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Full Length Research Paper

Microbial quality of some non-sterile pharmaceutical products sourced from some retail pharmacies in Lagos, Nigeria

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Eight drugs on sale in five community pharmacies in parts of Lagos State were evaluated for microbial quality using standard procedures. A total of 40 samples were screened and 24 bacteria isolates, comprising 12 cocci and 12 bacilli were isolated. The cocci comprise 9 staphylococci and 3 micrococci while the bacilli constitute of 10 *Bacillus* species and 2 clostidia. Fungal isolates included *Saccahromyces* species, filamentous fungi; *Aspergillus*, *Penicillium*, and *Microspora* mostly from tablets in the multidose pack. Total bacterial counts for tablets in multidose and blister packs ranged from 1.0×10^2 to 9.0×10^2 CFU/g and 0 to 2 CFU/g respectively. Total yeast counts for tablets in multidose packs ranged from 1.4×10^2 to 9.0×10^2 while those of blister packs were between 0 to 6 CFU/g. Higher values of both the bacterial and fungal counts were obtained from drugs in multidose packs including ascorbic acid, paracetamol, gelucil, folic acid and multivitamin. The antimicrobial susceptibility tests showed that the S*taphylococcus aureus* were susceptible to amoxicillin and cotrimoxazole but resistant to other antibiotics tested, indicating a common source contamination.

Key words: Microbial quality, non-sterile, pharmaceutical products.

INTRODUCTION

The microbial quality of pharmaceutical products may be influenced by the environment in which they are manufactured, the raw materials used in their production with the exception of preparations which are terminally sterilized in their final containers. The microbial flora of the final product may represent the contaminant from the raw material, the equipment with which it was produced, the atmosphere and the operator (Denyer and Baird, 1990). It may also come from the final container into which it was packed (Brannan and Dille, 1990).

Sources of contamination of pharmaceutical products include manufacturing equipment, the environment, water used during the manufacturing process which may be contaminated by sewage and if not well treated, could result in microbial contamination by such organism as *Proteus* spp., *E. coli* and other Enterobacteria. Yeasts and Actinomycetes have been isolated in water that was to be used in manufacturing process (Legnani, 1998). Contamination from process operators must be considered a significant hazard because during normal activity, loss of skin scales by shedding is about 10^4 /min. A proportion of these skin scales will be contaminated by species of the normal flora (Martinez, 2002).

The products prone to microbial contamination are those with moderate to high water activity and availability, products containing sweeteners and products in multidose containers (Atata et al., 2007). This is the main reason why preservatives are added to these types of product formulations. Although, dry formulations are less susceptible to contamination, spoilage of solid dosage forms have been reported (Itah et al., 2004; Akerele and Godwin, 2002; Martinez, 2002; Obukwe et al., 2002). Syrups containing high concentration of sugar (approx. 85%) resist bacteria growth due to the exosmotic effect on microorganisms. Products containing more than 15%

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alcohol such as elixir and spirits are self-preserving (Lucia and Clontz, 1997).

The majority of contaminants of pharmaceutical products and ingredients are bacteria, yeast and filamentous fungi (mould). Some of these contaminants may be pathogenic while others grow as commensals even in the presence of preservatives and spoil products. They have a wide range of nutritive requirements and environmental condition suitable for their proliferation (Lucia and Clontz, 1997). Pharmaceutical preparations will support the growth of many bacterial and fungal contaminants if they contain ingredients degradable by microorganism. The simple nutritional requirement and metabolic adaptability of many common saprophytic spoilage microorganisms enable them to utilize many of the components of medicines as substrate for biosynthesis and growth (Somerville, 1981).

In this study the microbial quality of some pharmaceutical products available for sale in some pharmaceutical premises in Lagos, Nigeria were evaluated to possibly isolate and identify contaminating organisms and to establish the antimicrobial susceptibility patterns of the isolates and then make recommendations on how to improve the safe handling of such products in pharmaceutical premises, based on the antimicrobial susceptibility test results.

MATERIALS AND METHODS

Eight different batches of tablets of ascorbic acid, chloroquine, multivitamin, paracetamol, folic acid B-complex, flagyl and gelusil produced in Nigeria were procured as packaged by their manufacturers and used for this study.

