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MICROBIOLOGICAL LOAD OF SELECTED ORAL LIQUID PHARMACEUTICALS

[1]EMEJURU M.C., [1]OJIEGBE G.C., [1]AZI S., [2]NWOSU N.B.
Department of [1]Medical Laboratory, Ebonyi State University, Ebonyi State, Nigeria. [2]Medical Microbiology, St Charles Hospital, Borromeo-Onictsha, Anambara State, Nigeria.
Corresponding author: *macchyke01@yahoo.com*

ABSTRACT

The microbiological quality of 24 samples of oral pharmaceuticals comprising antacids, cough and paracetamol syrups purchased randomly from different drug stores operating in Abakaliki metropolis were assessed. They were analyzed by pour plate method. Their microbial load was determined using the viable cell count method. The resulting contaminating microorganisms were isolated and characterized by standard methods. The results revealed fungal and bacterial contaminations in 16 and 19 samples respectively. Contaminant bacteria include *Bacillus spp., Staphylococcus spp., E.coli, Proteus spp, Klebsiella spp.* and *Pseudomonas spp.* with *Staphylococcus spp.* being the most predominant bacterial contaminant, while fungi contaminants were basically *Mucor* and *Aspergillus* species. The pH values of the analyzed drugs ranged from 5 to 9. The variations in the stated pH of sampled products were however, not justified in this work; thus queries the stated drug pH and why certain isolated organisms could grow on such pH outside their normal habitats' pH. This study has shown therefore, that some oral pharmaceuticals sold in drug stores maybe heavily contaminated by varying microbial agents.

Keywords: *Oral, Pharmaceuticals, Bacteria, Fungi, Contamination.*

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INTRODUCTION

At the beginning of the 21st century, microbial contamination of non-sterile products became one of the major reasons for product recalls and production shutdown (Jimenez, 2004). So far, microbial contamination of non-sterile pharmaceutical liquid products is rapidly becoming a matter of worldwide concern (Sykes, 1971), considering the fact that oral liquid pharmaceuticals are expected by their nature, to achieve maximum degree of sterility (Baird and Petrie, 1981). Unfortunately, Adeshina *et al*. (2009) submitted that oral liquid drug formulations such as aqueous solutions, suspensions, emulsions, and syrups used for children are at a greater risk of microbial contamination during consumption due to its sweetening-agent content, reconstitution methods, improper storage, and handling defects, which may ultimately contribute to secondary bacterial and fungal infections in these patients. This indicates that microbial contamination of routine drugs is becoming a public health challenge.

The United States Pharmacopoeia (USP) (1980) had suggested that liquid pharmaceuticals should not exceed 103 colony forming units per milliliter (cfu/ml) of bacteria, while yeast and mold should not exceed 102cfu/ml. Similarly, the National Agency for Food and Drug Administration and Control (NAFDAC) Hand Book (2000) had outlined the standard microbiological specifications for the certification of syrup oral suspensions. Thus, typical viable and fungal counts for bacterial and yeast cells must not exceed $1.0x10^3$cfu/ml and $1.0x10^2$cfu/ml for bacterial and fungal growth respectively.

Furthermore, Mohammad et al. (2012) had admitted in their work that the presence of certain micro-organisms in non-sterile pharmaceutical products adversely affects the therapeutic activity of the product and even becomes detrimental to the health of the patient.

With the exception of preparations which are terminally sterilized, the micro-flora of final products may represent the contamination from the raw materials used in their formulation, the equipment used, the atmosphere, the operator of the process or from the final containers into which it was packed.

Indeed, as antacids, cough syrups and paracetamol syrups are the three widely used oral liquid formulations (amongst others) in Nigeria (Tukur *et al.*, 2012), their microbiological safety is an important public health concern considering the fact that they are heavily commercialized and consumed by many people.

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This study therefore, is designed to evaluate the microbial load in some selected oral liquid pharmaceuticals in comparison with the permissible levels of contaminants officially allowed for such commercially available products.

MATERIALS AND METHODS

Study area: This study was carried out in Abakaliki Metropolis, the Capital City of Ebonyi State, situated in the Southeastern geopolitical zone of Nigeria. Geographically, Abakaliki is located between $6^{0}15^{1}18^{11}$N and $8^{0}05^{1}$ 55^{11}E. The state owned University (Ebonyi State University), the Federal Teaching Hospital, and other governmental/non-governmental institutions are amongst the institutions located in Abakiliki.

