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Comparative Quality Assessment of six commercial Brands of Metronidazole Infusions Sold in Abuja, Nigeria

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Abstract

Influx of substandard drugs as reported by the drug regulatory agency in Nigeria has necessitated the need to assess the quality of frequently used drugs of which metronidazole infusion is not exempted. Metronidazole infusion is mostly prescribed for pre and post-operative surgery to prevent infections from developing. This study was carried out to assess the quality of six commercial brands of metronidazole infusions sold in Abuja, Nigeria. Six brands of metronidazole infusions were randomly purchased from some Pharmacy stores in Abuja for chemical and microbiological assays. Five (83.3%) of the samples had percentage contents ranging from 95-110% which is within BP specification. The pH values of the whole samples were within BP specification of 4.5 to 6.0 which range from 4.9 to 5.8. The result of the microbiological analysis indicated zero colony forming unit (cfu) from all the samples, this is in conformity with official specification for parenteral formulations. Similarly, all the brands passed pyrogen test. Based on the percentage content result, five brands of the samples analyzed have been found to be fit for human use while one failed.

Keywords: Metronidazole, Infusions, Percentage content, Quality

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INTRODUCTION

Metronidazole is an antimicrobial that belongs to a class of drugs called nitroimidazole. Its parent compound is known as 5-Nitroimidazole with general formula of $O_2NC_3H_2N_2H$. The nitro group at position 5 on the imidazole ring is the most common positional isomer. Metronidazole is used for both pre and post-surgical cases especially in gastrointestinal and gynecological conditions to prevent infection [1,2]

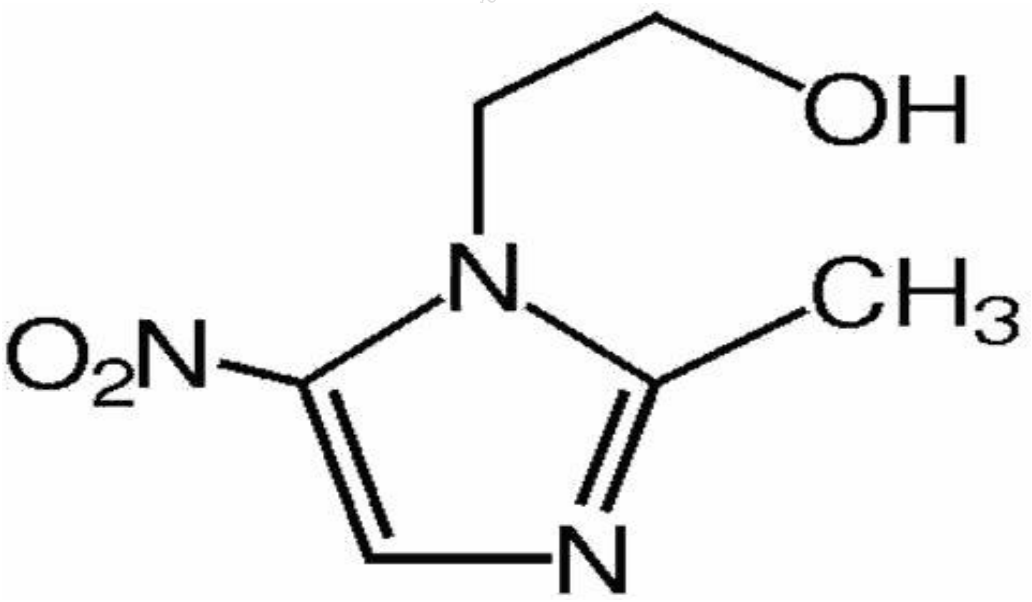

Fig 1. Structure of metronidazole: 1-(2-hydroxyethyl)-2-methyl-5-nitroimidazole

Metronidazole is an antibiotic and antiprotozoal agent [3]. It is used either alone or with other antibiotics to treat pelvic inflammatory disease, endocarditis, and bacterial vaginosis. It is effective for dracunculiasis, giardiasis, trichomoniasis, and amebiasis. It is the drug of

choice for a first episode of mild-to-moderate

Metronidazole exerts antibacterial effects in an anaerobic environment against most obligate anaerobes. Metronidazole is ineffective against both aerobic and facultative anaerobic bacteria. Once metronidazole enters the organism by passive diffusion and activated in the cytoplasm of susceptible anaerobic bacteria, it is reduced; this process includes intracellular electron transport proteins such as ferredoxin, transfer of an electron to the nitro group of the metronidazole, and formation of a short-lived nitroso free radical. The alteration of the metronidazole molecule, creates a concentration gradient that is maintained to promote the drug's intracellular transport. The reduced form of metronidazole and free radicals can interact with DNA results in inhibition of DNA synthesis and degradation leading to death of the bacteria [5].