Sample collection

Respective batches of samples of tablets of ascorbic acid, chloroquine, multivitamin, paracetamol, folic acid, B-complex, flagyl and gelusil in multidose (1000 tabs) containers as well as those in blister packs (10 tabs × 10 sachets) were purchased from five different randomly selected pharmaceutical premises in Akoka, Fola Agoro, Abule Oja and University of Lagos campus area of Lagos.

Sample processing

Ten tablets of each of the pharmaceutical products were crushed in a sterile mortar using a sterile pestle and one gram each of the respective pharmaceutical products was weighed out aseptically and dissolved in 9 ml of sterile distilled water. These were plated out on nutrient agar, Sabouraud Dextrose Agar (SDA), MacConkey agar and mannitol salt agar plates in duplicate and labelled accordingly. Nutrient agar, MacConkey agar and mannitol salt agar plates were incubated at 37℃ 24-48hr for bacteria gro wth while the SDA plates were incubated at 25 to 28℃ for fungal gro wth (Akerele and Godwin, 2002). The colonies observed after incubation were sub-cultured on nutrient agar for purity and identification.

Identification of colonies

The bacterial isolates were identified using standard biochemical tests (Sood, 2005). The yeast cells and the fungal isolates were identified microscopically based on their cellular morphology and differentiation. The latter were stained with lactophenol- in- cotton blue dye. The total viable aerobic bacterial and fungal count was calculated from the number of colonies that appeared on the plates.

Antimicrobial susceptibility testing

The disk diffusion method of antimicrobial susceptibility testing described by Kirby-Bauer (1959) was used to assay for the susceptibility of the pathogenic isolates. The antibiotics used were amoxicillin (25 µg), cotrimoxazole (25 µg), nitrofurantoin (30 µg), gentamicin (10 µg) and tetracycline (30 µg). Others are nalidixic acid (30 µg), ofloxacin (30 µg) and augumentin (30 µg). Zones of inhibition were interpreted according to the National Committee for Clinical Laboratory Standards (NCCLS), (2005). Organisms were reported as either resistant, intermediate or sensitive.

RESULTS

The result of the bacteriological examination of batches of tablets dispensed from multidose containers and those of blister packs showed some considerable variations in microbial load. A total of 24 bacteria species were isolated and these include 12 (50%) cocci and 12 (50%) bacilli. The cocci comprised of 5 (20.8%) *Staphylococcus ceprae,* 2 (8.3%) *Staphylococcus aureus,* 1 (4.2%) *Staphylococcus capacea,* 1 (4.2%) *Staphylococcus capitis,* 2 (8.3%) *Micrococcus viridians,* and 1 (4.2%) *Micrococcus sedantarous*. The bacilli comprised of 4 (16.7%) *Bacillus spheraecis,* 2 (8.3%) *Bacillus macerans,* 2 (8.3%) *Bacillus circulans,* 1 (4.2%) *Bacillus lentus,* 1 (4.2%) *Bacillus brevis* and 2 (8.3%) *Clostridium bifermentas* (Table 1). The total bacterial count obtained from the tablets of the multidose packs ranged from 1.0×10^2 to 9.0×10^2 CFU/g (Table 2) while that of fungi ranged from 1.4×10^2 to 7.2×10^2 CFU/g (Table 2). The total bacterial count for tablets in blister packs ranged from 0 to 3 CFU/g (Table 3) and that of fungi 0 to 6 CFU/g (Table 3). Higher bacterial counts were obtained from tablets in multidose packs for ascorbic acid, gelusil, folic acid and multivitamin tablets (Table 2) while higher fungal counts were obtained on chloroquine, gelucil, folc acid and paracetamol (Table 2). The antimicrobial susceptibility testing of the two *S. aureus* isolates revealed that they were sensitive to gentamicin and cotrimoxazole but resistant to nitrofurantoin, amoxicillin, tetracycline, nalidixic acid, ofloxacin and augumentin (Table 4).

DISCUSSION

The batches tablets sampled from the five community pharmacies were found to be contaminated with varying degrees of bacteria and fungi among which were *S. aureus* which is considered pathogenic, *Micrococcus* and *Clostridium* species. Fungi which include *Aspergillus*, *Penicillium* and *Microsporum* were also isolated. The

Table 1. Occurrence of different species of bacteria among samples.