According to Nigeria Population Commission (NPC) census (2006), Abakaliki has a population of about 149,683 with the larger population being civil servants. The state is notable for her agricultural prowess and renowned for the production of salt; hence the state acronym "salt of the nation".

Sampling technique: Twenty-four (24) properly sealed and packed oral liquid drugs of antacids, cough syrups, and paracetamol syrups (2 samples each of 4 different brands) produced by NAFDAC approved manufacturing pharmaceutical companies, were randomly purchased from various operating drug stores in Abakaliki metropolis. They were checked for their batch numbers, production date, expiry dates and certified intact.

The samples were then labeled for the purpose of this work and stored in a cool and dry laboratory shelf at room temperature (24-25^{o}C) as recommended by the manufacture.

LABORATORY ANALYSIS

Preliminary assessment (Macroscopy): The NAFDAC number, colour, and taste of the samples were assessed. Drug pH and preservatives/constituents were also noted (Table 1).

Enumeration of microbial contaminants (Microscopy): Inoculation by pour plate method was carried out after 1 in 100 serial dilutions of the various samples were carried out. 1ml of each of the diluted sample was then aseptically aspirated into each media (Nutrient Agar, MacConkey Agar, and Sabouraud Dextrose Agar) respectively. The media was poured aseptically into the sterile Petri dishes for each media at 40-45^{o}C and then swirled following which they were allowed to solidify for incubation at the appropriate temperature (Nutrient and MacConkey Agar plates at 37^{0}C for 24-48 hours; while Sabouraud Dextrose Agar plates at 25 ± 2^{0}C for 3-5days) for the enumeration of total viable and fungal counts respectively.

Typical colonies of microbial growth on plates were counted at the end of incubation and the total number of counts were multiplied by 10^{-2} (dilution factor) to get the total viable count for all samples as described by Fawole and Oso (1988) and Ochei and Kolhatkar (2008).

The results obtained were after wards, compared with the standard microbiological specifications for certification of syrups and with the microbiological permissible levels as judged by USP (1980) and NAFDAC Handbook (2000).

Characterization of isolates: The bacterial isolates were identified by Gram staining techniques and other biochemical tests (catalase, coagulase, oxidase, indole, methyl red and motility test by hanging drop method) as described by Fawole and Oso (1988) Cheesbrough (2000), Baker et al. (1998) while fungal isolation was based on growth rate and colonial morphology as described by Ochei and Kolhatkar (2008).

Sterilizations of glassware's and materials used for this research work were achieved by the use of direct heat and hot air oven as described by Isu and Onyeagba (2002).

RESULTS

Results from the microbiological analysis showed that out of the 24 samples of different brands of three oral pharmaceuticals (antacid, cough syrup and paracetamol syrup), 19 (79.17%) yielded growths while 5 (20.83%) yielded no growths (see tables 2 and figure 1).

Also, a number of bacteria organisms were isolated from the various samples as shown in tables 3 below.

For fungal isolates, 16 (66.67%) out of the 24 sample analyzed yielded growths while the rest 8 (33.33%) yielded no growth as shown in tables 4 below. Most recognizable fungal isolates were of *Mucor* and *Aspergillus* species. The growth pattern (in percentages) was graphically illustrated in figure 2 below.

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Table 1: Preliminary Assessment of the samples

Sample/brand	NAFDAC No	pH	Colour	Taste	Constituents/Preservatives used
Antacids					
Emtricil	04-1455	9.0	White	Sour	AMS, MT, MC, SB, S
Gestid	04-0480	8.0	Pink	Sour	Dried AH Gel, MH, Activated PMS.
Mist mag	04-3165	9.0	White	Sour	MT, Light MC.
Stopacid	04-4841	8.0	White	Sour	MH, Dried AH gel,
Cough syrups					
D-koff	04-2751	5.0	Amber	Sweet	DPH, BH, AC, SC, Methanol
Emzolyn	04-0266	5.0	Amber	Sweet	DPH, AC, SC, Methanol
Benylin	04-0887	5.0	Pink	Sweet	DPH, SC, PH Eur
Codrux	A4-7704	5.0	Pink	Sweet	DPH, AC Methanol SC.
Paracetamol syrups					
Albemol	A4-7709	5.0	Pink	Sweet	Paracetamal BP
Acepol	A4-0704	5.0	Pink	Sweet	Paracetamal BP
Emcap	04-7339	5.0	White	Sweet	Paracetamal BP
MB 5	04-0247	5.0	Pink	Sweet	Paracetamal BP

