RE-Appraisal of Quality of Available Commercial Tetracycline Sold in Metropolitan Kano

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Abstract
The chemotherapeutic role of tetracycline as a broad-spectrum antibacterial and even antiprotozoal has been utilized for the past sixty years now. Its promise potentials made industrial manufacturers and its commercialization a versatile business. This antibiotic was commercially obtained in two formulation or brands. Tetravine and Lono, 25mcg/ml of each brand was obtained from 250mg/ml or 250,000mcg/ml from prepared antibiotic sensitivity disk. Antimicrobial susceptibility test of each exhibited different levels of antimicrobial activities based on their inhibitions and standard control antibiotics, tetravine showed zone of inhibition against Escherichia coli, Pseudomonas aeruginosa, Streptococcus pyogenes and Klebsiella pneumoniae as 11mm, 6mm, 16mm and 17mm with standard control as 15mm, 9mm, 20mm and 17mm respectively. Lono also showed its activities against E. coli, P. aeruginosa, S. pyogenes and K. pneumoniae as 17mm, 11mm, 9mm, 12mm with standard control as 20mm, 16mm, 12mm, and 18mm respectively. E. coli and S. pyogenes were highly susceptible while P. aeruginosa and K. pneumoniae were moderately susceptible, indicating that tetracycline was effective against enteric and respiratory tract infections.

Keywords: Tetracycline, antimicrobial quality, potency, Kano.
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Introduction
Antibiotics are specific chemical compounds derived from or produced by micro-organisms that even in small amounts can selectively inhibit the life processes or growth of other micro-organisms (Pelczar et al., 1993). Tetracycline is a yellow, odorless, crystalline powder. It is stable in air but exposure to strong sunlight causes it to darken. Its potency is affected in solutions of pH below 2 and is rapidly destroyed by alkali hydroxide solutions. (Alekshum et al., 2007). Tetracycline is very slightly soluble in water, freely soluble in dilute acid and in alkali hydroxide solutions, sparingly soluble in alcohol, and practically insoluble in chloroform and in ether. Each capsule for oral administration contains 250mg or 500mg tetracycline hydrochloride. In addition, each capsule contains the following inactive ingredients. It contains colloidal silicum dioxide, pregelatinized starch and stearic acid. (Klain and Rafal, 2007).

Tetracyclines are primarily bacteriostatic and exert their antimicrobial effect by the inhibition of protein synthesis. Tetracycline is active against a wide range of Gram-negative bacteria (E. coli, Shigella species, Bacteroides species, Vibrio cholerae, Haemophilus influenzae, Klebsiella species, Enterobacter aerogenes, Brucella species), and Gram-positive organisms (S. pyogenes, S. pneumoniae, Enterococcus species) as well as other organisms such as Chlamydia trachomatis (Johns–Hopkine, 2001). A tetracycline disk may be used to determine microbial susceptibility to drugs in the tetracycline class. If the Kirby-Bauer method of disk susceptibility testing is used, a 30mcg tetracycline disk could give a zone of at least 19mm when tested against a tetracycline susceptible bacterial strain (Lippin-Cott et al., 2009).

The chemotherapeutic role of tetracycline as a broad spectrum antibacterial and even antiprotozoal has been utilized for the past sixty years now. That promise made industrial manufacturer and its commercialization a versatile business. As the result of that, issues surrounding the faking of the drug
are now high, for this fact, the possibility of adulterated brands is great in the country.

It is therefore the objective of the present study to assess the quality of two brands of tetracycline (Tetravine and Lono) as this is part of the WHO and NAFDAC protocol to ensure a regular surveillance of these kinds of drugs (Alekshum et al., 2007).

Materials and Methods
Test micro-organisms
Clinical isolates of the test microorganisms were obtained from the Microbiology laboratory of the Department of Biological Sciences, Bayero University Kano and Aminu Kano Teaching Hospital, Kano, Nigeria.

Standard Assayed antibiotics
The test antibiotics used were two brands of tetracycline (Tetravine and Lono) purchased from reputable pharmacy store located within Kano metropolis. Standard antibiotic sensitivity disks were also purchased from scientific supply stores in the Kano metropolis.

Preparation of the antibiotic concentrations
Filter papers (Whatman No. 1) were obtained and disks of 6mm each were punched using standard methods. These were wrapped in foil paper and sterilized in the oven at 160°C for four hours. The sensitivity disks were prepared according to NCCLS (1999) subcommittee these standards to contain the concentrations 25mcg equivalent to the standard and the standard commercial disks with concentration was 25mcg. The two brands were diluted to obtain the concentrations of the commercial standard disks using sterile distilled water. To arrive at 25mcg from 250mcg of each brand of tetracycline, 250mg was converted to 250,000mcg, by dissolving in 10ml of sterile distilled water, 25,000mcg was obtained, and a 1:10 dilution was carried out to give concentration of 2,500mcg. The 100 sensitivity disks already sterilized were put into the above solutions. Each disk absorbed 25mcg and was the required standard commercial concentration.

Sensitivity testing
Preparation of the growth medium
A quantity (12.5g) of Muller – Hinton agar was weighed and dissolved in 320 ml of distilled water. This was autoclaved at 121°C for 15mins at 15 lb pressure. After autoclaving was brought and of about 15mins, was poured into each sterile Petri dish of about 16 plates and allowed to set for inoculation.

Inoculum preparation and standardisation
The clinical isolates were subcultured onto nutrient agar slants. Peptone agar broth was prepared and the different identified isolates were inoculated into the broths, incubated at 35°C. A turbidometer was used to monitor the turbidity of the broth cultures. Immediately, the turbidity exceeded the barium sulphate standard, the incubation was stopped. Inoculation of the test plates within 20 minutes of the growth reaching final turbidity, each of the isolates was inoculated into a different Mueller-Hinton agar plates of 16 by spread plate method (Chesebrough, 2000).

