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EVALUATION OF MICROBIOLOGICAL PURITY OF SOME BRANDS OF TETRACYCLINE SOLD IN KANO STATE, NIGERIA

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ABSTRACT
Nineteen (19) brands of tetracycline belonging to the five groups of the tetracycline family were analysed for microbiological purity by tests for detection of bacterial contaminants and determination of bacterial and fungal loads. Bacterial and fungal contaminants were isolated and identified by combinations of culture, Gram – staining, microscopy and biochemical tests. Twelve (12) samples (63.16%) were found to be microbiologically pure, while the remaining seven (7) were each contaminated with at least one type of microorganism. The results are discussed in relation to limits and specifications recommended by the British Pharmacopoeia and the United States Pharmacopoeia.

Keywords: Microbiological purity, tetracycline, contaminants, bacterial load, fungal load, microbiological limits

INTRODUCTION

Just like food substances, pharmaceutical products can serve as media for growth and proliferation of microorganisms. A drug may be microbiologically contaminated from the raw materials used in its formulation, equipment from which it was made, from atmosphere of the production plant, from the person operating the process, from the final container into which it was packed or during the course of storage, transportation and even marketing. Some of the contaminants may be pathogenic, whilst some others may grow even in the preservative (antibiotic) added. Antibiotics which are used in treating bacterial diseases and in the preservation of pharmaceutical products have often been found to be contaminated by microorganisms, because not all antibiotics are active against all microorganisms, as such microbes can survive or even multiply in both syrup and capsules (Hugo and Russel, 1998). For this reason, microbial limit tests are nowadays employed for qualitative and quantitative estimation of specific microorganisms present in drugs (British Pharmacopoeia, 1993).

Tetracyclines are a family of widely used broad spectrum antibiotics with activity against Gram positive and some Gram negative bacteria as well as borreliae, rickettsiae, chlamydiae and mycoplasmas. Tetracyclines are bacteriostatic. They inhibit protein synthesis by binding specifically to the 30S ribosome thereby preventing access of amino acid (AA) – tRNA to the acceptor site on the m – RNA ribosome complex which in turn prevents the addition of AA to the growing polypeptide chain (Pelczar *et al.,* 2005). The tetracyclines are divided into five groups chlortetracycline, oxytetracycline, tetracycline, doxycycline and minocycline. Chlortetracycline, oxytetracycline and tetracycline are produced by *Streptomyces aureofasciens, S. rimosus* and *S. texas,* respectively. Minocycline and doxycline are semi-synthetic derivatives (Williams, 1982).

Contaminated antibiotics when consumed, injected or applied to the body may cause serious medicament – borne infections. Also, the infection they are intended to treat would continue unabetted. Thus, such antibiotics constitute a very serious public health hazard and the present work was carried out in order to determine the extent of this hazard posed by tetracyclines marketed in Kano. This is important as tetracyclines are among the most widely used antibiotics in Kano state and indeed, Nigeria.

MATERIALS AND METHODS

Collection of Samples

Nineteen (19) samples of tetracycline, consisting of five brands each of tetracycline, oxytetracycline and doxycycline and two brands each of chlortetracycline and minocycline were purchased from the Abubakar Rimi Market, Kano and Pharmaceutical shops randomly selected within metropolitan Kano. Pharmaceutical data like brand name, batch number, manufacturing date, expiry date, manufacturing firm and country were documented as recommended by the British Pharmacopoeia (1993). The samples were stored in cool and dry condition before being used.

Pretreatment of Samples

Samples were pretreated in order to revive microorganisms that may be present in the antibiotics following standard procedures outlined in the British Pharmacopoeia (1993) and by the World Health Organization (1983).

Detection and Enumeration of Microbial Contaminants

Tests for the enumeration of viable aerobic mesophilic bacteria as well as tests for detection of *Salmonella spp., Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus* and Enterobacteriaceae were carried out using pour plate method described in British Pharmacopoeia (1993).

Detection and enumeration of fungal contaminants were performed by the Standard Plate Count (SPC) method outlined in British Pharmacopoeia (1993).

Gram Staining and Microscopy

The method of Cheesbrough (2002) was adopted to help identify bacterial isolates.

