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Microbiological Quality of Some Selected Brands of Tablets and Syrups Produced in Nigeria

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Abstract Microbiological quality of some selected brands of tablets and syrups produced in Nigeria was carried out. The drugs were purchased from different chemist stores at different towns in the South West of Nigeria. The samples were serially diluted and plated on Nutrient and Saboraud Dextrose Agar by Pour plating method. The plates were incubated at 25 ^{o}C and 35 ^{o}C for Fungi and Bacteria isolation respectively. The isolated organisms were characterized and identified by using morphological, cultural and biochemical means. A total of 180 samples comprised of 80 syrups from 8 brands and 100 tablets from 10 brands were analysed. Bacterial counts ranged from 10^1 cfu/ml/g to 10^4 cfu/ml/g. A total of 167(93 %) samples conformed to microbial load specification with less than 10^3 cfu/ml/g microbial counts, only 13(7 %) samples were found out of specifications. syrups showed higher microbial counts than tablets. The organisms isolated from the samples include the following; *Erwinia ananas, Erwinia carotovora, Bacillus subtilis, Lactobacillus casei, Candida spp.* Only Candida species are of medically important isolates found. It can be concluded that majority of non sterile pharmaceutical products produced in Nigeria conform to the specified microbiological standard and fit for therapeutic application.

Keywords Nonsterile pharmqaceuticals, Microbial quality, Microbial limit.

Introduction

Pharmaceutical products are used in a variety of ways, in the prevention, treatment, and diagnosis of disease. However many diseases have been associated to the use of pharmaceutical products, leading to high level of morbidity and mortality. Several reports describing clinical hazards attributable to microbiologically contaminated pharmaceuticals have been published [1-3]. The major health concern is when such microbial contaminants exceed acceptable limits of 10^3 cfu/ml as specified in United States Pharmacopoeia [4]. The presence of microorganisms in drugs in excess of the limit (10^3 and 10^2 cfu/ml/g for Bacteria and Fungi respectively), not only makes them hazardous from the infectious point of view, but may also change the physical, chemical and organoleptic properties of drugs, alter the contents of active ingredients, or convert them to toxic products. Microbial infections are not only the result of physical presence of microorganisms, but also their metabolites or toxins that become harmful even if they are found in minute quantities [5].

Pharmacopoeial monographs primarily exist as standards against which pharmaceutical products are tested. It is recognized that it would be impractical with existing methodologies — to test pharmaceuticals exhaustively for all potentially pathogenic contaminants. In the United States Pharmacopeia [6], there are only 95 monographs that include microbial limits. Fifty-one of these require absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa* in 20 g or 10 ml, 20 required absences of *Escherichia coli* or *Salmonella* spp., or *Escherichia coli* and *Salmonella* spp., in 10 g or 10 ml. The restrictions on *S. aureus* and *P. aeruginosa* apply to topical products, because these microorganisms are typical of types that could cause infection when products are used on open wounds or abraded skin, or gastrointestinal infections when used orally.

The restrictions on *Escherichia coli* and *Salmonella* spp. are applicable to oral products because these microorganisms are typical of types that could cause gastrointestinal infections. The pharmacopoeial restricted species have been chosen as indicators, at least in part, because of the availability of robust techniques for their isolation and recognition. The possibility of other objectionable microbiological contaminants in non-sterile products cannot be disregarded. When contamination is discovered, its significance must be evaluated conservatively, considering the formulation of the product, its method of delivery, the contaminant, and the type of patient undergoing treatment.

For instance, in 1994 a U.S. company responsibly and voluntarily withdrew 3.6 million units of albuterol sulfate inhalation solution from the market on confirmation of contamination with *Pseudomonas fluorescens*. Bergey's Manual of Determinative Biology recognizes *Pseudomonas fluorescens* as being more likely to be associated with soil and water than with specific pathogenicity to humans. A team of independent microbiologists set up at the time of the recall concluded that *Pseudomonas fluorescens* has "very rarely been found to be the causative agent of illness." The reason for the recall was concern that this microorganism could cause lung infections, which could be particularly serious in people with cystic fibrosis, chronic obstructive lung disease or with compromised immune systems.