The use of alcohol with metronidazole can cause psychotic reactions. These symptoms include confusion, hallucinations or delusions. Metronidazole should not be taken within 2 weeks of taken disulfiram [6]. Lithium

Clostridium difficile in colitis [3,4].

potentiates the side effects of metronidazole resulting in higher risk of QT interval prolongation leading to irregular heartbeat [7]. The use of metronidazole with warfarin or other blood thinners can lead to higher risk of bleeding [4]. Metronidazole is available as intravenous infusion, oral and topical formulations. Intravenous therapy may be used to correct electrolyte imbalances, to deliver medications, for blood transfusion or as fluid replacement to correct, for example, dehydration. Intravenous therapy could also be used for chemotherapy [8]. The bioavailability of the medication is 100% in IV therapy that is, absolute bioavailability compares the bioavailability of the active drug in systemic circulation following other routes of administration [9]. Substandard/or counterfeit drugs has variable bioavailability leading to either treatment failure or organ damage [10].

Pyrogen tests, Rabbit Pyrogen Test (RPT)) is one of the safety tests [11]. Rabbits are used because their body temperature increase when

pyrogen is introduced by parenteral routes. Parenteral pharmaceutical preparations must be free of pyrogenic contamination as these substances can induce a life-threatening systemic inflammation in the recipient [11]. A pyrogen is any substance, products of growth of microorganisms or parts of dead cells or metabolic products that cause a fever when administered into the body. Its sources include equipment, solvents, packaging materials, washing/rinsing tubing, excipients, solute, environment etc. A pyrogen cause induction of a febrile response (elevation of body temperature, fever) which can be fatal in humans and animals. Pyrogens are classified into two groups; endotoxins, originating from Gram-negative bacteria and non-endotoxin pyrogens (NEPs), which originate from Gram-positive bacteria, viruses or fungi. Basically, there are two widely used methods for testing the presence of pyrogens in products; the rabbit pyrogen test (RPT) and the bacterial endotoxin test (BET) based on Limulus Amebocyte Lysate (LAL). However, an alternative *in vitro* pyrogen test has been developed in recent years, the Monocyte Activation Test (MAT), has been developed to detect and quantify endotoxin and NEP contaminations [12, 13]. The assay measured cytokine production of human peripheral blood mononuclear cells (PBMC). This test was performed to see the presence or absence of pyrogens in all aqueous parenteral preparations [13].

The aim of the study was to assess the chemical content and microbial quality of metronidazole present in six commercial brands of metronidazole infusions from different pharmacy outlets within Abuja. Drug faking or counterfeiting possess great threats to public health and the economy of the country. It is an ill wind that blows nobody good [8, 14]. A study conducted by Poole in Nigeria in 1989 indicated that 25% of samples studied were fake, 25% genuine and 50% inconclusive. The National Agency for food and Drug Administration and Control (NAFDAC) reported that 13-15% of drugs in Nigeria are substandard or counterfeit [23].

MATERIALS AND METHODS

Sample Collection

Six brands of metronidazole 500 mg/100 mL solution for infusions were purchased from different pharmacy outlets within Abuja. Each brand was assessed for the presence of National Agency for Food and Drug Administration (NAFDAC) number, manufacturing date, expiry date and batch number. The standard metronidazole was obtained from Sigma Aldrich chemical. The samples were coded and the samples were analyzed before the expiration dates

Chemicals, Reagents and Equipments

Metronidazole Reference Standard (RS) (Sigma Chemical), hydrochloric acid (Sigma-Aldrich), buffer Tablets 4.0, 7.0 and 9.2, distilled water, volumetric flasks, measuring cylinders, beakers, FTIR (Thermo Scientific Nicolet), Jenway UV-Vis Spectrophotometer (6065), Analytical plus weighing balance (Ohaus AP250D), pH meter (Eco Tester) Ultra-sonicator (Bransol), Test tubes, Curvets(1cm), Tryptone soy agar, Incubator, Autoclave (280E), clinical thermometer, Micro pipette, Petri dishes, sterile membrane filters (0.45 µm), membrane filtration unit.

