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EVALUATION OF SOME SELECTED NON STERILE PHARMACEUTICAL PRODUCTS FOR BACTERIAL AND FUNGI OF CLINICAL IMPORTANCE

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ABSTRACT

This study investigated, identified, and quantified microbial contamination of 12 non-sterile pharmaceuticals products frequently made available to Akala Primary Health Care Centre Ibadan, for the possibility of detecting harmful and non-pathogenic microorganisms. Though sterility is not a requirement in official compendia for non-sterile pharmaceuticals, their bioburdens should not exceed the acceptable limit. The representative, syrups, tablets, capsules, and disinfectants from the dispensing unit were selected as guided by the conventional protocol for the study type. Bacteria and fungi of clinical potential were isolated and enumerated using standard microbiology procedures. Ten (10) of the twelve (12) non-sterile pharmaceutical products examined elicited microbial contamination beyond USP acceptable bio-burden standard. The isolates of bacteria identified comprised *Pseudomonas aeruginosa, Staphylococcus aureus,* and *Escherichia coli* elicited varied resistance to gentamicin, ciprofloxacin, tetracycline, and erythromycin but susceptible to augmentin, amoxicillin, cloxacillin, and chloramphenicol, while the *Candida albicans* fungi isolated, were susceptible to ketoconazole and fluconazole at every concentration exposed. The differences in means for CFU/mL and zones of inhibition among the microorganisms isolated were considered, data collected were analyzed using SPSS 15 and the graph was plotted using Graph Pad prism 8.1 Version 5 for Windows. Ten of the twelve (83%) of non-sterile pharmaceuticals products examined were presumably contaminated which could be an indication of improper handling, poor dispensing, poor repackaging, and or non-adherence to Good Manufacturing Practices. Therefore, training and educating the dispensers, as well as patients, on the proper handling and use of medicines to reduce or prevent microbial contamination are hereby advocated.

KEYWORDS: **Evaluation; Non-sterile pharmaceutical products; Bacteria; Fungi.**

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INTRODUCTION

Non-sterile pharmaceutical products are preparations that are produced under conditions that minimize microbiological contamination, but the processes are not monitored in the same way as during the production of sterile products. These products do not directly enter the bloodstream, and some of these products for everyday treatments include tablets, capsules, syrups, creams/ointments, and medical devices are noninvasive [1].

The presence of some microorganisms in non-sterile preparations may have the potential to reduce or even inactivate the therapeutic activity of the product and has the capacity to adversely affect the health of the patient. Manufacturers have therefore to ensure a low bioburden of finished dosage forms by implementing current guidelines on Good Manufacturing Practices during the manufacture, storage, and distribution of pharmaceutical preparations [2].

The presence of microbes in drugs not only makes them hazardous from the infectious standpoint, but may also change the physical, chemical, and organoleptic properties of the drugs, alter the contents of active ingredients or convert them to toxic products or metabolic poisons. Thus, a medicine may be considered microbiologically spoiled in this situation, depending on its intended use. The presence of even a low level of acutely pathogenic microorganisms, higher levels of opportunist pathogens, or toxic microbial metabolites that persist even after the death of the original contaminants may render the product ineffective [3].

Microbial infections are not only the result of the physical presence of microorganisms but also their metabolites/ toxins that become harmful even if they are found in minute quantities. Most of the *Escherichia coli* strains that are commonly found in the lower intestine of warm-blooded organisms are harmless, but some serotypes are pathogenic and can cause serious infections. *Staphylococcus aureus* is a member of the normal flora of the body, which is frequently found in the nose, respiratory tract on the skin, and the lower reproductive tract of women. Although *Staphylococcus aureus* is not always pathogenic, it is a pathogenic strain that often promotes infections by producing virulence factors such as potent protein toxins that bind and inactivate antibodies [4].

Pseudomonas aeruginosa is found in soil, water, skin flora, and most man-made environments around the world. It can cause disease in plants and animals including humans. It is a multi-drug resistant organism, recognized for its ubiquity, it has intrinsically advanced antibiotic resistance mechanisms, and its association with serious illnesses made the organism a source of concern. The organism is considered opportunistic insofar as serious infection often occurs because of existing diseases. It has a potential plasmid that could develop resistance to disinfectants [5].

