Bacteriological Assessment Of Some Selected Antacid Suspension Products In Nigeria

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ABSTRACT: This study was carried out to ascertain the bacteriological quality of some brands of antacid suspensions produced in Kwara State and Ogun State, Nigeria. Three different brands of antacid suspensions were assessed. The samples were examined for the presence of bacteria using standard techniques. The samples examined include, Magsil, Biomag and Mist Mag. The enumeration of total viable count (TVC) was done using pour plate method. The study showed that Magsil has the highest TVC of 5.0 x 10^3 CFU/mL, Biomag had a TVC of 3.0 x 10^3 CFU/mL while Mist Mag has the lowest TVC of 2.0 x 10^3 CFU/mL. The results obtained showed that none of the brands of these antacid suspensions conformed to the international standard. The bacterial isolated were identified to be *Corynebacterium* sp, *Enterococcus* sp, *Lactobacillus fermenti, Staphylococcus aureus, Staphylococcus epidermidis, and Streptococcus* sp. However; no objectionable organisms such as *Salmonella typhi* and *Escherichia coli* was detected. Proliferation of bacterial contaminants can lead to product spoilage, recalls and outcomes that are detrimental to health and business.
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1. INTRODUCTION

Antacid is any substance, generally a basic salt which contract stomach acidity in other words, antacids are acid neutralizer (Malik *et al*., 1991). Suspension is heterogeneous fluid containing solid particles that sufficiently large for sedimentation. Usually, they must be larger than one micrometer. Suspension is a mixture of two chemicals with the character that does not rapidly settle out (Denyer and Baird, 2007). One of the major concerns with formulating an antacid liquid is the preservative efficacy (Hoq *et al*., 1991). Liquid antacid preparations are generally susceptible to microbial contamination. The pH of any aqueous solution is critical to controlling the microbial growth within the solution. Generally, acidic solutions (pH 3-6) are less susceptible to microbial growth than alkaline solutions of pH 8-9 (Hoq *et al.,* 1991).

Antacid is used to treat the symptoms of too much stomach acid such as stomach upset, heat burn, and acid indigestion. It is also used to relieve symptoms of extra gas such as bloating, belching, and feelings of pressure and discomfort in the stomach and gut (Hugo and Rusell, 2007). Aluminum and magnesium antacids work faster and better than tablets or capsules (Hugo and Rusell, 2007). The medication works only on existing acid in the stomach. It does not prevent production. It may be used alone or with other medications that lower acid production (Malik *et al*., 1991). The clinical use of antacids is based on their ability to neutralize stomach acid and increase the pH of gastric secretions. Although antacids do not neutralize all gastric acids, increasing gastric pH from 1.3 to 2.3 neutralizes 90% and increasing pH to 3.3 neutralizes 99% of gastric acid (Hugo and Rusell, 2007). In general, liquid antacid suspensions are preferred to tablets or powders since they are more rapidly and effectively soluble and have a greater ability to react with and neutralize gastric acid (Hugo and Rusell, 2007).

The quality of pharmaceuticals cannot be compromised as these constitute a group of products ingested into the human and animal systems by routes such as oral, parenteral, topical etc (Tella et al., 2011). These groups of products therefore have direct bearings on our well being and there is therefore an absolute need to guarantee their quality, safety and efficacy (Tella et al., 2011). Drugs therefore have to be designed and produced such that when patients receive them for management of their ailments, they do not produce any adverse side reactions on such patients or their unborn babies (Tella et al., 2011).

It has been well established that microorganisms have a vital role to play in the degradation of spoilage in antacid suspension products. Microorganisms are extremely versatile and adaptation in their ability to synthesize the degradable enzymes contributes largely in spoilage of pharmaceutical products. Proliferation of bacterial contaminants can lead to product spoilage, recalls and outcomes that are detrimental to health and business. The aim of this study was to assess the microbial quality of some selected antacid suspension products in Nigeria, so as to ascertain its bacteriological fitness for use, base on laid down standard for specification.

