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# Potency of Preservatives in Selected Drug Mixtures in Ibadan, Oyo State, Nigeria.

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***ABSTRACT:*** *Seven pharmaceutical preparations comprising Mist Kaolin, Chemiron blood tonic, Multivitamins syrups (A and B), Chloroquine syrups (A & B), and Antacid mixture were purchased in Ibadan, Oyo State, Nigeria and analyzed to ascertain their level of microbial contamination before use. Four of the seven drugs were found to contain microorganisms while three were free from microbial contaminations. Thirteen microorganisms consisting of 11 bacteria and 2 yeasts were isolated from the drugs: three Bacillus species; B. subtilis, B. lichenformis, B. pumilis. Four Pseudomonas species, P. syringae, P. putida, P. stutzeri and P. mendocina. Other bacteria isolated are; Hafnia alvei, Lactobacillus desidiosus, Micrococcus varians and Sporolactobacillus. Two yeasts isolated were Saccharomyces graminis and S. rouxii.. Sorbic acid and sodium metabisulphite were tried on the isolates, no growth was recorded at their safe limits. It was then concluded that the preservatives used in these drugs at the stated safe limits are not effective and that appropriate quality control measures should be taken to eliminate microbial contaminations and or recontamination of drugs so as to retain the potency of the drugs.*

***KEYWORDS:*** *Concentrations, Contaminants, Drugs, Microbes, Preservatives, Safe limits.*

## I. INTRODUCTION

A preservative is a naturally occurring or synthetically produced substance that is added to products such as foods, pharmaceuticals, paints, biological samples, wood, etc. to prevent decomposition by microbial growth or by undesirable chemical changes. Preservatives can be divided into two types, depending on their origin; natural preservatives which are naturally occurring, everyday substance such as salt, honey and wood smoke and synthetic preservatives which refer to preservatives that are synthetically manufactured [1]. Preservatives in drugs have two functions; protecting the consumers from microbial contamination and maintaining potency and stability of drugs [2]. [3] put it as being essential in formulations provided the drugs will be used more than once from the same container. [4] defined preservatives in medicaments as being compulsory to protect the consumer from being infected and help in maintaining the integrity of the products. This, they stressed as very important in multidose injectable products, in sterile products – generally. This is necessary as the sterile products could be applied topically to open wounds and successive doses would be withdrawn from the same container. With preservative in most formulations, this prevents the growth of microbes if they are accidentally introduced into the formulation. [5] [6] [7][8]. Several chemicals have been employed as preservatives in pharmaceutical industries. The ultimate is to prevent microbial contamination of their products. Among the chemicals used are the alcohols, parahydroxybenzoic acid, esters, quarternary ammonium compounds, phenolics, sodium benzoates, parabens etc. [9][10] [11]

According to [12][13] ideal preservatives are expected to be:

1) Effective at low concentration against a wide variety of microorganisms;
2) Soluble in formulation at the required concentration;
3) Non-toxic and non-sensitizing externally and internally at the concentration required;
4) Compatible with a wide variety of drugs solubilizing and dispensing agents;
5) Free from objectionable odour, taste or colour;
6) Active with long-term stability over a wide range of pH and temperature;
7) Inexpensive based on the cost of products.

Preservatives are substances that commonly added to various foods and pharmaceutical products in order to prolong their shelf life. The addition of preservatives to such products, especially to those that have higher water content, is essential for avoiding alteration and degradation by microorganisms during storage [14][15][16] **.**Other ingredients are also utilized in preparing the desired dosage form of a drug substance. Some of these agents may be used to achieve the desired physical and chemical characteristics of the product or to improve its appearance, odor and taste. In each instant, the added ingredient must be harmless in the amount used; does not exceed the minimum quantity required to provide its intended effect; its presence does not impair

the bioavailability, the therapeutic efficacy or safety of the official preparation, and does not interfere with analysis and tests prescribed for determining compliance with the pharmacopeias standards **[17]**. Preservatives should not by any means penetrate or react with the closure or containers. A preservatives has its limitations which is expected to adhere to, therefore pharmacists use the one that suits the composition of the medicament being produced. Apart from meeting the requirement of the formulation, the industrial pharmacist also ensures that the preservatives is of the choice that is being governed by the practice of the company or by the customs of the particular country. The inclusion of antimicrobial preservative is essential in certain mixtures since they will support the growth of spoilage microorganisms, for example mixtures with only drug in water. This is a good source of contamination. Drugs may undergo sweetening process by a way of adding sweetening and flavouring agents. Those often used in pharmaceutical industries include sorbitol, glycerol, sucrose and propylene glycol some of which are observed to have antimicrobial activity inherent in them. [17].