Candida albicans is opportunistic pathogenic yeast that is a common member of the human gut flora. It does not proliferate outside the human body. It is detected in the gastrointestinal tract and mouth in 40-60% of healthy adults. It is usually a commensal organism but can become pathogenic in immune-compromised individuals under a variety of conditions. Some of these toxin-related illnesses include acute gastroenteritis, abdominal discomfort, and diarrhea. Symptoms vary from mild gastric distress to death, depending on individual susceptibility to the toxin, amount of ingested toxin, and general health of the victim [6].

Solid dosage forms (capsules or tablets) and likewise (syrups and disinfectants) are prone to microbial spoilage or degradation. The more serious problem associated with microbial contamination of solid dosage forms, is the absence of obvious signs of spoilage. Therefore, there is a need to be informed the microbial content of all drugs and medicines, whether they are sterile or nonsterile. This study evaluated the microbiological quality of some selected non-sterile pharmaceutical products obtained from Akala Primary Health Care Centre (APHCC) in Ibadan, Oyo State, Nigeria.

MATERIALS AND METHODS

Sample Collection

Three representative samples each of capsules, syrups and tablets that are made up of Jawaron, Diflucan, Basslox, M&B paracetamol, Ascorex, Flagyl, Stugeron, Paracetamol and Natdrix were randomly selected from designated containers on the shelve in pharmaceutical dispensing unit of the health centre. These capsules, syrups, tablets, and disinfectants constitute a large proportion of the pharmaceutical products in the community market, are dispensed in all health facilities, and are prone to microbial contamination under improper storage conditions. The aliquots of Savlon, Izal, and Harpic liquid disinfectant were drawn from a specific vessel that was used for the extemporaneous preparation of the disinfectant taking into consideration their ingredient composition, manufacturing date, batch number, directions for use, and expiry date.

Sample Preparation

A quantity of 2 grams collected from each brand was ground and/or dispersed in 10 mL of sterile normal saline by gentle agitation. Similarly, 2 mL of the representative syrup and disinfectant solution were each dispersed in 8 mL of sterile normal saline and made up to a volume of 10 mL. All dispersions were left to settle for five minutes to dislodge possible microbial cells and to separate the solid particles and supernatants to be used in further tests. Sterile normal saline was used as a negative control.

Isolation and Quantification of Microbial Contaminants

Exactly 1 mL aliquots from the supernatant were spread plated on Cetrimide nutrient agar (CNA), MacConkey agar (MCA), mannitol salt agar (MSA) and Saboraud dextrose agar (SDA) for the enumeration of aerobic viable bacteria and fungi, respectively. The NA and MCA plates were incubated at 37^0C for 24–48 hours while SDA plates fortified with 0.05 mg of chloramphenicol were incubated at room temperature (25±3^0C) for 3–5 days. The biochemically identified colonies were then counted and expressed as colony forming units per milliliter (cfu/mL) of samples. All counts were done in duplicate using the Quebec colony counter. Distinct colonies were subcultured repeatedly on media used for primary isolation to obtain pure culture.

Determination of Antibiogram

Antimicrobial susceptibility profiles of the bacterial isolates were determined using the agar diffusion method of Kirby Bauer. Three to five colonies of the overnight culture of the strains were inoculated into a tube containing Tryptone Soy Broth and were incubated for 24 hours at 37^0C. The inoculums were standardized by adjusting the broth cultures until the turbidity matched the 0.5 McFarland standards. A sterile cotton swab was dipped into the standardized suspension, drained and used for inoculating 20 mL of Mueller Hinton agar (Oxoid, UK) on a 100-mm disposable plate. The inoculated plates were aseptically air-dried for 30 minutes, and antibiotic discs were impregnated on the agar plates using flamed forceps. The discs were gently pressed on the agar medium to ensure maximum contact. Discs containing the following antibiotics were used: Augmentin (30 µg), amoxicillin (25 µg), erythromycin (5 µg), tetracycline (10 µg), cloxacillin (5 µg), cotrimoxazole (25 µg), chloramphenicol (30 µg), gentamicin (10 µg) and ciprofloxacin (5 µg). The plates were incubated aerobically at 37^0C for 24 hours before measuring diameters of the zones of inhibition.

