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Microbiological Quality of Selected Non-Sterile Pharmaceutical Products Retailed in Anyigba, Kogi State, Nigeria

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Abstract

This study was designed to check for the microbiological quality of non-sterile products retailed in Anyigba. The use of contaminated non-sterile pharmaceutical products can cause hazards in a majority of ways like economic loss to the industrialists, alter the therapeutic effect of the drug and affect the health of a patient. A total of 10 samples were collected; the isolated organisms were characterized and identified by using morphological, cultural and biochemical tests. The organisms isolated were *Staphylococcus aureus, E.coli* and *Pseudomonas* spp, *Aspergillus* spp, *Mucor*spp, *Saccharomyces* spp and Rhizopus spp. The percentage of the isolate includes *S. aureus* 37 (45.7%), E.coli 16 (19.7%) Pseudomonas spp 28 (34.5%) to safeguard the product from contamination, it is important to ensure that good manufacturing practices such as raw material testing, equipment sanitization and automation, microbial testing of water, training of personnel, post marking surveillance, monitoring of environment among others were employed. The focus should also be on surveillance and effective monitoring of the distribution and marketing of pharmaceutical products. Local regulatory agencies such as the Standard Organization of Nigeria (SON), and the National Agency for Food,drug administration and Control (NAFDAC) should always ensure that all pharmaceuticals released into the market for sales and consumption should conform to specifications and are fit for their intended use.

Keywords: *Staphylococcus aureus, E.coli; Pseudomonas* spp; SON; NAFDAC.

1. Introduction

Non-sterile drugs are pharmaceutical products that are not completely free from viable microorganisms or contamination due to the environment in which there are produced and the raw materials used in their formation. Examples of non-sterile drugs: solutions, ointments, creams, powders, capsules and tablets [1].

The pharmaceutical industry is an important element of health all over the world, [1]. Pharmaceutical products are divided into sterile and non-sterile products. Non-sterile pharmaceuticals are not produced by aseptic processes and therefore, are not expected to be free from the degree of contamination in non-sterile products is regulated and is based on the acceptance criteria for microbiological quality established in pharmacopeia monographs [1].

The poor qualities of medicines are not only a health hazard but also a waste of money for both government and consumers [2]. Therefore, the maintenance of quality with continuous improvement in facilities is very important in pharmaceutical industries [2]. To achieve the quality objective, it is necessary to control all stages of drugs, which covers all matters, which individually or collectively influence the quality of a product, including raw materials, manufacturing the process and the evaluation of the finished product [3].

One of the control stages is the assessment of the microbiological quality of medicinal products [4]. Syrups are viscous oral liquids that may have one or more active ingredients in solution which usually contain large amounts of sucrose or other sugars to which certain polyhydric alcohols may be added to inhibit crystallization or to modify solubilization, taste and other properties [1]. Sugarless syrups may contain sweetening and thickening agents with 95% ethanol being a preservative solvent that incorporates agents, in addition, antimicrobial agents are also added to syrups [1]. The presence of microbes in syrups is a great public health concern globally [1].

Contamination of pharmaceutical preparations with microorganisms irrespective of whether being pathogenic or non-pathogenic can bring about changes in the drugs' physical characteristics, including the breaking of emulsions, fermentation of syrups, and appearance of turbidity or deposit; besides producing possible odours and colour changes. The source of contamination may be from start-up materials, water used during manufacturing, operational equipment, the untidy surrounding environment and through workers, the pharmaceutical manufacturing and packaging environment, raw materials as well as the manufacturing water may attribute to the microbiological spoilage of the finished products [2, 5, 6]

The presence of a high number of non-pathogenic microorganisms in pharmaceutical products is objectionable as the organisms may deteriorate active ingredients and interfere with the desired activity of the product or generate toxic metabolites [5]. Since non–sterile pharmaceuticals are not produced by aseptic processes and, thus not expected to be free from microbial contaminations which can lead to significant economic loss to the industry as well as morbidity and mortality of the consumers [7].