Non-sterile pharmaceutical products are generally formulated to prevent any microorganisms from increasing in number during their shelf lives by being adequately preserved. This may be intrinsic to the solid dosage form. An example in solid dosage forms, such as tablets or powder inhalations, is the lack of sufficient water to allow microorganisms to multiply over time. Conversely, nonsterile aqueous dosage forms, in which there is sufficient, water to potentially allow microorganisms to multiply, are usually formulated to incorporate antimicrobial preservatives. In addition to these formulation-related factors, there are regulatory requirements governing the standards of hygiene applicable to the manufacture of non-sterile pharmaceuticals. Such regulations may restrict the numbers and types of microbial contaminants that could be initially present on the product. Although microbial populations are present in all types of habitats, there are several major limiting factors that affect microbial distribution, survival, and proliferation in the environment.

Nonsterile pharmaceuticals are manufactured under aseptic conditions, but the processes used during production are not as strictly monitored as in sterile products. For instance, in sterile manufacturing, water, air, and environmental monitoring are performed on a routine basis preventing sterility failures and system breakdown.

However, pharmaceutical companies follow different strategies during the manufacturing of non-sterile products. For instance, some companies perform environmental monitoring of production facilities and equipment sporadically, while others perform it on a regular basis or none at all [7-8]. Microbial identification of environmental isolates from nonsterile manufacturing environments varies from company to company. In some cases, companies pursue microbial identification by only a gram stain reaction, e.g., gram negative or positive. Other companies take the identification one step further when the environmental isolate is completely identified by genera and species such as gram-negative rod, *Pseudomonas aeruginosa.* Because of the infrequent and inconsistent monitoring of equipment, personnel, and environment, microbial limits testing of raw material and finished product is a critical step for the quality control analysis of nonsterile pharmaceuticals. The results of theses analysis form the bases for determining microbiological fitness of nonsterile pharmaceuticals. The aim of this work, therefore is to determine microbiological quality of some of the nonsterile pharmaceutical products produced in Nigeria.

Materials and Methods

Sample Collection

This study was carried out on some selected tablets and syrups of some pharmaceutical companies in Nigeria. A total of 180 samples comprise of 100 tablets packs from 10 brands and 80 bottles of syrups from 8 brands were bought from chemists, in the south west region of Nigeria. Three different batches of a product of tablets and syrups were purchased from a chemist. These packaged samples were collected aseptically and transported to the laboratory for microbiological analysis.

Microbiological Analysis of Samples

Glass materials such as conical flasks, test tube, MaCcartney bottles, measuring cylinders used were washed with detergent and thoroughly rinsed with water. They were allowed to dry and were sterilized in the hot air oven before used. Media used include; Nutrient Agar, Saboraud Dextrose Agar, and Peptone water. These media were prepared according to the manufacturer's instructions.

Bottles of syrups analysed were aseptically opened. By using sterile syringes and needles, 1ml was taken, while in the case of tablets, 1 g was weighed, dissolved and serial diluted in 9 ml of sterile peptone water up to 10^{-4}. Each

dilution was plated out by pour plating method in Nutrient and Saboraud Dextrose agar; the plates were incubated for 24 to 72 hours at 35 ^{o}C and 25 ^{o}C for the isolation of bacteria and fungi respectively.

The organisms developed on the plates after incubation were counted and multiply by their corresponding dilution factors to get the actual numbers of microorganisms in 1 ml or 1 g of each sample diluted and plated on agar. Different organisms on the plates were isolated in pure culture and fully characterized; culturally and biochemically. The identified bacteria and fungi were recorded.

Results

Table 1: Average microbial loads of the tablets and syrups produced by each of the eight companies sampled and analysed.