2. MATERIALS AND METHODS

2.1. Sample collections

Samples were collected from different pharmaceutical industries. A total of three samples were collected which are; Mist Magnesium- Tuyil pharmaceutical industries limited, Biomag- Bioraj pharmaceutical limited, Magsil- Leady-pharma industries limited and samples were taken to the laboratory for analysis. Table 1 shows the brands of Antacids collected and their respective manufacturing industries.

Table 1: Different brands of Antacids collected

Brand Name	Pharmaceutical Industry
Magsil	Leady-pharma Industries Limited, Ogun state
Biomag	Bioraj Pharmaceutical Kwara state
Mist Mag	Tuyil Pharmaceuticals Kwara state

2.2. Enumeration, Isolation and Identification of Isolates

All the chemicals and reagents used were of analytical grade, obtained from Sigma chemical co. Ltd, England. The media used for the bacteriological analysis of antacid include Plate Count agar (PCA), Nutrient agar (NA), Mac Conkey agar (MCA), blood agar (BA) and MRS broth of de Man, Rogosa & Sharpe (1960) diffusate + 2 % (w/v) agar. All the media used were weighed out and prepared according to the manufacturer's specification, with respect to the given instructions and directions and sterilized at 121°C for 15 min. at 15lb pressure. A serial dilution method was used. The sterility of each batch of test medium was confirmed by incubating one or two uninoculated plates along with the inoculated plates. The uninoculated plates were always examined to show no evidence of bacterial growth. Any uninoculated plate that showed evidence of bacterial growth was discarded. All the samples and the test organisms were replicated on different media and the plates were then incubated at 37°C for 24 - 48 h. Discrete colonies were sub-cultured into fresh agar plates aseptically to obtain pure cultures of the isolates. Colonies identifiable as discrete on the Mueller Hinton Agar were carefully examined macroscopically for cultural characteristics. All isolates were subjected to various morphological characterization and gram stained to determine their gram reaction. Biochemical tests were carried out as described by Jolt *et al.* (1994) to determine the identity of the bacteria isolates with reference to Bergey's Manual of Determinative Bacteriology. The isolates were identified by comparing their characteristics with those of known taxa, as described by Jolt *et al.* (1994), Cheesbrough (2006) and Oyeleke and Manga (2008).

3. RESULTS

A study to assess the bacteriological quality of some selected antacid suspension products in Nigeria was carried out to ascertain their bacteriological safety for use, base on laid down standard for specification. Table 2 shows the total viable count (CFU/ml) in Antacid Suspensions. It showed that Magsil had the highest microbial count of $5.0X10^3$ (CFU/ml) while Mist Mag had the lowest microbial count of $2.0x10^3$ (CFU/ml) as shown in Table 2.

Table 2: Total Viable Count (CFU/ml) in Antacid Suspensions

Brand Name	Total Bacterial Count (CFU/ml)
Magsil	$5.0X10^3$
Biomag	$3.0x10^3$
Mist Mag	$2.0x10^3$

Table 3 shows the distribution of bacterial Isolates in the antacids suspensions evaluated. It was observed that all the bacterial isolates recovered in this study were present in Biomag while *Enterococcus* sp was not found in Mist Mag. However, *Enterococcus* sp and *Corynebacterium* sp was not found in Magsil (Table 3).

Table 3: Distribution of Bacterial Isolates in the Samples

Isolates	Biomag	Magsil	Mist Mag
Corynebacterium sp.	+	_	+
Enterococcus sp.	+	-	-
Lactobacillus fermenti	+	+	+
Staphylococcus aureus	+	+	+
Staphylococcus epidermidis	+	+	+
Streptococcus sp.	+	+	+

Key: + = Present; - = Absent

4. DISCUSSION

There are some oral liquids in which microbiological contamination can present significant health hazards. Some oral liquids, such as nitration suspension are used in infants and immune-compromised patients, and microbiological contamination with organisms, such as Gram-negative organisms, are objectionable (Garg and Gupta, 2008). There are other oral liquid preparations such as antacids in which *Pseudomonas sp*. contamination is also objectionable (Garg and Gupta, 2008). For other oral liquids such as cough preparations, the contamination with *Pseudomonas sp*. might not present the same health hazard (Garg and Gupta, 2008). Obviously, the contamination of any preparation with Gram-negative organisms is not desirable (Garg and Gupta, 2008). In addition to the specific contaminant being objectionable, such contamination would be indicative of a deficient

process as well as an inadequate preservative system (Garg and Gupta, 2008). The presence of a specific *Pseudomonas sp*. may also indicate that other plant or raw material contaminants could survive the process (Garg and Gupta, 2008).