Preparation of the sensitivity disks
The method described by Praif and Fekeyy (1986) was used. Sterilized filter paper disks (6.0 mm diameter each) were impregnated with the dilution of 25mcg of the test antibiotics in duplicates. With the aid of a sterile forceps, the impregnated disks were carefully placed on the inoculated plates and firmly pressed onto the agar with the sterile forceps to ensure complete contact with the agar. The disk was distributed evenly at 20mm distance and in manner as to be no closer than 10mm from the edge of the Petri-dish. The standard antibiotic disks as control were also placed on separate plates seeded with the test organisms. The plates were covered with tops, inverted and incubated immediately at 37°C for 24 hours. The standard positive commercial disks included gram-negative, gram-positive and broad spectrum disks, while the negative
control disks were impregnated with sterile distilled water. For *S. pyogenes*, Mueller Hinton agar with 5% sheep blood was used. After incubation, the zones of clearance of organisms around the disks were measured and recorded.

**Results**

The microbiological characteristics of the test organisms are presented in Table 1. The isolates were confirmed to be *S. pyogenes*, *E. coli*, *P. aeruginosa* and *K. pneumoniae*. The results of antibiotic susceptibility tests are presented in Table 2. From Table 2, however, significant differences were macroscopically observed between the zones of inhibition of the two brands of tetracycline and their standard control antibiotics against the test organisms. Tetracycline Lono showed higher zone of inhibition against *K. pneumoniae*, *P. aeruginosa* and *E. coli* than that of tetravine, while the tetracycline tetravine showed higher zones of inhibition against *S. pyogenes* than lono. From the same Table 2, both brands showed high susceptibility against *E. coli* and *S. pyogenes* and low susceptibility against *P. aeruginosa* and *K. pneumoniae*.

### Table 1: Morphological and biochemical characteristics of the test bacteria

<table>
<thead>
<tr>
<th>Isolate No.</th>
<th>Gram reaction</th>
<th>Mobility</th>
<th>Glucose</th>
<th>Serose</th>
<th>Lactose</th>
<th>Malose</th>
<th>Catalase</th>
<th>Oxidase</th>
<th>Citrate</th>
<th>Urease</th>
<th>MR</th>
<th>UP</th>
<th>Indole</th>
<th>Bacitracin</th>
<th>Confirmed organisms</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
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<td></td>
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<td></td>
<td></td>
<td><em>E. coli</em></td>
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<td>2</td>
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<td></td>
<td></td>
<td><em>K. pneumoniae</em></td>
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<td>3</td>
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<td></td>
<td></td>
<td><em>P. aeruginosa</em></td>
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<td>4</td>
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<td></td>
<td></td>
<td><em>S. pyogenes</em></td>
</tr>
</tbody>
</table>

Key: + = Positive  
- = Negative

### Table 2: Susceptibility profile of the bacterial isolates

<table>
<thead>
<tr>
<th>Isolates</th>
<th>Brands</th>
<th>Zones of inhibition</th>
<th>Control standard (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. coli</em></td>
<td>Tetravine</td>
<td>25mcg 11</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>Lono 25mcg</td>
<td>17</td>
<td>20</td>
</tr>
<tr>
<td><em>K. pneumoniae</em></td>
<td>Tetravine</td>
<td>25mcg 11</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>Lono 25mcg</td>
<td>12</td>
<td>18</td>
</tr>
<tr>
<td><em>S. pyogenes</em></td>
<td>Tetravine</td>
<td>25mcg 16</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>Lono 25mcg</td>
<td>9</td>
<td>15</td>
</tr>
<tr>
<td><em>P. aeruginosa</em></td>
<td>Tetravine</td>
<td>25mcg 6</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>Lono 25mcg</td>
<td>11</td>
<td>16</td>
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</tbody>
</table>
**Discussion**

The zones of inhibition shown by the two brands of tetracycline against the test organisms indicated their potencies (Chesebrough, 2000; Pelczar and Reid, 1998). The potencies have to do with the active ingredients contained in each of the antibiotics since the test were compared to the standards. Other factors that could have affected their potencies were not investigated; however, the differences in efficacy between the two brands constitute a grave danger to health. From the results obtained, it can be observed that the two brands of tetracycline sold in Kano showed significant or great variation in their different activities against the test organisms when compared to the standard antibiotics. This may indicate that some of the tetracyclines sold in Kano may be fake or adulterated (Adejoh, 2000). The negative health impact of fake drugs cannot be over-emphasized. These poor quality or substandard drugs could be responsible for the increasing number of resistant strains of microorganisms in the country. The general concept is that the active ingredients in this antibiotic may be less than what is indicated on the drugs labels and it is of serious concern, because the quality of a drug is dependent on the correctness of its active ingredient (Immaculata and Abraham, 1990).

**Conclusions and Recommendations**

When compared to the standard antibiotic used in this study, it is obvious that the two brands of tetracycline had reduced potency against the test microorganisms. There is therefore, need for all pharmaceutical products manufacturers to make sure that the recommended active ingredients and other ingredients and expiration dates are ascertained before the drugs are distributed for consumption by the public. Lono is more preferable to tetravine when dealing with etiologic agents like *P. aeruginosa*, *K. pneumoniae* and *E. coli*. Tetravine was more effective against *S. pyogenes* than that of Lono. Both brands could therefore be used for patients that have both enteric and respiratory infections as may be recommended by a physician.

**References**