Organism	Total (%)
Staphylococcus ceprae	5 (20.8)
Staphylococcus aureus	2 (8.3)
Staphylococcus capacea	1 (4.2)
Staphylococcus capitis	1 (4.2)
Micrococcus sedantarous	1 (4.2)
Micrococcus viridians	2 (8.3)
Total number of cocci	12 (50)
Bacillus spheraecis	4 (16.7)
Bacillus macerans	2 (8.3)
Bacillus circulans	2 (8.3)
Bacillus lentus	1 (4.2)
Bacillus brevis	1 (4.2)
Clostridium bifermentans	2 (8.3)
Total number of bacilli	12 (50)
Total number of isolates	24 (100)

Table 2. Total bacterial and fungal counts (CFU/g) of tablets from multidose packs.

Tablet	Pharmacy A	Pharmacy B	Pharmacy C	Pharmacy D	Pharmacy E
Ascorbic acid	5.0×10^2 3.0×10^2	1.0×10^2 1.5×10^2	5.0×10^2 8.0×10^2	7.0×10^2 3.6×10^2	1.5×10^2 3.0×10^2
Paracetamol	3.0×10^2 5.0×10^2	6.0×10^2 7.0×10^2	6.2×10^2 2.3×10^2	6.0×10^2 5.0×10^2	1.0×10^2 5.2×10^2
Gelucil	4.0×10^2 6.0×10^2	7.0×10^2 4.8×10^2	3.3×10^2 7.2×10^2	7.0×10^2 3.0×10^2	3.0×10^2 3.0×10^2
Folic acid	2.5×10^2 4.0×10^2	9.0×10^2 5.0×10^2	6.0×10^2 3.0×10^2	9.0×10^2 7.0×10^2	2.5×10^2 1.4×10^2
B-complex	3.0×10^2 3.0×10^2	3.0×10^2 3.0×10^2	3.0×10^2 3.0×10^2	3.0×10^2 7.0×10^2	3.0×10^2 2.3×10^2
Flagyl	2.0×10^2 3.0×10^2	1.4×10^2 2.3×10^2	3.3×10^2 4.0×10^2	1.4×10^2 5.0×10^2	1.0×10^2 4.8×10^2
Multivitamin	1.0×10^2 3.0×10^2	7.0×10^2 5.0×10^2	2.8×10^2 4.1×10^2	7.0×10^2 3.0×10^2	1.5×10^2 3.0×10^2
Chloroquine	2.0×10^2 6.0×10^2	3.0×10^2 9.0×10^2	3.0×10^2 3.0×10^2	3.0×10^2 3.0×10^2	3.0×10^2 2.0×10^2

Figures in the first row are values of total bacterial counts (CFU\g) while those in the second row are values of total fungal counts (CFU\g) of tablets.

dominant surface contaminants were yeast cells which includes *Saccharomyces*. The incidence of Bacillus isolated was higher among B-complex tablets while *Staphylococcus* species had common occurrence in

Table 3. Total aerobic bacterial and fungal count (CFU/g) of tablets in blister packs.

Tablet	Pharmacy A	Pharmacy B	Pharmacy C	Pharmacy D	Pharmacy E
Ascorbic acid	0 3	0 0	0 0	0 0	0 1
Paracetamol	2 5	1 0	0 3	2 2	0 2
Gelucil	1 6	1 1	1 1	0 0	2 1
Folic acid	2 4	2 0	2 1	0 0	0 2
B-complex	0 3	0 0	0 1	0 0	0 2
Flagyl	1 3	2 0	0 1	1 1	1 2
Multivitamin	1 3	0 0	1 0	0 0	0 0
Chloroquine	1 6	1 0	0 1	0 0	0 0

Figures in the first row are values of total bacterial counts (CFU\g) while those in the second row are values of total fungal counts (CFU\g) of tablets.

Table 4. Antimicrobial susceptibility patterns of the *Staphylococcus aureus* isolates to selected antibiotics.