Biochemical Tests

To confirm preliminary identification of bacterial contaminants revealed by colonial morphological features, Gram staining and microscopy, six biochemical tests were performed. Oxidase, coagulase, indole and catalase tests were conducted using the methods of Cheesbrough (2002) while citrate utilization and urease tests were carried out according to the procedure described by Harley and Prescott (1999).

RESULTS AND DISCUSSION

A great majority of the tetracyclines sold in Kano are foreign while the rest are manufactured and packaged in Nigeria under license of overseas – based pharmaceutical firms. All the samples collected for the study have been registered by the National Agency for Food, Drug Administration and Control (NAFDAC) and none had expired at the time of sampling. Brands of all the five groups of tetracyclines are readily available in Kano metropolitan pharmaceutical chemists and shops (Table 1).

Of the nineteen (19) brands of tetracycline tested, seven (7, 37%) were contaminated with atleast one type of microorganism (Table 2). Two brands of tetracycline group were contaminated, one with *E. coli* and the other with *E. coli,* and *Salmonella spp.*. Likewise, two brands of oxytetracycline were contaminated, one with aerobic mesophilic bacteria and fungi, and the other by aerobic mesophilic bacteria only. *E. coli, Salmonella spp.* and enterobacteriaceae were isolated from a brand of chlortetracycline and viable aerobic bacteria were detected in a brand of minocycline. Only one brand of doxycycline was found to be contaminated with fungi.

The results for bacterial and fungal counts indicate tetracycline a, tetracycline d and chlortetracycline a had 1.3×10^3, 4.4×10^3 and 1.2×10^3 cfu/g of enterobacteriaceae, respectively. Oxytetracycline a, oxytetracycline c and minocycline b contained 9.0×10^2, 5.5×10^2 and 1.2×10^3 cfu/g of viable aerobic mesophilic bacteria, respectively. Fungal counts of 8.2×10^2 and 4.0×10^3 cfu/g were isolated from oxytetracycline a and doxycycline b samples, respectively (Table 3).

The level of contamination of the seven brands of tetracycline analysed has exceeded the specifications and limits of the British Pharmacopoeia (1988) and the United States Pharmacopoeia (2001). According to these pharmacopoeias, *Salomonella spp* and *E. coli* must not be detected in one gramme samples of antibiotics. In addition, the number of colony forming units per gramme (cfu/g) of enterobacteriaceae, aerobic mesophilic bacteria or fungi should not exceed 5×10^2.

In terms of % microbiological purity, tetracycline d and chlortetracycline a, each with 57.14% had the least purity (Table 4). Of the five tetracycline groups, doxycycline had the highest purity (80%) while chlortetracycline and minocycline, each with 50%, had the least. The overall microbiological purity of tetracyclines sold in Kano was found to be 63.16% (Table 5).

Microbial contamination of seven of the samples examined could be attributed to poor manufacturing practice, such as improper sterilization of water or raw materials used in the production of the antibiotics, lack of equipment sanitation as reported by Hugo and Russel (1988) and USFDA (2006). Resistance may also be responsible for some the contaminations as three different specific mechanisms of tetracycline resistance have been identified, namely tetracycline efflux, ribosome protection and tetracycline modification. These mechanisms have been observed both in aerobic and anaerobic Gram negative and Gram positive bacteria. To date, about sixty one (61) tetracycline resistance genes have been sequenced (Roberts, 1994; 1996; Schappinger, 1996; Yamaguchi, 1997; Taylor and Chan, 1996; Hanahan, 1983).

CONCLUSION

A majority of the brands of tetracycline sold in Kano are of satisfactory microbiological purity and are therefore fit for use in treating bacterial infections. However, a significant number harbour microbial contaminants beyond acceptable limits rendering them unsafe and unfit for human consumption.

Recommendations

To overcome the problem of sale of substandard tetracyclines in Kano, it is recommended that:

i). Post marketing microbiological purity surveys be regularly carried out by state agencies like NAFDAC in order to ensure safety and quality of this antibiotic in particular and other drugs generally.

ii). Pharmaceutical companies producing microbially contaminated tetracyclines be encouraged by government to improve on their manufacturing practice by conducting quality control tests on finished products.