KEY: AMS - Aluminum Magnesium Silicate; MT - Magnesium Trisilicate,; MC - Methicone.; MH - Magnesium Hydroxide; Dried AH Gel - Dried Aluminum Hydroxide Gel; Activated PMS - Activated Polymethylsiloxane ; DPH- Diphenhydramine Hydrogen chloride; BH- Bromohexine Hydrogen chloride; AC - Ammonium Chloride; SC- Sodium Citrate

Table 2: Bacterial Growth Analysis on Samples and their Percentages

Sample/brand	No. sampled	No. of growth	No. of no growth	% growth
Antacids				
Emtricil	2	2	-	100
Gestid	2	-	2	0
Mist mag	2	2	-	100
Stopacid	2	1	1	50
Cough syrups				
D-koff	2	2	-	100
Emzolyn	2	2	-	100
Benylin	2	1	1	50
Codrux	2	2	-	100
Paracetamol syrups				
Albemol	2	1	1	50
Acepol	2	2	-	100
Emcap	2	2	-	100
MB5	2	2	-	100

Table 3: Bacteria's isolated from each of the samples

Sample/brand	No. of brands with growth	Organisms isolated
Antacids		
Emtricil	2	*Staph.* spp.
Mist Mag	2	*E. coli, Klebsiella spp., Proteus spp.*
Stopacid	1	*Klebsiella spp., Proteus spp., Bacillus spp., E.coli.*
Cough syrups		
D- koff	2	*Staph spp*
Emzolyn	2	*Pseudomonas spp.,E.coli, Proteus spp.*
Benylin	1	*Staph. spp.*
Codrux	2	*E.coli.*
Paracetamol syrups		
Albemol	1	*Staph. Spp.*
Acepol	2	*Staph. spp., Pseudomonas spp.*
Emcap	2	*Proteus spp.*
MB 5	2	*Staph spp.*

Table 4: Fungal Growth Analysis on each of the Samples and their Percentages

Sample/ brand	No. sampled	No. that showed growth	No. that showed no growth	% Growth
Antacids				
Emtricil	2	2	-	100
Gestid	2	1	1	50
Mist Mag	2	2	-	100
Stopacid	2	1	1	50
Cough syrups				
D-koff	2	2	-	100
Emzolyn	2	-	2	100
Benylin	2	1	1	50
Codrux	2	1	1	50
Paracetamol syrups				
Albemol	2	2	-	100
Acepol	2	1	1	50
Emcap	2	2	-	100
MB 5	2	1	1	50

DISCUSSION

This study aligns with the argument by Sheikh *et al*., (1988) that sterility is not a requirement in the official compendia for oral pharmaceutical dosage forms. It also implies that contamination may occur during manufacturing, packaging, and/or handling by the consumer (Baird, 2004; Ibezim et al., 2002). Of course, this raises concern since some drug forms, even though stored in favorable environment, can serve as substrates for micro-organisms (Sapra *et al.*, 2012). More so, variations in the stated pH of sampled products were not highly justified, thereby querying the authenticity of such stated (but questionable) pH; contrary to established normal habitat's pH. Indeed, the ability of certain organisms (as seen in this study) to grow at such stated pH, makes it more worrisome. This implies that certain drug constituents (in addition to their normal roles), are microbial growth enhancers. Interestingly, Baird and Petrie (1981) states that liquid antacids often contain ingredients of high basic pH, which readily support the growth of a variety of micro-organisms, when appropriate precautions are not taken.

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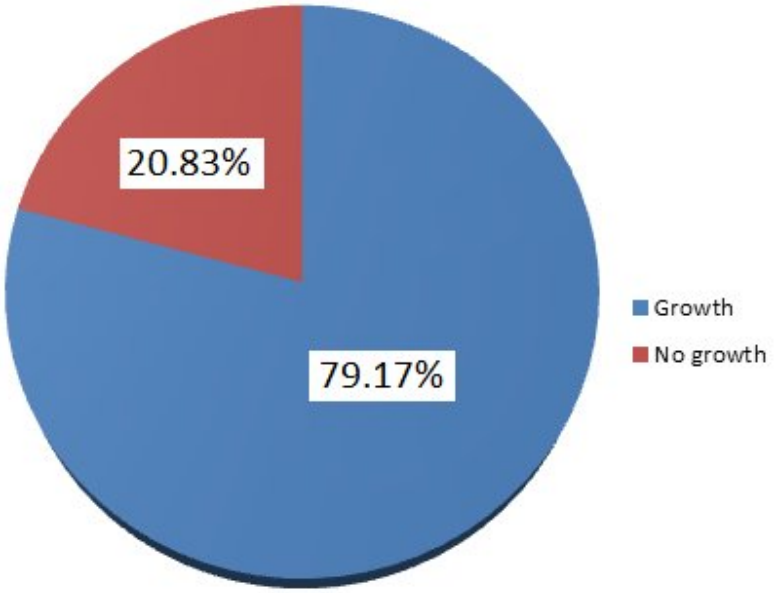