2. Materials and Method

2.1. Inclusion Criteria

This research strictly examines the quality of non-sterile pharmaceutical drugs in the form of tablets, and syrup with various routes of administration and compositions.

2.2. Exclusion Criteria

The microbiological quality of sterile drugs will not be inclusive

2.3. Sample Collection

The samples to be used include 10 non-sterile drugs which include: Panadol extra, Nifedipine,Ampicillin, Vitamin C, Paracetamol, Diclofenac, Metronidazole,Emzolyn cough syrup,Tutolin Cough Syrup, Moduretic randomly selected from different pharmaceutical retails in Anyigba.

2.4. Isolation and Quantification of Microbial Contaminants

Selective and nonselective culture media will be used for quantification and isolation of the microbial contaminants, which are nutrient agar, Saboraud dextrose agar, and MacConkey agar. A 1 mL aliquot of each suspension will be directly plated onto the sterile media and incubated for 24–48 hours at 37^0c [8]. Pure and single microbial colonies will be subcultured onto solid and liquid media, and incubated at 37°C for 24 hours, and the substantive isolates will be finally stored at 4°C until further use.

2.5. Identification of Microbiological Contaminants

Isolated microbial contaminants will be subjected to standard microbiologic identification tests [9]. This includes;

2.5.1. For Bacteria

Gram Staining, Indole test, Citrate utilization test, Catalase test, Coagulase test, Methyl- Red Test, Urease Test and motility test

2.5.2. For Fungi

Isolated organisms will be subjected to standard microbiologic identification tests based on cultural characteristics and colony growth morphologies.

3. Result

3.1. Colony Forming Unit (CFU/ml/g) of the Samples on Nutrient Agar and MacConkey Agar

Table 1 shows the bacteria load of the samples analyzed on Nutrient agar and Macconkey agar. Sample A had the highest bacteria load of 1.8 $x10^3$cfu/ml/g and Sample H have the lowest bacteria load of 1 $x10^2$.

Table-1. Colony forming unit (cfu/ml/g) of the samples on Nutrient agar and MacConkey agar

Sample	NA(cfu/ml/g)	MAC(cfu/ml/g)
A	1.8 x 10^3	-
B	3 x 10^2	-
C	4.2 x 10^2	-
D	1.2 x 10^3	-
E	6.3 x 10^2	-
F	-	-
G	-	2 x 10^2
H	-	1 x 10^2
I	-	-
J	-	-

Key: (-) = no growth,
NA - Nutrient Agar, MAC - MacConkey Agar,
A – *Panadol extra*, B – *Vitamin* C, C – *Nifedipine*, D – *Ampicillin*,
E – *Metronidazole*, F – *Diclofenac*, G – *Paracetamol*, H – *Moduretic*, I – *Emzolyn*, J – *Tutolin*

3.2. Colonial Morphology, Gram Reaction and Biochemical Characteristics of Bacteria Isolates Sample

Table 2 shows the colony morphology of the different isolates according to their shapes,colour,elevation,edge,consistency,and colony surface.The gram reaction shows the isolate's ability to retain the primary dye (crystal violet) which classifies them as gram-positive and gram-negative organisms.Also, different biochemical test was used for the identification of the various isolates.

Table-2. Colonial Morphology, Gram Reaction and Biochemical Characteristics of Bacteria Isolates from Samples

	Morphology						Biochemical test								
Isolate	Shape	Colour	Elevation	Edge	Consistency	Colony surface	Gram reaction	Cat	Ind	Mot	Coa	Met	Ur	Cit	Probable organisms
1	Circular	Milky	Raised	Entire	Moist	Smooth	+	+	-	+	+	+	+	+	*S. aureus*
2	Circular	Yellow	Raised	Entire	Moist	Smooth	-	-	+	+	-	+	-	-	*E.. coli*
3	Circular	Milky	Raised	Entire	Moist	Smooth	+	+	-	-	+	+	+	+	*S. aureus*
4	Circular	Milky	Raised	Entire	Moist	Smooth	+	+	-	-	+	+	+	+	*S. aureus*
5	Circular	Greenish	Flat	Irregular	Moist	Smooth	-	+	-	+	-	-	-	+	*Pseudomonas*spp
6	*	*	*	*	*	*		*	*	*	*	*	*	*	
7	Circular	Milky	Raised	Entire	Moist	Smooth	+	+	-	-	+	+	+	+	*S. aureus*
8	Circular	Milky	Raised	Entire	Moist	Smooth	+	+	-	-	+	+	+	+	*S. aureus*
9	*	*	*	*	*	*	*	*	*	*	*	*	*	*	
10	*	*	*	*	*	*	*	*	*	*	*	*	*	*	

