated brackets, the lower fiducial assay limit frequently was outside this range, so that even these samples could not be said to have fulfilled the minimum requirement. In the evaluation of the results of this assay however, allowance was made for the fact that the assay procedure used was not the official Pharmacopoeial assay.

Table 2: Properties of capsule samples

Sample code	Weight variation test (BP 1988) 250mg ± SD)	Ampicillin content (Mean % of	Relative bioavailability (%)	Wilcoxon test	Absorption rate constant (hr-1) (Mean ± SD)
A1	Pass	93.67 ± 4.81	79.60	NS	0.64 ± 0.27
A2	Pass	88.79 ± 3.05	72.36	NS	0.45 ± 0.21
A3	Pass	83.94 ± 6.19	67.43	S	0.61 ± 0.26
A4	Fail	93.94 ± 6.62	ND	ND	ND
A5	Pass	96.93 ± 8.22	83.61	NS	0.56 ± 0.37
A6	Pass	99.64 ± 5.63	78.24	NS	0.47 ± 0.27
Reference solution	ND	100	100		0.81 ± 0.65

NS = Not significant; S = significant at P≤ 0.05

The BP 1988 does not require ampicillin capsules to comply with a dissolution assay. The results of the test in this study demonstrated that there was some variation in the performance of the different capsule samples as shown in Figure 1.

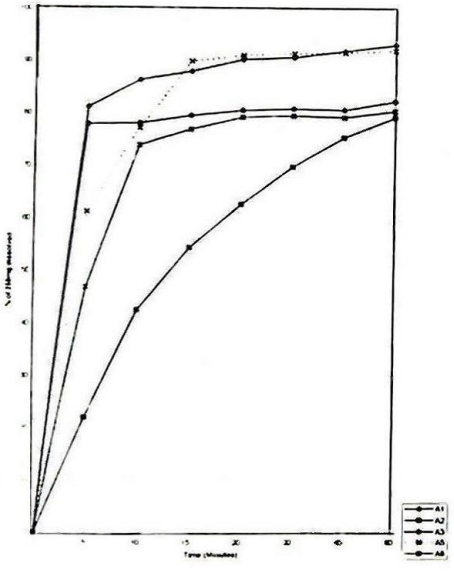

Fig. 1: Dissolution profiles of ampicillin capsule samples.

Considerable lag was observed in the dissolution of all samples except A1 and A3. Particularly noteworthy was the fact that one of the capsule samples, A2, consistently left an undissociated mass at the end of each dissolution test. Microbiological assay revealed that ampicillin content of the mass was 12.76 ± 2.22%.

Table 3: Analysis of variance

Source of variation	Sum of squared deviations	Degrees of freedom	Mean square deviation	Observed f-value	p=0.05
Cumulative amount of ampicillin excreted in urine (mg)					
Among subjects	8,658.63	7	1,236.95	1.84	NS
Among capsule samples	3,175.82	5	635.16	0.94	NS
Residual	23,544.97	35	672.71		
Total	35.379.42	47			
Absorption rate constant of ampicillin (hr^{-1})					
Among subjects	0.525	7	0.075	0.50	NS
Among capsule samples	0.690	5	0.138	0.92	NS
Residual	5.230	35	0.149		
Total	6.445	47			

Analysis of variance of the cumulative amount of ampicillin excreted in urine by the volunteers as well as the absorption rate did not reveal significant differences in these parameters between the capsule samples or the subjects (Table 3). However, as noted in Table 2, analysis of individual results by the Wilcoxon signed rank test demonstrated that the bioavailability of sample A3, as computed from cumulative urinary excretion of ampicillin was significantly below what was obtained with the solution. There was some correlation between cumulative excretion and chemical content of the capsules ($P \leq 0.10$). Although correlation between cumulative excretion and dissolution parameters did not reach statistical significance, there was significant correlation between the amount of drug dissolved at all sampling times in the dissolution test and the absorption rate constant ($P \leq 0.05$).

Discussion

The pharmaceutical quality of most of the capsule samples was below the standards permitted by the British Pharmacopoeia 1988 [17]. The weight variation seen with sample A4 was so wide that no capsule could be considered to be truly representative of the batch. Clinically, this could bring about a significant fluctuation in dose and pharmaceutically, no test carried out on capsules from this sample could be regarded as reliable. As the sample was purchased from a roadside stall of the type where different batches, brands and occasionally drugs are often stored mixed together in a single container, it is not unlikely that the defect originated at the dispensing point. All the capsules were identical in appearance and no sub-batches could be distinguished, therefore, the sample was not employed in further tests.

The failure of two samples to comply with the BP 1988 content requirement was a further lapse in quality detected during the study that could have clinical consequences. Previous reports of amino-penicillins, which did not comply with pharmacopoeial requirements being marketed in Nigeria, have been documented and the current study demonstrates that the condition has remained unchanged [7, 8]. The capsule content for A1, a freshly prepared batch obtained directly from the manufacturers was 93.7% of label claim, barely above the BP1988 minimum limit of 92.5% and

indeed the assay range was outside the permitted boundary. It is doubtful if such a borderline preparation will maintain its Pharmacopoeial acceptability throughout the life-span of the product [22] particularly if it is stored at ambient tropical temperature and humidity for extended periods. The adverse consequences of the release of borderline products by manufacturers' are likely to be augmented by inappropriate post manufacture handling by distributors and dispensers resulting in further loss of content by deterioration.