Media

Soybean Casein digest broth (SCDB) by Oxoid, USA; Mueller Hinton Agar by Oxoid, USA; Nutrient Agar by Oxoid, USA. All the media were prepared according to Manufacturers recommendation.

METHODS

Identification

Identification test for Metronidazole infusion was done based on BP 2016. 20 ml of Metronidazole infusion measured and 9 g sodium chloride was transferred into separating funnel and shake for 5 minutes. Then, 20 ml of acetone was added to it and the mixture was allowed to separate. The upper layer was evaporated to dryness in an evaporating dish and 10 mg of the sample was taken in KBr plate to FTIR instrument for the identification test [15].

Assay

A 10 ml infusions equivalent to 50 mg of metronidazole was pipetted in to a 100 ml volumetric flask and made up to volume with 0.1M Hydrochloric acid. From the resulting solution, 10 ml was pipetted in to a 250 ml volumetric flask and made up to volume with 0.1M hydrochloric acid. The absorbance of the final solution was taken at the maximum wavelength of 277 nm according to British Pharmacopeia 2016 specification. The content of metronidazole, $C_6H_9N_3O_3$ present in each sample was calculated taking 375 as the value of A (1%, 1 cm) at maximum at 277 nm [15].

pH determination

The pH meter was calibrated using buffers 4.0, 7.0 and 9.2 which were prepared with standard buffer tablets. The pH of the samples were measured.

Microbial analysis

The membrane filtration method according to United State Pharmacopoeia was employed to determine the sterility of the samples [1]. The surface of the metronidazole infusion bottles containing the sample was disinfected with 70 % ethanol before transferring to the biosafety cabinet. A sterile 0.45 µm (pore size) membrane filter was placed in its proper place in the membrane filtration unit using sterile forceps. Fifteen milliliters of sterile tryptic soy broth was used to wet the membrane filter. Thereafter, the whole content (100 mL) of the infusion was transferred aseptically through the membrane filter (one membrane filter per bottle) for vacuum filtration. The filtration system was rinsed thrice with sterile Tryptic soy broth after each session to neutralize and wash the membrane. At the end of filtration, the membrane filter was carefully lifted with sterile forceps and placed in SCDB and incubated at 20 - 25^{o}C respectively aerobically for 14 days. The incubated medium was observed for turbidity daily and recorded accordingly.

Pyrogen Test

Experimental Animals

Three healthy adult rabbits of both sexes and same variety weighing not less than 1.5 Kg were

used for each of the six brands of metronidazole infusion samples. The animals were housed individually in an area of uniform temperature and maintained in a 12 hours light/dark cycle in an air-conditioned room with a temperature less than 20 ºC with free access to water and food ad libitum. The animals were fasted prior to the test. Animals that showed temperature variance of 0.6°C or a baseline temperature greater than 39.8°C were not used for the test [11].

Fresh animals were used for the study. The animals were conditioned for three days. Food was withheld from the animals 2 hours before the commencement of the study but access to water was allowed. A baseline temperature of the animals was measured in duplicate using a clinical thermometer inserted into the rectum of the rabbits to a depth of not less than 5 cm and the mean was calculated. After 90 minutes, a given volume of each brand of metronidazole infusion was administered intravenously (IV) through the marginal ear vein at a dose of 0.5 mL/kg body weight. The temperature of the animals after the intravenous injection was measured and recorded at an interval of 30 minutes and continued for 3 hours to obtain six different readings. The response of the rabbit to the sample was determined by subtracting the mean of the baseline temperature from the maximum temperature after administration of the sample [11].

