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Microbial and Physicochemical qualities of selected Co-trimoxazole and Metronidazole formulations in South Eastern Nigerian

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ABSTRACT:

The physical, chemical and microbiological qualities of nine brands of co-trimoxazole suspensions ($C_{1...9}$) and eight brands of metronidazole syrups ($M_{1...8}$) sourced from different locations in South-Eastern Nigeria were evaluated to determine their conformity with stipulated standards. The microbiological qualities of 87.5% of the metronidazole brands tested contain viable microbial load within acceptable limit while that of one brand (M_1) fell below stipulated standard. Out of the nine brands of co-trimoxazole suspension tested, 66.7 % have bacteria loads within acceptable limit while those of C_1, C_3 and C_4 are above acceptable limit. Only one brand out of the eight brands of the metronidazole syrup tested (M_6) contained objectionable microbe; *Salmonella sp,* while only one brand out of the nine brands of the co-trimoxazole syrup (C_1) tested contained an objectionable microbe; *Escherichia coli*. Brands M_5, M_6 and M_7 of the metronidazole syrup contained moulds, but these are within acceptable limits. None of the brands of the co-trimoxazole contained moulds. The physical and chemical properties of the analyzed drug samples complied with BP standards.

Key words: Physicochemical quality, Microbial quality, Co-trimoxazole, Metronidazole,

INTRODUCTION:

Drugs and pharmaceutical products are manufactured based on stipulated standards. These standards are regulated by the regulatory authorities. The standards are

achieved through well articulated Current Good Manufacturing Practice (CGMP). Maintaining CGMP will ensure the formulation of products of acceptable standards in contents of active ingredients, good physical and chemical stability and acceptable microbiological quality. For oral dosage formulations, official standards required that viable microbial counts should not exceed $1.0x10^3$cfu/ml and the products should not contain enteric microorganisms. [1] Deviation from these standards attracts serious sanctions from the regulatory authorities.

Adulterated and substandard pharmaceutical products which pose serious challenge to good health are often produced in compromised environments. These environments predispose the products to risks of contamination. Microbial contamination of pharmaceutical dosage forms may lead to spoilage resulting in physical and chemical changes and risk of infection to the user. [2, 3]

Paediatric Co-trimoxazole suspensions and Metronidazole syrups represent two important medicaments used in paediatrics for the control and treatment of bacteria and protozoan infections. [4, 5] Their high demand make them targets for adulteration and faking by dubious manufacturers with attendant physical, chemical and microbiological qualities being compromised. Frequent surveillance and quality evaluation of these pharmaceutical products will indicate if the fight against adulteration and production of unwholesome products is succeeding or otherwise.

In this research therefore, the physical, chemical and microbiological qualities of selected Co-trimoxazole suspensions and Metronidazole syrups sourced from local markets in selected states of South-East Nigeria; Anambra, Enugu and Imo States are evaluated. The findings of the research will help to redirect the regulatory bodies properly towards checking adulterated and unwholesome drugs.

MATERIALS AND METHODOLOGY:

A total of eight brands of metronidazole syrups and nine brands of co-trimoxazole suspensions were sourced from the local outlets between October 2009 and February 2010. The samples were taken to the laboratory and stored in a dry place and analyses performed within 24 hours of sample collection. Standard microbiological media for isolation of bacteria and fungi were used.

Physical examinations of the samples were performed. The organoleptic characters were evaluated using a 5-man panel of assessors. The character evaluated was

appearance/colour. The pH of the samples was evaluated using standard procedures after calibration of the digital pH meter with potassium dihydrogen phosphate buffer. [6] The contents of active ingredients of the samples was evaluated spectrophotometrically (Unicon UV-2102 PC, USA) using a standard calibration curve prepared from the respective pure drug samples.

One (1) ml of each drug sample was introduced into 5 ml of the sterile peptone water for enrichment. Thereafter, 1 ml of these enriched samples was dispensed into a sterile Petri-dish and 20 ml of sterile Nutrient agar maintained at 45^{o}C was introduced into the plates, mixed by gentle rocking and the inoculation allowed to solidify. The plates were incubated at 37^{o}C for 24 hours and total number of discrete colonies counted. Similarly, 1 ml of the enriched drug samples was inoculated into 20 ml of the MacConkey (Coliforms) and Sabouraud's dextrose agar (mould and yeast). The MacConkey agar inoculation was allowed to solidify and then incubated at 37^{o}C for 24 hours. The Sabouraud's dextrose agar inoculation was also allowed to solidify and incubation done at room temperature for seven days. Total colonies count was also performed on these incubated samples.