Antibiotic	No. of Isolate	No. (%) Sensitive	No. (%) Resistant
Gentamicin	2	2 (100)	0 (0)
Cotrimoxazole	2	2 (100)	0 (0)
Nitrofurantoin	2	0 (0)	2 (100)
Augumentin	2	0 (0)	2 (100)
Amoxicillin	2	0 (0)	2 (100)
Erythromycin	2	0 (0)	2 (100)
Tetracycline	2	0 (0)	2 (100)
Ofloxacin	2	0 (0)	2 (100)

paracetamol, flagyl, chloroquine and ascorbic acid tablets (Table 2). In general, paracetamol and ascorbic acid were the most contaminated followed by folic acid and multivitamin tablets. Flagyl, B-complex and chloroquine tablets were the least contaminated. The tablets in the multidose packs were more contaminated than those in the blister packs. Akerele and Godwin (2002) reported the isolation of Bacillus species as the major contaminant of pharmaceutical products. Their study however, showed the presence of *Streptococcus* species and *Enterobacteria*. Obuekwe et al. (2002) had reported the isolation of *Klebsiella aerogenes*, *Pseudomonas aeruginosa* and *S. aureus* in coated and non-coated tablets. Itah et al. (2004) had reported the isolation of *Aerobacter aerogenes* and *Proteus mirabilis* from bacteriological examinations of tetracycline capsules, paracetamol and flagyl tablets from the open markets and buses in Uyo, Akwa Ibom State of Nigeria. The fact that many of the species isolated by previous investigators were not isolated in this study may suggest a more stringent quality control measures by the manufacturing companies. The presence of microbial contaminants in

non-sterile pharmaceutical products can reduce their potency and reduce the therapeutic activity of the products with their potential adverse effects to the patients taking the medicines (Nakajima et al., 2005).

The result of the antimicrobial susceptibility testing of the two *S. aureus* isolates may indicate that both organisms could be from common source. This present study indicate that the source of contamination of non sterile pharmaceutical products on sale in some outlets in Lagos is largely from the environment.

REFERENCES

Akerele JO, Godwin CU (2002). Aspects of microbial contamination of tablets dispensed in hospitals and community pharmacies in Benin City, Nigeria. Typ. J. Pharmaceu. Res*.,* 1 1: 23-28.

Atata RF, Ibrahim YKE, Akanbi AA, Abdul IF, Sani A, Ahmed RN (2007). Microbiological quality of some brands of intravenous fluids produced in Nigeria. Afri. J. Biotechnol*.,* 6 19: 2197-2200.

Brannan DK, Dille JC (1990). Type of closure prevents microbial contamination of cosmetics and pharmaceuticals. Appl. Environ. Microbiol*.,* 56: 1476-1479.

Denyer SP, Baird RM (1990). Guide to microbiological control in pharmaceutical chichesters In: Hugo B and Russel AD (eds) *Pharmaceutical Microbiology*, 5th ed. Blackwell Science Ltd., London, pp. 391-402.

Itah AY, Udokpoh AE, Ofum MU (2004). Bacteriological quality of some pharmaceutical products marketed by vendors in Uyo, Nigeria. Afr, J. Hlth. Sci., 11 3-4: 128-133.

Lucia C, Clontz C (1997). Microbial contamination and control In: *Microbial limits and bioburden test: validation approaches and requirements*. CRC Press, pp.1-7.

Martinez JE (2002). Microbial bioburden on oral solid dosage forms. Pharmaceu. Technol., 26: 58-70.

Nakajima K, Nanok K, Yamamoto K, Yamagushi N, Tani K, Nasu M (2005). Rapid monitoring of microbial contamination on herbal medicines by fluorescent staining method. Lett. Appl. Microbiol., (40) 2: 128-1323.

National Committee for Clinical Laboratory Standards (NCCLS) (2005). Performance standards for antimicrobial disk susceptibility test, edition 4. *NCCLS Document*, M2-14, Villanova, P. A. 19085.

Obuekwe IF, Ogbimi AO, Obuekwe CO (2002). Microbial contamination of pharmaceutical products in a tropical environment. Pakistan J. Sci. Ind. Res., 45 5:341-3440.

Sood R (2005). Microbiology and bacteriology In: *Textbook of Medical Laboratory Technology*, 1st ed. Jaypee Brothers Medical Publishers (P) Ltd. New Delhi, pp. 1061-1118.