Key Cat = Catalase Met = Methylred(-) = positive, Ind = Indole Ur = Urease (-) = negative (*) = no growth, Mot =motility , Cit = Citrate , Coa= Coagulase

3.3. Microbial Counts of Non-Sterile Drugs Samples

Table 3 shows the accepted microbial limits of bacteria for non-sterile Pharmaceutical products [10] Sample A wasmicrobiologically unacceptable because the Total Viable Bacteria Count exceed the limits 10^3cfu/g/ml. Sample B was also microbiologically unaccepted because the specified organism *E.coi* is present, Sample D was microbiologically unacceptable because the Total Viable Bacteria (TVBC) exceed the accepted. While other samples were microbiologically acceptable.

Table-3. Microbial Counts Of Non-Sterile Drugs Samples

Drug Sample	TVBC	Specified microorganisms (*E. coil*)	Comments
A	1.8×10^3	Absent	Microbiologically unacceptable
B	3×10^2	Present	Microbiologically unacceptable
C	4.2×10^2	Absent	Microbiologically Acceptable
D	1.2×10^3	Absent	Microbiologically unacceptable
E	6.3×10^2	Absent	Microbiologically Acceptable
F		-	-
G	2×10^2	Absent	Microbiologically Acceptable
H	1×10^2	Absent	Microbiologically Acceptable
I	-	-	-
J	-	-	-

Accepted microbial limits of bacteria for non-sterile oral drugs [10]
KEY: TVBC = total viable bacteria count (-) = no growth
($*10^2$ cells/ml) = the Acceptable microbial limits of bacteria for syrup
($*10^3$ cells/g) = the Acceptable microbial limits of bacteria for the tablet.

3.4. Frequency Occurrence of Bacteria Isolate from Non-Sterile Pharmaceutical Products

Table 4 shows the frequency of occurrence of bacterial isolates. *Staphylococcusaureus* had the highest frequency with 37(45.7%) and *E.coli* had the least frequency with 16(19.7%)

Table-4. Frequency Occurrence of Bacteria Isolate from Non-Sterile Pharmaceutical Products

Isolates	% A	%B	%C	%D	%E	%G	%H	Total
S.aureus	10 (58.8)	6 (42.9)	4 (40	3 (30)	0	6 (60)	8 (66.6)	37 (45.7%)
E. coli	2 (11.8)	5 (35.7)	2 (20)	3 (30)	1 (12.5)	1 (10)	2 (16.7)	16 (19.7%)
Pseudomonas spp.	5 (29.4)	3 (21.4)	4 (40)	4 (40)	7 (87.5)	3 (30)	2 (16.7)	28 (34.5%)

E– Metronidazole, F – Diclofenac, G – Paracetamol, H– Moduretic,
I – Emzolyn,J – Tutolin.
Key: A – Panadol extra, B – Vitamin C,C – Nifedipine, D– Ampicillin,

3.5. Colonial Morphology of Fungi Isolated From Non-Sterile Pharmaceutical Products

Table 5 shows the colonial morphology of fungi isolated from the non-sterile pharmaceutical product according to their spore, septate or non-septate hyphae, texture, and description of the colony on media. The probable organisms isolated include Aspergillus spp, Mucor spp, Penicillium spp, Rhizopus spp and Saccharomyces spp.