Sub-culturing of the incubated samples was done. Isolates from the MacConkey agar plates were inoculated into Salmonella-Shigella agar while isolates from the nutrient agar plates was inoculated into Mannitol salt agar (Staphylococcus spp) and the plates incubated for 24 hours. Microscopic and biochemical characterization of the isolates were performed using standard procedures. [7-9]

RESULTS AND DISCUSSIONS:

The manufacturing and expiry date of the Metronidazole syrups and Co-trimoxazole suspensions were as shown in table 1. There were growths of organisms in five out of eight brands of metronidazole tested. The total viable count for the five brands M_1, M_4, M_6, M_7 and M_8 were 20.0x10^2, 10.0, 30.0, 27.0 and 1.0x10^3 cfu/ml respectively. There were no growths in M_2, M_3 and M_5 brands of the metronidazole syrups. Coliforms growth was recorded in only one out of the eight brands evaluated (M_6), with the count being 7.0 cfu/ml. Three out of the eight brands showed yeast growth, with counts of 1.0 cfu/ml for M_5, 3.0 cfu/ml for M_6 and 1.0 cfu/ml for M_7 as shown in table 2. The total viable counts for the Co-trimoxazole brands showed that brand C_1 had 4.4 x10^3 cfu/ml. There are no growths in C_2, C_5, C_6, and C_7 while in brands C_3, C_4, C_8 and C_9, the counts were 2.2 x 10^4, 3.0 x 10^3, 22.0 and 14.0 cfu/ml respectively. Coliforms counts were obtained in brand C_1 of the Co-trimoxazole with a count of 1.5 x 10^3 cfu/ml. There

are no Coliforms growths in the rest of the brands. Yeast counts were not observed in any of the brands of the Co-trimoxazole suspensions as shown in table 3. The organisms obtained from the sub-culturing into various selective media and identified by standard biochemical test were shown in table 4 for the Metronidazole syrups and Co-trimoxazole suspensions. In the Metronidazole syrup, only brand M_6 contains *Salmonella sp*, while in the Co-trimoxazole suspensions, only brand C_1 showed presence of *Escherichia coli*.

The brands appearances range from light yellow through yellow to light brown. The pH values range from 3.9 to 4.2, while the specific gravities range from 1.8516 to 1.9965. The content of active ingredients for the brands of Metronidazole ranges from 197.42 to 198.20 mg/5 ml of the syrup as shown in table 5.

The appearances of the Co-trimoxazole brands varied from pink, light brown to white. The pH ranges from 5.7 to 6.25, while the specific gravities range from 1.8475 to 1.9996. The content of active ingredients ranges from 198.4/40.6 to 199.3/41.2 mg/5 ml of the syrups as shown in table 6.

The current fight against adulterated and unwholesome drugs warrants frequent evaluation of marketed pharmaceutical products to ensure their conformation to stipulated standards. Quality of drugs can be compromised in their content of active ingredients, inappropriate physical characters and contamination with non permissible microbial bioloads.

Microbial contamination of pharmaceutical products has been recorded in tablet dosage forms [10], suppositories [11] and anti-malarial, cough paediatric liquid preparations [1] and water for injection. [12]

Consequently, out of the eight brands of Metronidazole tested, seven brands (87.5%) had bacteria loads of less than $1.0x10^3$cfu/ml. In the Co-trimoxazole suspension, six out of the nine brands (66.7%) tested had bacteria loads less than $1.0x10^3$cfu/ml. This implies that less than half of the tested samples (12.5 % for Metronidazole and 33.3 % for Co-trimoxazole) are contaminated with microbial bioloads higher than the stipulated standards. Pharmaceutical oral formulations with bioloads of $1.0x10^3$cfu/ml or less conform to official requirements for the microbiological quality of oral liquid dosage forms. [1] The presence of these microbes has the potential to reduce or even inactivate the therapeutic activity of the product. [2] However, Muhammed and Umoh had

indicated that paracetamol and cough syrups tested in Northern Nigeria all scaled through the microbiological standard tests. [13]

Thus, the results suggest inappropriate Current Good Manufacturing Practice (CGMP) on the part of the Pharmaceutical Company. Pharmaceutical products can be contaminated during handling and storage. [14] The presence of Coliforms; *Salmonella sp* in brand M_6, *Escherichia coli* in brand C_1, is suggestive of contaminations from improper water treatment systems and contaminated raw materials. [15] Adeshina *et al*, [2009] after Committee of Official Laboratory and Drug Control Services and a section of the International Pharmaceutical Federation 1975, has noted that the existence of these Coliforms make such preparation unfit for human consumption. [1]The presence of fungi in some of the brands; M_5 M_6 and M_7, however, suggest contamination from air and packaging materials. [15] The results showed that more of the Co-trimoxazole suspension brands contain greater units of total viable bacteria counts than the Metronidazole syrup brands, suggesting that the high sugar content of the syrups may have contributed to the microbial reduction. Moreover, the suspensions contain suspending agents of natural origin which may harbour microbes. The important physical (pH) and chemical property (content of active ingredients) measured conform to the stipulated standards [6], except for slight difference in pH of the Co-trimoxazole which will not pose any danger to the consumer.