The bacteriological status of liquid antacid suspension of three (3) pharmaceutical industries in Nigeria was studied. Two of the samples were from Ilorin, Kwara state and the third one, Ogun state. However, the results obtained in this work which is between $2.0x10^3$ to 5.0×10^3 CFU/mL, compared to the microbiological standard which is 1000 cfu/ml does not conform with the standard, although, it met with the pharmaceutical standard which allows 10000 cfu/ml.

Previous studies showed that a wide range of bacterial contaminants have been isolated from various antacid suspensions, including *Pseudomonas aeruginosa* and *Staphylococcus aureus* (Malik *et al*., 1991). Organisms that should be totally excluded from products are objectionable; they include acute pathogens such as *Escherichia coli, Salmonella typhi* and *Pseudomonas aeruginosa*. However, according to the findings of this study, high level of opportunistic pathogens was discovered from the antacid suspensions. The bacterial species isolated were: *Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus* sp., *Lactobacillus fermenti, Enterococcus* sp. and *Corynebacterium* sp.

These microorganisms could have resulted from contaminated batches of raw materials, lapse in normal plant cleaning procedures, a particular infective or careless operator, sporadic detachment of large microbial growth from within the plants, the emergence of more aggressive species and changes in production procedures which allow growth during manufacture (Rawlins, 1996). The study also shows that *Staphylococcus* spp. account for most of the bacterial contamination. This can be easily traced to the fact that it is a normal flora of the skin with a very high chance of contaminating a wide variety of non-sterile pharmaceutical products.

Staphylococcus belongs to the family Staphylococcoceae which contains four genera of which the most important is the genus *Staphylococcus (*Willey et al., 2008). *Staphylococcus* species can be divided into pathogenic and non-pathogenic strains based on the enzyme coagulase. *S. aureus* tests coagulase positive while *S. epidermidis* tests negative. *Staphylococcus* spp are normally associated with the skin, skin gland and mucus membranes of warm blooded animals (Willey et al., 2008). In this study, the major species that were isolated were *S. epidermidis* and *S. aureus*. *S. epidermidis* is a common skin resident that is sometimes responsible for endocarditis and infections of patients with lowered resistance examples are wound infections, surgical infections and so on (Willey et al., 2008). *S. aureus* is most important staphylococcal pathogen and causes boils, abscesses, wound infections, pneumonia, toxic shock syndrome and other diseases. One of the most virulent factors of *s. aureus* is the enzyme coagulase which causes blood plasma to clot (Willey et al., 2008)

Corynebacteria species occur commonly in nature in the soil, water, plants, and food products. The non-diptheriod *Corynebacterium* can even found in the mucosa and normal skin flora of humans and animals. It can be deduced that this organism would have contaminated the suspensions via the environment or the operating personnel. *Corynebacterium diphtheriae* is a pleomorphic gram-positive rod that is isolated from the nasopharynx and skin of humans. The organism is easily grown in the laboratory on media containing 5% sheep blood. *C. diphtheriae* produces a potent exotoxin and is the causative agent of diphtheria, one of the most widespread bacterial diseases in the pre-vaccine era. Laboratory-associated infections with *C. diphtheriae* have been documented, but laboratory animal-associated infections have not been reported (Pike, 1976; Geiss et al., 1997). Inhalation, accidental parenteral inoculation, and ingestion are the primary laboratory hazards (CDC, 2009).

Enterococcus faecalis is a major cause of nosocomial infections in humans and has been linked to severe extra-intestinal infections in poultry. A zoonotic potential has been suggested (Olsen et al., 2011). It has been well established that microorganisms have a vital role to play in the degradation of spoilage in antacid suspension products (British Pharmacopoeia, 1980, 1993).