Table 1: Pharmacopoeial Details of Some Brands of Tetracycline sold in Kano

Antibiotic	Brand code	Manufacturing date	Expiry date	Manufacturing Country
Tetracycline	a	May 2006	May 2010	Ghana
	b	Feb. 2005	Feb. 2009	China
	c	Mar. 2004	Mar. 2008	China
	d	Jan. 2004	Jan. 2008	USA
	e	Mar. 2004	Mar. 2009	Nigeria
Oxytetracycline	a	Mar. 2006	Mar. 2010	Nigeria
	b	Jan. 2006	Jan. 2009	Malaysia
	c	Oct. 2004	Oct. 2008	Nigeria
	d	Jun. 2006	Jun. 2009	China
	e	Nov. 2003	Nov. 2006	India
Doxycycline	a	Jul. 2006	Jul. 2010	Nigeria
	b	Jun. 2004	Jun. 2007	Pakistan
	c	Apr. 2003	Apr. 2006	Nigeria
	d	May 2004	May 2007	China
	e	May 2005	May 2008	Malaysia
Chlortetracycline	a	Mar. 2005	Mar. 2008	Ghana
	b	Aug. 2005	Aug. 2008	India
minocycline	a	Oct. 2003	Oct. 2007	Ireland
	b	Nov. 2004	Nov. 2008	India

Table 2: Bacterial and Fungal Contaminants in Tetracycline Samples Sold in Abubakar Rimi Market, Kano (2007)

Antibiotic	Brand code	*Salmonella spp*	*E. coli*	*P. aeruginosa*	*S. aureus*	Enterobacte-riaceae	*Viable Aerobic Bacteria*	*Fungi*
Tetracycline	a	-	+	-	-	+	-	-
	b	-	-	-	-	-	-	-
	c	-	-	-	-	-	-	-
	d	+	+	-	-	+	-	-
	e	-	-	-	-	-	-	-
Oxytetracycline	a	-	-	-	-	-	+	+
	b	-	-	-	-	-	-	-
	c	-	-	-	-	-	+	-
	d	-	-	-	-	-	-	-
	e	-	-	-	-	-	-	-
Doxycycline	a	-	-	-	-	-	-	-
	b	-	-	-	-	-	-	+
	c	-	-	-	-	-	-	-
	d	-	-	-	-	-	-	-
	e	-	-	-	-	-	-	-
Chlortetracycline	a	+	-	-	-	+	-	-
	b	-	-	-	-	-	-	-
minocycline	a	-	-	-	-	-	-	-
	b	-	-	-	-	-	+	-

Key:
+ = Microorganisms detected, - = No microorganisms detected

Table 3: Mean Bacterial and Fungal Counts

Antibiotic	Enterobacteriaceae (CFU/g)	Aerobic Counts (CFU/g)	Fungal Counts (CFU/g)
Oxytetracycline a	-	9.0×10^2	8.2×10^2
Oxytetracycline c	-	5.5×10^2	-
Minocycline b	-	1.2×10^3	-
Doxycline b	-	-	4.0×10^3
Tetracycline a	1.3×10^3	-	-
Tetracycline d	4.4×10^3	-	-
Chlortetracycline a	1.2×10^3	-	-

Table 4: Microbiological Purity of Brands of Tetracycline Sold in Abubakar Rimi Market, Kano (2007)

Antibiotic	Brand code	No. of Tests Positive	% Microbiological Purity
Tetracycline	a	2	71.43
	b	0	100.00
	c	0	100.00
	d	3	57.14
	e	0	100.00
Oxytetracycline	a	2	71.43
	b	0	100.00
	c	1	85.72
	d	0	100.00
	e	0	100.00
Doxycycline	a	1	85.72
	b	0	100.00
	c	0	100.00
	d	0	100.00
	e	0	100.00
Chlortetracycline	a	3	57.14
	b	0	100.00
minocycline	a	0	100.00
	b	1	85.72

Table 5: Microbiological Purity of Members (Groups) of Tetracycline Family Analysed

Group	No. of Samples Tested	No. of Samples Contaminated	% Microbiological Purity
Tetracycline	5	2	60.00
Oxytetracycline	5	2	60.00
Doxycycline	5	1	80.00
Chlortetracycline	2	1	50.00
Minocycline	2	1	50.00
		Grand % Microbiological Purity	63.16

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