Inhibition of species like *Staphylococcus aureus, Bacillus sutilis, Pseudomonas aeruginosa* and moulds were once studied and tried with the sweetening and flavouring agents. *Aspergillus niger, Penicillin notatum* and *Candida albicans* have also been tried and found to be inhibited in aqueous solution of either glycerol or propylene glycol, containing various concentrations of sorbitol, the inhibition was attributed to the osmotic pressure of the solutions. Sugars at different concentrations have also been used as inhibitory agent. Report has it that 60% invert sugar could inhibit *Aspergillus niger* and at 50% no sharp reaction was noted. Mixtures are made to contain high concentration of glycerol or propylene glycol to serve as preservative since they contain sweetening or flavouring agents .Tablets, have their physical stability of their suspension agents or emulsifying agents. Some of the agents namely, acacia, tragacanth are soil borne, carbohydrates, hence liable to microbial attack. Cellulose derivations such as methylcellulose under certain condition also support growth and lose their pharmaceutical usefulness [18][19][20][21]. Moreover, methylcellulose forms complexes with a number of preservatives notably the parabens. Synthetic, non-ionic surface active agents i.e. spans and tweens are used in pharmacy as solubilizing agents and emulsifying agents.

Microorganisms like *Pseudomonas aeruginosa, Aspergillus niger* could grow in solutions and dispersions of such substances and produce esterases which split ester linkages. This create an adverse effect on the formulation since the splitting of the surface active agents will seriously on the physical property of the drug hence the need for effective preservative. Among the preservatives used in pharmaceutical industries are the following: Parabens, or the esters of para-hydroxybenzoic acid, [22][23][24]They have been in use since 1944.

## II. MATERIALS AND METHODS

Seven pharmaceutical products in form of syrups and mixture were purchased across seven pharmaceutical shops in Ibadan, Oyo State, Nigeria. All the drugs were manufactured in Nigeria. They include chloroquine syrup A (anti-malaria); Chemiron Blood syrup; Mist kaolin mixtures (anti diarrhea); chloroquine syrup B (antimalaria); Multivitamin syrup (A) ; Multivitamin syrup (B); Magnessium trisiliticate mixure (antacid mixture) as shown on Table 1.The seven oral formulations were tested to ascertain the level of microbial contamination.

### 2.1 Media Preparation for Primary Isolation

The media were Plate Count Agar (PCA); Nutrient Agar (NA); Yeast Peptone Dextrose Agar (YPDA);

### 2.2 Plating Technique

Direct dilution of the samples were carried out. This was done as scantly colonies were got on plates when serial dilution was first carried out. The direct culturing of the drugs were carried out by pippeting 1m1. of the mixtures and syrups asceptically on sterile Petri-dishes. Pour plate technique was used. The plates with PDA and NA were incubated at $35^0$C for 2-3 days. While plates with YDA were incubated at $27^0$C for 3-4 days. Bacteria and yeasts isolated were identified in their unique colonies. They were subcultured by streaking out on Nutrient Agar and yeasts dextrose agar. Bacterial cells on NA took 2-3 days at $35^0$C and yeasts isolated took 4-5 days at $27^0$C before their pure cultures were stored on slants containing Nutrient agar for bacteria and yeasts peptone dextrose agar for yeasts and were stored at $4^0$C. These slants served as stock culture.

### 2.3 Morphological Studies

The cultural characteristics of all the isolates were examined under the microscope using oil immersion (X100) objective. The growth pattern, the colour or pigmentation and colony edges on the plates were recorded after 24hours of incubation for bacterial growth and 48 hours of incubation for the yeasts at $35^0$C and $27^0$C respectively.

**2.4 Staining Reaction**

The following reactions were carried out to know the gram reaction and spore formation technique of the isolates.

**2.5 Gram's Staining Reaction**

Smears of 24hour old isolates were on slides and heat fixed. Crystal violet was first applied and allowed to remain for 1 minute and washed off with water. Grams iodine was added. This stayed for another 1 minute and was later washed off. The smear was decolorized with alcohol, washed with water and counter stained with 1% Safranin. Smear was them blot dried with clean filter paper and observed under (X100) oil immersion lens of the microscope.

**2.6 Spore Staining**

This staining procedure was carried out to confirm the presence or absence of spores produced by the isolates. Spores production was induced by adding 0.5ml of 0.4% solution of Manganese sulphate (MnSO4 $4H_20$) per litre of medium .Smear from the medium was heat fixed and flooded with Malachite green. The slides were heated until the stain started to boil. The stain was then washed off and the smear was counter-stained with safranin. Bacterial spores stained green while vegetative cells stained red on examination under the microscope. The shapes and positions of endospore were observed. Morphological characteristics of the yeast isolates was done by making a thin smear on a glass slide stained with cotton blue in lactophenol. This was observed under light microscope using the oil immersion objective.