Determination of antifungal Susceptibility profile

A Whatman® Grade 1 qualitative filter paper (Merck, Germany), 5 mm discs were punched, five different concentrations of ketoconazole and fluconazole (0.1, 0.05, 0.02, 0.01, and 0.005 µg/mL) each were prepared for the isolated *Candida albicans.*

Statistical Analysis

Data collected was analyzed using SPSS 15 and graph was plotted using Graph Pad prism 8.1 Version 5 for Windows.

RESULTS

A total of twelve (12) nonsterile pharmaceutical products obtained from APHCC made up of Jawaron, Diflucan, Basslox, M&B paracetamol, Ascorex, Flagyl, Stugeron, Paracetamol and Natdrix, Salvon, Izal and Harpic were examined for bacterial and fungal contaminants with their manufacturing, and expiry date and batch number into consideration as shown in Table 1.

The results as indicated in Table 2 showed 10 of the 12 products with contaminant of varied density. *Pseudomonas aeruginosa* grew in 10 of the 12 products while *Escherichia coli* was found in 1(Izal) of the 12 products.

The antibiogram of the isolates of bacterial obtained elicited varied degree of susceptibility and resistance. The isolates were Susceptible to Augmentin, amoxicillin, cloxacillin and chloramphenicol but elicited resistance to gentamicin, ciprofloxacin, tetracycline, and erythromycin as showed Figure 1.

The antifungal susceptibility of the *Candida albicans* to ketoconazole and fluconazole was susceptible to 5 varied concentrations examined as shown in Figure 2. All the isolates obtained were sensitive to Augmentin, amoxicillin, cloxacillin and chloramphenicol but were resistant to gentamicin, ciprofloxacin, tetracycline, and erythromycin at varied diameter of zones of growth inhibition.

The isolate of *Candida albicans* were sensitive to ketoconazole and fluconazole concentrations.

DISCUSSION

The study examined twelve different nonsterile pharmaceutical products that are routinely prepared, dispense or easily made available for therapeutic use. Ten of the 12 products examined were found to contain contaminants in varying density with the

Table 1: Sources of analyzed pharmaceutical products

Product type	Brand name/ Active ingredients	Batch number	Manufacturer	Manufacture date	Expiry date
Capsule	Jawaron (iron, vitamins, minerals)	0200163	Jawa International ltd	May 2020	April 2023
Capsule	Diflucan (fluconazole)	B603202	Pfizer	Nov 2021	Oct 2026
Capsule	Basslox (ampicillin +cloxacillin)	B031046	Maydon Pharmaceutical Ltd	June 2021	Nov 2023
Syrup	M&B paracetamol syrup	R220088	May &Baker Ltd	Jan 2022	Dec 2024
Syrup	Ascorex (expectorant)	10220216	Glenmark Pharmaceutical Ltd	Jan 2022	Dec 2024
Syrup	Flagyl syrup(metronidazole)	RC220224	M&B Nig PLC	April 2022	March 2025
Tablet	Stugeron (cinnarizine)	21FQ137-R1	Janssen	June 2021	May 2024
Tablet	Paracetamol	L114b	Emzor	Feb 2022	Feb 2025
Tablet	Natdrix (indapamide)	29381	Servier	Nov 2021	Nov 2023
Disinfectant	Savlon	0282204	Vervaadig deur	Jan 2020	Jan 2026
Disinfectant	Izal	P130002	Peters hygiene	Sept 2021	Nov 2024
Disinfectant	Harpic	022X	Reckitt&Benckisers Nig. Ltd	Feb 2022	Feb 2024