Bacterial endotoxin abounds everywhere. The Gram negative bacteria exist in particulate matter, in air, water and soil (Schaumann, et al., 2008). Endotoxin is also an impurity in sterile pharmaceuticals especially Large Volume Parenterals (LVPs) and it has to be tested for in the products meant for intravenous administration (Radhakrishnan, 2010). In an on-going research, Salawu et al. (2010) have demonstrated that delay in sterilization of parenteral solutions of up to 48 hrs could lead to production of highly pyrogenic solutions, provided the solution had been contaminated with Gram negative organism like *Escherichia coli* before the delayed sterilization (Tella et al., 2011). In their report the resultant increase in the population of the contaminating bacteria before sterilization caused an intolerable rise in pyrogen level even after sterilization (Tella et al., 2011). Such a product in real production must be discarded after the production cycle had been completed. This was because only sterilzed product can be admisnistered to rabbit for pyrogen tests (Tella et al., 2011).

Microbial quality or resistance to microbial growth has to be retained according to the specified requirements (Allen, 2002; European Pharmacopoeia 6.1, 2007; Helin-Tanninen, 2008). Products containing sufficient water to permit bacteria or fungi growth are vulnerable to spoilage (Hodges, 2007). Products contaminated with pathogenic organisms may be an infection hazard (Hodges, 2007;

Helin-Tanninen, 2008). The occurrence of microbiological growth in aqueous medicines can affect the organoleptic characteristics of the product, producing turbidity, bad odour or taste (Costello et al., 2007; Ghulam et al, 2007; Helin-Tanninen, 2008). Deterioration of the product due to bacterial or mould growth can either render the product unacceptable, harmful or toxic to the patient (Barnes, 2007; Costello et al., 2007; Hodges, 2007; Helin-Tanninen, 2008). The presence of micro-organisms and their metabolites can even impair the chemical or physical stability and the drug solubility by affecting the pH (Costello et al., 2007; Hodges, 2007; Helin-Tanninen, 2008). For example, air contamination of a cellulose syrup mixture or unhygienic use of the product can also lead to microbiological contamination (Nahata et al., 2003; Costello et al., 2007; Helin-Tanninen, 2008).

There are pharmacopoeial requirements for microbiological quality of oral preparations, i.e. not more than 10^3 bacteria and not more than 10^2 fungi per gram or per millilitre, and the absence of *Escherichia coli* (European Pharmacopoeia 6.1, 2007; Helin-Tanninen, 2008). Hygienic manufacture, sterilization and suitable preservatives are used to prevent the presence or growth of microorganisms in the product (Billany, 2007; Helin-Tanninen, 2008). The factors impacting on the hygienic manufacture of medicines are air, building, equipment, work surfaces, raw materials, both ingredient and cleaning water, formulation and personnel (Hodges, 2007; Helin-Tanninen, 2008). Health, hygiene, clothing and training of the personnel may all have an impact on product contamination (Helin-Tanninen, 2008).

5. CONCLUSION

Microorganisms are extremely versatile and adaptations in their ability to synthesize the degradable enzymes contribute largely in spoilage of pharmaceutical products (British Pharmacopoeia, 1980, 1993; Tella et al., 2011). Proliferation of bacterial contaminants can lead to product spoilage, recalls and outcomes that are detrimental to health and business. Emphasis should be placed on degradation/stability studies of drugs because improper storage and distribution of pharmaceuticals can lead to their physical deterioration and chemical decomposition resulting in reduced activity and occasionally, in the formation of toxic degradation products (Tella et al., 2011). Producers should pay more attention to good manufacturing practices and adhere to guidelines given by relevant government authorities such as the National Agency for Food, and Drug Administration and Control (NAFDAC). Compromise in the quality of pharmaceutical products is dangerous to health of consumers. Quality control measures should be reviewed and enforced until microbial contaminants are reduced to its barest minimum. It is also advised that proper attention should be paid to maintenance of hygienic conditions, stability testing and manufacturing processes. Effective preservatives should also be employed. Conclusively, home users should be adequately educated or informed on usage and storage of products to minimize the introduction of contaminants.

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