Table 1: Co-trimoxazole and Metronidazole syrup sample

Samples	Man. Date	Exp. Date	Sample	Man. Date	Exp. Date
M_1	05/09	05/12	C_1	03/09	03/12
M_2	05/07	04/10	C_2	05/09	04/12
M_3	01/09	01/11	C_3	03/08	03/11
M_4	08/09	08/11	C_4	08/09	07/11
M_5	02/08	02/11	C_5	08/09	09/11
M_6	03/09	03/12	C_6	02/09	02/12
M_7	06/09	05/11	C_7	06/08	06/11
M_8	08/08	07/11	C_8	04/08	03/11
			C_9	03/08	03/11

Table 2: Growth of Isolates from different brands of Metronidazole

Metronidazole Brand	Total count (cfu/ml)	Coliform count (cfu/ml)	Yeast/Mold count (cfu/ml)
M_1	20.0×10^2	---	---
M_2	---	---	---
M_3	---	---	---
M_4	10	---	---
M_5	---	---	1
M_6	30	7	3
M_7	27	---	1
M_8	1.0×10^3	---	---

Table 3: Growth of Isolates from different brands of Co-trimoxazole

Co-trimoxazole Brand	Total count (cfu/ml)	Coliform count (cfu/ml)	Yeast/Mold count (cfu/ml)
C_1	4.4×10^3	1.5×10^3	---
C_2	---	---	---
C_3	2.2×10^4	---	---
C_4	3.0×10^3	---	---
C_5	---	---	---
C_6	---	---	---
C_7	---	---	---
C_8	22	---	---
C_9	14	---	---

Table 4: Coliforms isolates from different brands of Metronidazole Syrups and Co-timoxazole suspensions

Metronidazole	Organism	Co-trimoxazole	Organism
M_1	---	C_1	*Escherichia coli*
M_2	---	C_2	---
M_3	---	C_3	---
M_4	---	C_4	---
M_5	---	C_5	---
M_6	*Salmonella sp*	C_6	---
M_7	---	C_7	---
M_8	---	C_8	---
		C_9	---

Table 5: Physicochemical analyses of different brands of Metronidazole Syrup

Metronidazole Syrup Brand	Appearance	pH	Specific gravity[a]	Content of Active Ingredients (mg/5ml)
M_1	Yellow	3.9	1.8588	197.42
M_2	Light yellow	4.2	1.9714	197.42
M_3	Yellow	3.9	1.9893	197.58
M_4	Yellow	3.9	1.9965	198.18
M_5	Yellow	4.2	1.9685	197.52
M_6	Yellow	4.1	1.8516	198.20
M_7	Light brown	3.9	1.8516	197.92
M_8	Light yellow	3.9	1.8532	197.76

[a] Measured at 25°C

Table 6: Physicochemical analyses of different brands of Co-trimoxazole

Co-trimoxazole Syrup Brand	Appearance	pH	Specific gravity[b]	Content of Active Ingredients (mg/5ml) Sulphamethoxazole/Trimethoprine
C_1	Pink	6.0	1.9963	198.6/40.8
C_2	Light brown	6.25	1.9996	199.1/40.7
C_3	White	5.7	1.8757	199.1/41.0
C_4	Light brown	6.2	1.9888	198.6/40.8
C_5	Pink	6.1	1.9980	199.1/40.5
C_6	Pink	5.8	1.9944	198.6/41.4
C_7	Light brown	5.68	1.8609	198.5/41.0
C_8	Pink	5.8	1.8475	198.4/40.6
C_9	White	5.9	1.8810	199.3/41 .2

[b] Measured at 25°C

CONCLUSION:

Some of the brands of Metronidazole syrups and Co-trimoxazole suspensions evaluated contain microbiological bioloads well above stipulated limit of $1.0x10^3$cfu/ml (M_1 and M_8, C1, C_3 and C_4). Very few contain objectionable micro organisms (*Salmonella spp* in M_6 and *Escherichia coli* in C_1). Also few of the Metronidazole syrups contain moulds (M_5, M_6 and M_7) while none of the Co-timoxazole suspensions contain moulds. All the evaluated brands conform to the stipulated standards physically and chemically. The implication is that the production of these brands of pharmaceutical dosages is done with the required expertise. The short fall in the microbiological quality of the above

stated brands is likely as a result of breach in standard operating procedure and non validation of some of the resources involved in the production.

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