**2.7 Motility Test**

Motility of the isolates were determined using cavity-slide method. A drop of 24hours old bacteria suspension was placed on a cover slip and inverted over a depression of a cavity slide, so that the drop of bacteria suspension formed a hanging drop on the cover slip. The drop was observed for activity motile cells under (X100) oil immersion objective of a microscope.

**2.8 Biochemical Tests**

The isolates were made to undergo a number of biochemical tests as follows.

***2.8.1 Catalase Test***

This is a test to detect the presence or absence of catalase enzyme. The catalase enzyme catalyses the breakdown of hydrogen peroxide to release free oxygen gas and the formation of water. A few drops of 3% $H_20_2$ was added to a 24 hour old culture of isolates on slide. Evolution of a gas a a white froth indicated a catalase positive reaction while the absence of the effervescence or white froth showed negative reaction.

***2.8.2 Voges-Proskauer Test***

This is a test carried out to know if the isolates can produce acetymethylcarbinol from glucose. The medium used is glucose phosphate broth. The sterilized medium was distributed into test tubes in 5ml volumes and inoculated with the isolates, an uninoculated tube served as control.

***2.8.3 Methyl-red Test***

This test was used to determine whether the production of acid glucose has lowered and help pH at about 4.2 or below. The medium used is glucose-phosphate broth. This was distributed into screw caps and was sterilized at $121^0$C for 15 minutes. Isolated were grown in the medium for two days after which methyl-red test reagent was added. Uninoculated tubes served as control. Development of yellow colour was recorded as negative result.

***2.8.4 Starch Hydrolysis***

This test was carried out to determine the production of amylase by the isolates. Starch agar plates were made by dissolving and melting starch to gelatinization in a known volume of distilled water and adding to a melted nutrient agar to give a final concentration of 1% starch.

The medium was autoclaved and poured into sterile plates before solidifying. Single streak of isolates was done on plate and incubated at $37^0$C for 48 hours, after which plates were flooded with Gram's iodine. Unhydrolysed starch formed a blue colour with the iodine. Clear zones around the area of growth indicated starch hydrolysis and are the results of B-amylase activity while reddish brown zones around the area of growth indicated partial hydrolysis of starch i.e. result of e-amylase.

*2.8.5 Casein Hydrolysis*

This is a test for proteolytic activity by microorganisms. Skim milk was added to molten plate count agar medium and autoclaved at 10Ib sq. for 10 minutes. This made up to 1% skim milk used. The medium was poured into sterile plates, solidified and dried. The isolates were streaked on the medium in the plates and incubated at $35^0$C for 48 hours. Clearing zones around region of growth of the isolates was marked as an indication of casein digestion and a positive result.

***2.8.6 Gelatin hydrolysis***

This test is designed to test for the production of enzyme gelatinase. Gelatin broth was prepared by suspending 10g of gelatin in 100ml of nutrient broth to give a broth of 10% gelatin (w/v). the solution was steamed and distributed into screw cap tubes and autoclaved at $115^0$C for 15minutes. The isolates were grown in the medium for 7 days. Gelatin hydrolysis was tested for by immersing the tubes in an ice-berg for 10 minutes while uninoculated medium containing the same medium was similarly treated. The tubes were tilted at an angle while in ice-berg and after 10minuets they were removed. The tubes whose content had been liquefied showed gelatin hydrolysis, while tubes whose gelatin remained unhydrolysed were solidified and they were negative.

***2.8.7 Oxidase Test***

A few drops of the oxidase reagent (1% aqueous tetramethyl-p-phenylene diamine hydrochloride) was added onto an isolated colony from 24 hours old pure culture. A purple coloration produced within five-ten second on clean filter paper indicated oxidase positive cultures. A delayed reaction was recorded as negative.

***2.8.8 Indole Test***

Indole is a nitrogen-containing compound formed from the degradation of the amino acid tryptophan by various bacteria. Tryptone broth was prepared and distributed into screw caps tubes and sterilized at $121^0$C for 15mins. After cooling, the tubes were inoculated and incubated at $35^0$C for 48 hours. After which 3ml of Kovac's reagent was added to 6ml of the culture fluid.

The mixture was mixed by rotating the tube between the palms. A reddening of the alcohol layer within five minutes indicated indole production in the tubes. Control tube was set up and no reddening layer was observed.