Although the BP 1988 does not require dissolution tests for ampicillin capsules, the USP 1985 monograph [23] requires such a test and these tests are often carried out as part of a complete quality assurance protocol. One of the test samples in this study consistently produced an undissociated mass during the test in which was trapped a substantial portion of the drug. Compact masses of this nature are known to form when there is too much powder in the shell or if the formulation is stored under excessively humid conditions [24]. With respect to pharmaceutical quality therefore, only samples A1, A5 and A6 could be said to be acceptable. The samples, which were found to be of poor pharmaceutical quality, in addition to capsules purchased from road-side and patent medicine stalls, included the sample from a community pharmacy. Similar observations were made for tetracycline capsules in a previous study. As noted by Chidomere (1991)[25], even though they have specialized training, pharmacists in developing countries often lack access to quality control facilities. Since they often obtain their drugs from the same dubious sources as unauthorized distributors, purchasing drugs from authorized sources provides no assurance of quality as has been demonstrated in this case.

Statistical analysis by the Wilcoxon test demonstrated that one of the capsule samples (A3) produced a bioavailable amount significantly below what was seen with the solution . This sample also had the lowest ampicillin content of all the samples. Although absorption from solutions is not hampered by formulation factors that may affect availability from capsule formulations, the fact that this sample was the only one that gave results significantly less than did the solution, suggests that it is inferior to the other products in this respect. It is interesting to note that this sample released more drug in the dissolution test than two other formulations and this test cannot therefore be considered to be indicative of bioavailability in this instance. This is borne out by the low levels of correlation seen between cumulative excretion and dissolution data as well as by the fact that the bioavailability from most of the capsule formulations compared favorably with the results obtained with a solution for which a preliminary dissolution step is not required. By contrast, there was some correlation between cumulative excretion and chemical content of the capsules ($P \leq 0.10$). This is in line with the current position that bioequivalence can only be attributed to chemically equivalent products. Furthermore, it suggests that if chemical inequivalence can be overcome, bioinequivalence is unlikely to pose a problem with ampicillin capsules in this environment.

The differences in absorption rate constant between the different capsule samples and the reference solution observed in this study did not reach statistical significance. There was however good correlation between absorption rate and dissolution. This has been reported previously by Andrade *et al.* (1978) [11]. The correlation seen in this study, taking into cognizance the slow-to-peak dissolution profiles, suggest that dissolution may be a determinant factor in the absorption rate of the drug from formulations.

The samples that failed the BP 1988 assay requirement showed the lowest relative bioavailability. Above the BP 1988 limit however, chemical content was no indicator of bioavailability. It is pertinent to note that all the lapses in biological availability that were seen in this study could be attributed to poor pharmaceutical quality. The significant difference in the extent of absorption seen with sample A3 was likely to have been due to its low content of active drug while the decreased absorption rate from sample A2 could be attributed to its poor dissolution profiles. As both shortfalls could be detected employing *in vitro* tests prescribed in official compendia, there is no excuse for the dispensing of such products.

There have been reports of 'counterfeit' drugs being placed on the market in Nigeria and other Third World markets. It is unlikely that any of the substandard samples detected in this study fall into this category since they all contained significant proportions of the active ingredient. If it is to be presumed that all products met the minimum standard requirements when they were placed on the market by manufacturers, the results of this study suggest that these minimum standards are not sufficiently stringent to ensure the quality of the product is maintained throughout its shelf life when such a product is likely to be dispensed at points such as those sampled in this study. This is because the deleterious post-manufacturing handling that drugs are often subjected to in the tropical climate of Nigeria and other developing countries, makes it possible that heat- and moisture-labile drugs like ampicillin will deteriorate significantly before expiration. When products, such as sample A1, leave manufacturers premises barely fulfilling the minimum standards required to be present at use, it becomes even more likely that products at dispensing points will fail to meet these criteria. Even more worrisome is the fact that the products still had over two years of permitted shelf life, during which further breakdown could occur.

The mishandling of drugs by distributors makes it even more likely that drugs will deteriorate before consumption. The matter is not helped by the fact that a number of distributors (such as those in charge of patent medicine and road-side stalls) are not authorised to dispense antibiotics and lack the knowledge and special facilities for their correct storage. This results in mixed batches (such as A4), making drug recall totally impossible. However, as demonstrable from sample A3, purchasing the drug from an authorised source gives no assurance of quality. Furthermore, with the exception of the hospital pharmacy, none of the dispensers demanded a prescription or even inquired as to the prospective use for the large amount of capsules purchased.

In conclusion, the results of this study have demonstrated that biopharmaceutically inferior samples of ampicillin capsules are being routinely dispensed from authorised and unauthorised points in a Nigerian sub-urban town. The shortfalls in biological availability that were detected in the study were apparently a direct consequence of poor pharmaceutical quality that could have been the result of inappropriate handling of the drugs during distribution. The adverse consequences of such actions include therapeutic failure as well as the exacerbation of the already appalling situation of antibiotic resistance within the study area. It is recommended that both manufacturers and distributors take into cognisance the possibility of degradation of this and

similar drugs and take preventive measures to ensure that drugs of a acceptable quality are dispensed to patients. There is also need for regulatory control of the distribution process and monitoring of products at the dispensing point.

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