Company	No. of different Products analysed and their corresponding microbial loads.			
	Tablets (Packs)	Average Microbial load (cfu/g)	Syrups (Bottles)	Average Microbial load (cfu/ml)
A	26	$1.3x10^2$	16	$1.9x10^3$
B	26	$9.8x10^1$	13	$5.2x10^2$
C	-	-	14	$3.7x10^3$
D	-	-	13	$4.7x10^2$
E	24	$2.0x10^2$	-	-
F	12	$4.4x10^1$	-	-
G	-	-	13	$1.2x10^3$
H	12	$1.6x10^2$	13	$6.1x10^2$

Table 2: Isolated organisms and their frequencies of isolation from both tablets and syrups.

Isolated organisms	No. of packs of tablets with microorganisms	No. of Bottles of syrups with microorganisms	No. of times each organism was isolated (%)
Bacillus subtilis	5	16	21(20.6)
Erwinia ananas	2	9	11(10.8)
Erwinia carotovora	nil	5	5(4.9)
Lactobacillus casei	15	32	47(46.0)
Candida spp.	nil	18	18(17.6)
Total	22	80	102(100)

Discussion

The occurrence of microbial contamination has been well documented, and contaminants range from true pathogens such as *Clostridium tetani*, to opportunistic pathogens such as *Pseudomonas aeruginosa* [9]. The results of this study showed that, the microbial load in tablets sampled and analysed ranged from $4.4x10^1$ to $2.0x10^2$ cfu/g while in the syrups the range was $4.7x10^2$ - $3.7x10^3$ cfu/ml. Species of *Erwinia, Bacillus, Lactobacillus and Candidas* were the isolated microorganisms in the products.

The three major pharmacopoeias, U.S. (USP), European (EP), and Japanese (JP), have divided microbial limit testing into two: the quantitative test and qualitative test [10-12]. The quantitative test ascertains the numbers of microorganisms, bacteria, yeast, and mold present in a given pharmaceutical sample. The qualitative test determines the presence of specific pathogen indicators, e.g., *Salmonella* spp., *Staphylococcus aureus, Escherichia coli, P. aeruginosa*, and the Enterobacteriaceae family which might cause disease to consumers or indicate the presence of other pathogenic bacteria. These indicators are representative microbial species of different types of bacterial populations. For instance, *Salmonella* spp. and *E. coli* are gram-negative rods, capable of lactose fermentation, commonly found in fecal sources. *Salmonella* spp. is virulent pathogens associated to intestinal disorders, while E. coli in general is not a virulent pathogen. However, some strains of E. coli are known to be producers of toxins associated to gastrointestinal diseases. *P. aeruginosa* is a gram-negative nonfermentative rod, which is typically associated to opportunistic infections. *S. aureus* is a grampositive cocci commonly associated to skin, gastrointestinal, and toxic shock syndrome conditions. The Enterobacteriaceae family comprises genera such as *Escherichia, Salmonella, Shigella, Citrobacter, Enterobacter, Klebsiella, Proteus,* etc. Most of the members of this family, other than *Salmonella* spp. and *Shigella* spp., are opportunistic pathogens. They are widely distributed in the environment.

In this study, none of the objectionable microbial species except *Candida* species were isolated, the products therefore conformed to the microbiological standard specification and hence suitable for human consumption. Presently, the regulatory body such as NAFDAC has deemed non-sterile pharmaceuticals safe for use.

Although, the tablet and syrup samples produced by the pharmaceutical companies in Nigeria meet microbiological specification, the Quality Assurance department in these pharmaceutical companies should not relent in their duties in making sure that the products conform to the specification. In conclusion, the bodies such as Standard Organization of Nigeria (SON), National Agency for Food and Drug Administration and Control (NAFDAC), should always ensure that all pharmaceuticals both sterile and non sterile pharmaceuticals released into the market for sale and consumption should conform to the specifications.

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