Fig I: Pie- chart representation of percentage bacterial growth and no growth
NB: Summary: No. of growths = 19(79.17%) No. of no growths = 5(20.85%)

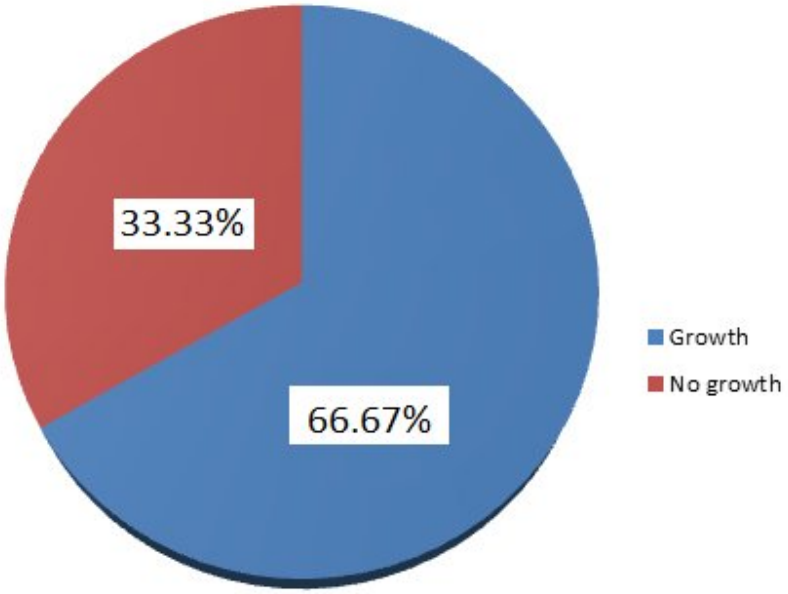

Fig 2: Pie- chart representation of percentage fungal growth and no growth
NB. Summary: No of growths = 16 (66.67%) No of no growths = 8(33.33%)

According to NAFDAC Handbook (2000), bacterial count should not exceed 1000 cfu/ml, but unfortunately, out of the 19 samples with growth, 8 (42.11%) samples exceeded this rule in Nutrient Agar, while 11 (57.89%) samples exceeded it in MacConkey Agar.

Given the heavy contamination observed in this study, predominantly *Staphylococcus aureus*, it is a worrisome trend as such organism secretes toxins that contribute to serious gastrointestinal distress (Denyer, 1990). In addition, the presence of *Escherichia coli* is a good indicator of faecal contamination probably

from the water used. This might be responsible for reported cases of infant diarrhea in some parts of Nigeria (Antai and Anozie, 1987).

Similarly, NAFDAC Handbook (2000) stipulates that fungi count must not exceed $1.0x10^2$cfc/ml, but the results on fungi count far exceeded this value in all the 16 samples with fungi growth. Given the high toxin producing nature of some Aspergillus spp, especially *Aspergillus flavus*, serious problems could result in man due to its high aflatoxin production- a known carcinogenic agent (Fortnum, 1986; Payne and Brown, 1998; Abbas *et al.*, 2004).

Over all, the findings of this study imply that water supply, raw materials and lack of personal hygiene have remained the major sources of contamination in pharmaceuticals. Also implicated, are the high levels of sweetening and preservative agents (Mahboob *et al.*, 2004). In fact, sweeteners of high sugar category in drugs, have been known to enhance microbial growth (Mahboob *et al*., 2004); hence the need for caution.

It is our recommendation therefore, that health care providers should monitor this trend, while NAFDAC is encouraged to tighten regulations in this regard.

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AUTHOR'S CONTRIBUTION

All the authors (Emejuru MC, Ojiegbe GC, Azi S. and Nwosu NB) contributed to the study from the design, data collection and analysis to the manuscript preparation and presentation of final draft.