Table 2: Isolates of Microbes (contaminants) from the sample products

Products				
	Pseudomonas aeruginosa	***Staphylococcus aureus***	***Escherichia coli***	***Candida albicans***
Jawaron	5	-	-	-
Diflucan	1.62×10^2	-	-	5.1×10^1
Basslox	4.5×10^2	-	-	2.2×10^2
M&B PCM syrup	2.4×10^2	-	-	-
Ascorex	2.3×10^2	2	-	-
Flagyl syrup	3.0×10^1	1.62×10^2	-	2
Stugeron	-	-	-	-
Emzor Paracetamol	2.0×10^1	-	-	-
Natdrix	-	-	-	-
Salvon	3	-	-	-
Izal	4.3×10^2	2	2	4
Harpic	1	-	-	-

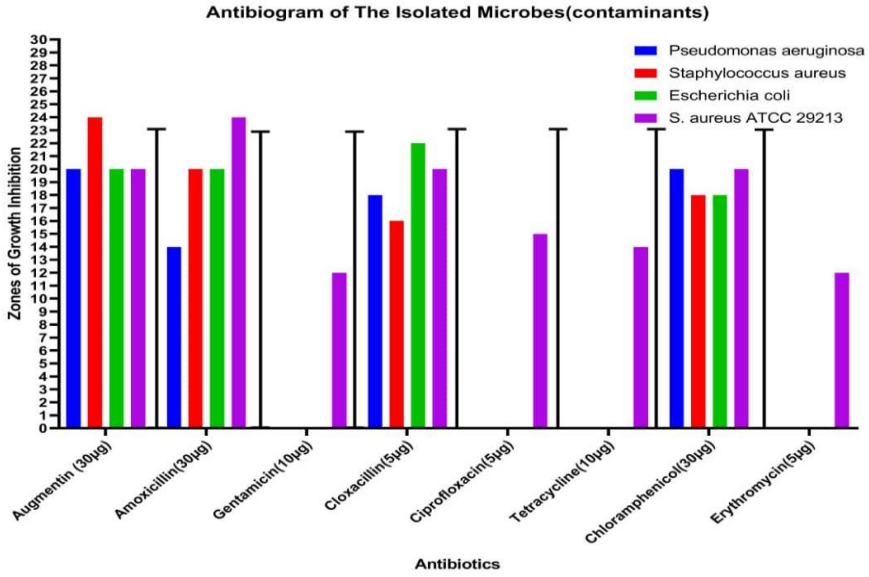

Figure 1: Antibiogram of the isolates of microbes(contaminants).

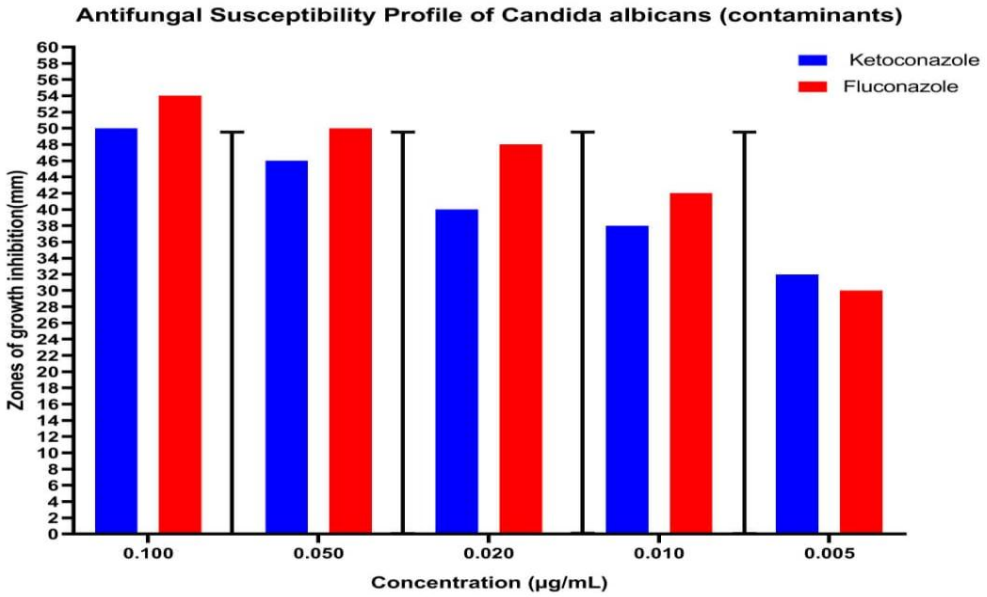

Figure 2: Antifungal Susceptibility Profile of *Candida albicans*.

exception of stugeron and natdrix that were found to support no growth of contaminants. This could be due to degree of adherence to quality control norms to preparation procedures and good manufacturing practice. This agrees with previous studies on the recovery and detection of fungal contaminants in some ointments and tablets after opening of the packages [7].