RESULTS AND DISCUSSION

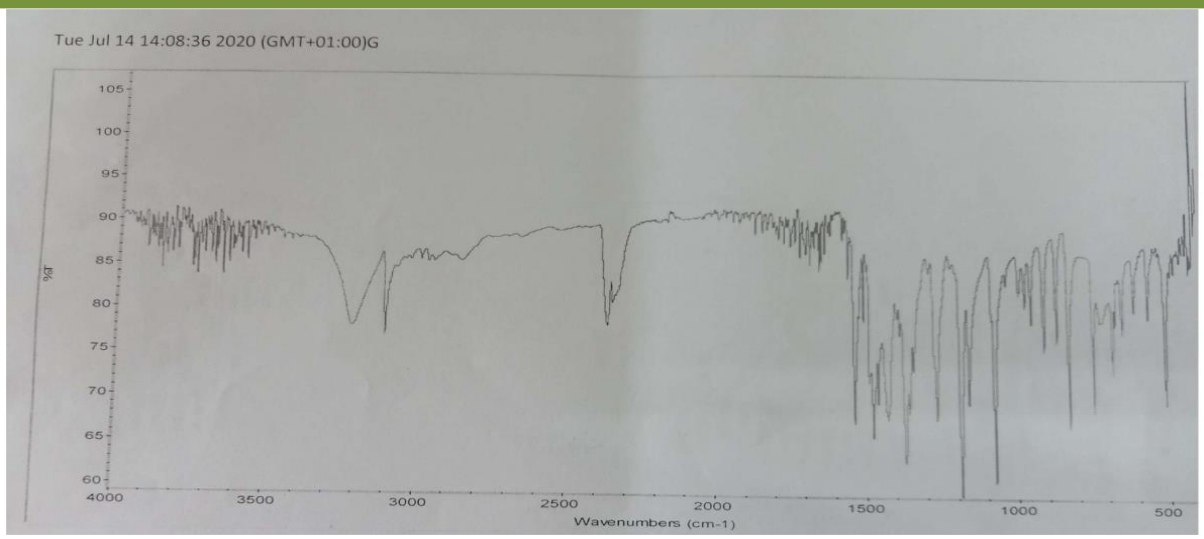

Figure 1. FITR Spectrum of metronidazole reference standard

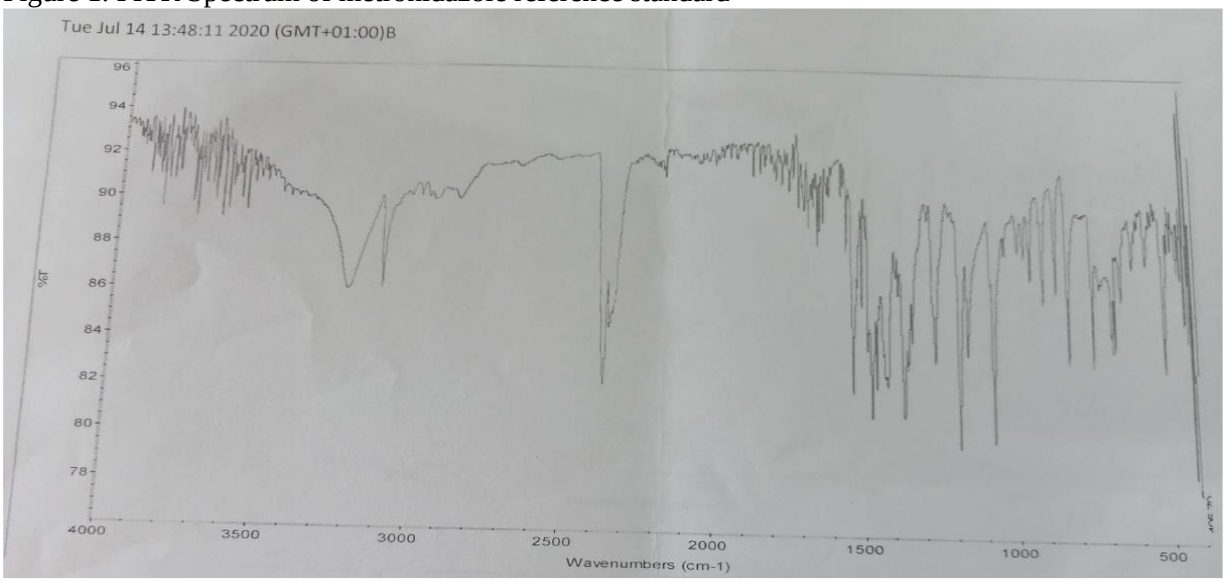

Figure 2. Representative FITR Spectrum of metronidazole in the sampled products

Table 1.0: Mean Percentage Content, Standard Deviation, pH and Sterility Test Results (n =3)