Table-5. Colonial Morphology Of Fungi Isolated From Non-Sterile Pharmaceutical Product

Isolate	Description of colony	Septate/Aspetate	Spore	Texture	Probable organisms
1	Greyish black spore with white margin	Septate	Sporulating	Powdery	*Aspergillus* spp
2	-	-		-	
3	Whitish cream raised and smooth colony	Aseptate	Sporulating	Velvety	*Mucor*spp
4	Green spores with white margin	Septate	Sporulating	Powdery	*Penicillium*spp
5	Whitish cotton –like with black spores	Aseptate unbranched	Round and black	Velvety	*Rhizopus*spp
6	-	-	-	-	-
7	Whitish cotton –like with black spores	Aseptateunbranched	Round and black	Velvety	*Rhizopus*spp
8	Small cream colonies that are raised and smooth	Aseptate	Budding cells	Velvety	*Saccharomyces spp*
9	-	-	-	-	-
10	-	-	-	-	-

Key: (-) = No Growth

4. Discussion

The result of this study showed that the microbial load in tablets is high while the syrup produces no growth. They include sample A (Panadol extra) is $1.8x10^3$, B (Vitamin C) is 3 $x10^5$, (Nifedipine) is 4.2 $x10^2$, D ampicillin 1.2 $x10^3$, E (metronidazole) 6.3 $x10^2$, G (Emozor para),2.0 $x10^2$, and H (moduretic) $1x10^2$ from work sample A, B and D are microbiologically unacceptable because the total aerobic microbial count is greater than the standard acceptable limit.

According to the acceptance criteria of non-sterile dosage from (European pharmacopoeia 2010). Total anaerobic microbial count (cfu/g/ml) is 10^3 and total yeast and mould count (cfu/g/ml) 10^2 specified that *E. coli* is present while samples F (diclofenac), I (cough syrup) and J(totulin cough syrup) have no contamination which may be due to proper hygienic practices, good manufacturing practices, proper storage and proper handling and packaging.

This work is similar to Akerele and Ukoh [11]; Obuekwe, *et al.* [12]. All the samples examined the standard limit for non-sterile preparation which agrees with the work.

Isolated from this work include *Staphylococcus Aureus, E. coli And Pseudomonas* while the fungi include *Aspergillus* spp, *Mucor*spp, *Saccharomyces* spp *and Rhizopus*spp. *Staphylococcus aureus* has the highest frequency of occurrence of 37(45.7%) This is the same as the finding of [14] the above list organism was also isolated, this work also agrees with the work of [13] who observed that *Staphylococcus aureus* was the bulk of microbial contamination of the result of this survey show contamination of drug sample which could be from varying sources this finding agrees with the report of Kabir and Dulal [14]. This could be a result of the water, environment, human resources, source from packaging as well as its natural surface flora, the skin and the respiratory tract could be the possible source of contamination, material and excipient and personnel [14]. These entire factors may account for the incident of drug contamination observed in this study.

[15] reported that *Staphylococcus aureus* and *E. coli* are the major contaminants of pharmaceutical products agree with this work.

Fungi isolated were *Aspergillus* spp, *Mucor*spp, *Penicillium* spp, *Rhizopus*sppand *Saccharomyces* spp Adetunji, *et al.* [16] and Hanlon and Hodges [17] carried out quality testing on non-sterile drugs and he also isolated *E.coli, Staphylococcus aureus* which was among the organism isolated in this work.

The fungi isolated are *Aspergillus niger, Mucor*spp, *Saccharamyces*spp *and Rhizopus*spp. Where the same as fungi isolated in the findings of Aha, *et al.* [18] also correlate with the finding of Ratajcza, *et al.* [3].

5. Conclusion

Most of the brand's non-sterile pharmaceutical products sold in outlets in Anyigba were found to contain various levels of microbial contaminants which may constitute a public health concern and economic problem. A significant number of microorganisms isolated from the sample were either from human sources or from airborne. Local regulatory agencies such as the Standathe rd Organization of Nigeria (SON), and the National Agency for Food, drug administration and Control (NAFDAC) should always ensure that all pharmaceuticals products released to the market for consumption and sales should conform to specifications and fit for their intended use.

Non-sterile preparations have less stringent requirements regarding the exclusion of microbes. They need not be sterile but it has to be shown that some specifically named organisms are not present in them [10].

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