Pseudomonas aeruginosa were isolated in 10 of the 12 isolates, while *Staphylococcus aureus* were found in 3 of the 12 sample products. *Escherichia coli* were found in only 1 of the 12 products while *Candida albicans* were obtained from 4 of the 12 isolates as shown in Table 2. The prevalence of *Pseudomonas aeruginosa* amongst the bacterial isolates examined in this study could be attributed to the nutritionally non-exacting nature and the intrinsic genetic armament of this organism that could easily survive in stringent conditions. While *Staphylococcus aureus* and *Escherichia coli* obtained in varied lower proportion could be due inherent composition of the products that dictated the survivability of the isolates. This is consistent with similar findings of Mehmood *et.al.* [8] which found that most microbial contaminants in the non-sterile pharmaceuticals are pseudomonads, staphylococci, enteric isolates, Gram negatives and Gram-positive isolates in varying products [8].

The microbial loads obtained from *Pseudomonas aeruginosa, Staphylococcus aureus* and *Candida albicans* exceeded the acceptable bio-burden loads of 10^2 aqueous and non-aqueous total aerobic microbial count indicated in the harmonized Pharmacopeia <1111> for the non-sterile pharmaceutical products [9].

Microorganisms of clinical potential become problematic when they outnumber the normal flora and could create health problems and economic loss. Antibacterial susceptibility profiles of the isolates exposed elicited varying zones of growth inhibition and were recorded against 8 different conventional antibiotics. Bacterial isolates examined in this study were susceptible to Augmentin, amoxicillin, cloxacillin, and chloramphenicol but elicited resistance to gentamicin, ciprofloxacin, tetracycline, and erythromycin. The patterns of susceptibility and resistance to antibiotics studied could be attributed to strain variation amongst the isolates, reaction to chemical components of each antibiotic, environmental-related factors, and or overuse of the antibiotics which corroborates with the study of Al-Charrakh *et al.* [10] on the frequency of the antimicrobial resistant bacteria isolated from oral and topical medicaments from Hilla [10].

The observed antimicrobial resistance in this present study calls for adherence to rational uses of antibiotics to prevent the epidemics of drug resistant microbes which when ingested could aggravate illness of the immune-suppressed individuals. However, *Candida albicans* isolated in this study, were susceptible to both ketoconazole and fluconazole, though they are opportunistic pathogens, with potentials to cause deterioration of health in immune-compromised individuals [11].

The presence of potentially pathogenic opportunistic microbes, in this study, cannot be treated with ignominy, because they may cause a significant deterioration in the health status of patients, particularly those who are immunologically compromised and majority of the microorganisms isolated from the samples were normal human flora, which are widely distributed in nature [12].

CONCLUSION

The results obtained from this study showed microbial contamination of 10 of the 12 nonsterile pharmaceutical products sampled from the Primary Health Care Clinic (PHCC) elicited contamination. beyond USP acceptable bio-burden standard. This is an indices of improper handling of pharmaceutical products in our hospital pharmacies, hence calls for more stringent measures to prevent possible detrimental effects. Failure to comply with strict observation of good manufacturing practice at any stage of production may greatly affect the microbiologic quality of the end products.

Limitation of the study

Failure to establish a direct correlation between the drug-resistant microbial contaminants isolated and their direct implications for patient health in the local PHCC settings might be one of the drawbacks of the present study and such finding would have elucidated the magnitude of pharmaceutical contamination - related microbial infections. Hygienic handling and proper storage of medicine and dispensing is therefore advocated.

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