Sample code	Amount declared (mg/ml)	Amount found (mg/ml)	Mean % content (%)	**Std dev**	**pH value**	**Microbial Sterility**	**Remarks**
MetA	5.0	5.6	112	±26.0	5.3	Absence of microbial growth	Failed % content
MetB	5.0	4.8	95	±4.0	5.6	Absence of microbial growth	Passed
MetC	5.0	4.9	98	±8.0	4.9	Absence of microbial growth	Passed
MetD	5.0	5.1	101	±6.0	5.8	Absence of microbial growth	Passed
MetE	5.0	5.0	100	±6.0	5.4	Absence of microbial growth	Passed
MetF	5.0	5.0	100	±11.0	4.9	Absence of microbial growth	Passed

Table 2.0 Pyrogen Test Results

S/No	Sample code	Rabbit Response (˚C)			Remarks
		1st	2nd	3rd	
1	MetA	0.10	0.35	0.45	Passed
2	MetB	0.00	0.50	0.00	Passed
3	MetC	0.00	0.00	0.30	Passed
4	MetD	0.00	0.00	0.00	Passed
5	MetE	0.00	0.25	0.55	Passed
6	MetF	0.05	0.10	0.00	Passed

The identification test for all the six brands was carried out according to British Pharmacopeia [15] method for identification of the metronidazole. The FTIR spectrums of the standard and the samples were similar, indicating the standard and the six brands of metronidazole analyzed in the present study have similar infrared (IR) spectrum absorption frequency, which in turn shows identity of Active Pharmaceutical Ingredients (API) of interest in the samples as shown in Figure 1 and 2. Accordingly, all the brands passed the identification test. Identification tests performed on Metronidazole in various countries demonstrate mixed results. The present study is in agreement with reports of identification test conducted in South West, Ethiopia, Zaria, Nigeria and Addis Ababa [17, 18, 19] in which all the brands analyzed passed the identification test. Contrary to this finding was the report of Taylor *et al*, [20] in a study carried out in Nigeria on 581 drugs, in which 5 of the samples are Metronidazole suspensions and 36 of the samples are Metronidazole tablets in which all the Metronidazole suspensions in the study failed identification test [20].

From the results obtained, the mean % content of samples MetA was 112%, more than the

specification of 95 to 110% [1], while the remaining samples MetB, MetC, MetD, MetE and MetF have mean % content of 95, 98, 101, 100 and 100% respectively. Comparing sample metA with other samples in respect to manufacturing date and expiry date. It can be deduced that 16.7% of the samples have excess amount of the active pharmaceutical ingredient (metronidazole) while 83.3% samples have the right amount as claimed by the manufacturers. The presence of excess or lower amount of API in a formulation can lead to severe adverse effects like liver toxicity, kidney failure and treatment failure and drug resistance respectively.

It was observed that the pH values of all the samples were within the specification of 4.5 to 6.0. (Table 1). Also, the microbial content test (MCTA) shows zero colony forming units (cfu) from all the samples, this is in conformity with standard operating procedure (SOP) for parenteral formulations. Microbial quality of the sterile dosage forms should be maintained in official specifications throughout the shelf life of the drug for the formulation to be stable and therapeutically effective. The results of our study agreed with the study by Angus, *et al* in which over 70% of the metronidazole infusion tested passed [22]. The pyrogen test was conducted according to Chul-Yong Park *et al* method [11]. The results of the samples analyzed were shown on Table 2, all the six brands of metronidazole infusions passed the test for pyrogen. Samples are considered to have passed, if the sum of the responses of three rabbits does not exceed 1.4 ˚C and none of the rabbit shows a temperature responses greater than 0.6˚C.

CONCLUSION

This study revealed that there are some incidences of counterfeit in one of the Metronidazole infusion in Abuja, thus, there is need for routine quality checks of pharmaceuticals so as to safeguard the health of Nigerian populace.

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