***2.8.9 Nitrate Reduction***

Nitrate is reduced by some microorganisms to nitrite, ammonia on free nitrogen. The method of Payne, (1973) was used. The medium contained 0.1% $KNO_3$ in peptone water. This was distributed into test tubes fitted with inverted durham tubes. The tubes were autoclaved cooled and inoculated with the isolates. Uninoculated tubes served as controls. The tubes were incubated at $35^0$C for 4 days. The presence of nitrite was determined by the addition into each tube of 0.5ml of 1% sulphanilic acid in 5N acetic acid. This was followed by 0.5ml of 0.6% dimethyl-naphthylamine in 5N acetic acid. The development of a red colour showed the presence of nitrite. Gas production was indicated by its accumulation in the durham's tubes.

***2.8.10 Sugar Fermentation Test***

The test is used to determine the ability of the bacteria and yeasts to utilize different sugars. This is useful in distinguishing the different strains of organisms. 10% solution of each sugar was separately prepared and 1m1 of each sugar was added to 9mls tubes of phenol red broth base which had been separately sterilized at $121^0$C for 10 minutes while the phenol broth was sterilized at $121^0$C for 15mins in screw capped tubes.Each tube was fitted with an inverted Durham tube. The tubes were inoculated with test organisms (bacteria and yeasts cultured) and incubated for 3 days at $35^0$C. Uuninoculated tubes served as controls. Acid production was indicated by a change of colour medium from red to yellow. Production of gas was collected in the inverted Durham's tubes .

***2.8.11 Growth at Different Temperature :***

Isolates were streaked on nutrient agar plates and incubated at desired temperatures. $10^0$, $15^0$, $30^0$C, $35^0$ and $40^0$ for 24 hours. Growth along the lines of streak was recorded as positive at the temperature.

***2.8.12 Growth of bacteria Isolates at Different Nacl Concentration***

This test was designed to test the ability of isolates tom withstand or survive in different Osmotic conditions. Tryptone water containing 4%, 6%, 8% and 10% of Nacl were prepared. 5ml of each medium was dispensed into test tubes and inoculated with the isolates. They weree incubated at $35^0$C for 48 hours. Increased turbidity in any of the tubes, was recorded as positive for growth at that concentration of sodium chloride.

***2.8.13 Oxygen Relationship***

Oxygen requirement of isolates were known through this method. 28g of nutrient agar was dissolved in 1 litre of water and boiled, the medium was distributed into screw capped tubes and sterilized at $121^0$C for 15mins. The medium was allowed to solidify. Each tube was inoculated with isolates by stabbing with an inoculating needle and were incubated at $35^0$C for 4 days. Surface growth only indicated that isolates were obligate aerobes while growth on the surface and along the line of stab showed that they are facultative anaerobes. Where there was growth at the bottom, indicated that they (isolates) were obligate anaerobes.

***2.8.14 Anaerobic Growth***

In order to test whether the isolates could grow under anaerobic conditions, they were streaked onto the surface of plate count agar and incubated for 7 days, in anaerobic gas-pak system at $35^0$C. Growth along the line of streak showed that they could grow under anaerobic condition and recorded positive.

***2.8.15 Citrate Utilization Test***

This test is based on the ability of some organisms to utilize citrate as a sole source of carbon. The medium used for this test was the Koser's citrate medium. The medium was prepared and pispensed into screw capped bottles and sterilized. The bottles were inoculated aseptically from 24 hours old peptone water culture. Incubation was done at $35^0$C for 24 hours, 48 hours and 72 hours. A positive result showed that growth of the isolates occurred in this simple medium, which was indicated by a change in the colour of the medium from green to blue. A negative result was with no change in the colour of the medium control of the experiment was also set up.

***2.8.16 Hydrogen Sulphide Production***

The production of hydrogen sulphide as a metabolic and product of growth was tested for an lead acetate medium., a positive brown or black colouration of the medium showing hydrogen sulphide production. For this test, nutrient broth was prepared sterilized and dispensed into sterile MacCartney bottles. Inoculation was done by aseptically transferring the 24 hours old test organisms from peptone water cultures. Then just before covering of the bottles, filter papers cuttings impregnated in lead acetate was inserted. The filter paper was not allowed to touch the medium inside the bottles. Incubation of the inoculated bottles was for 48 hours. Blackening of the lead acetate impregnated filter paper showed a positive result. Control set up under the same condition showed no blackening of filter paper.

**2.8.17 Urease Test**

Urease Christenson's urea slope is used for this experiment. This can be done by preparing glucose phosphate agar with o.4% phenol red and 40% urea added. Where urea is produced, the ammonia liberated makes the indicator to turn re. In this experiment liquid modification of Christenson's medium was sed. It assayed urea-splitting organisms and urea hydrolysis by other microorganisms. Urea broth base with the following compositions: Peptone: 1g/ltr., Dextrose: 1g/ltr., Sodium dihydrogen Phosphate: 0.8g/ltr., Disodium phosphate. 1.2g/ltr. Sodium chloride 5g/ltr., Phenol red 0.004g/ltr. Was mixed and prepared. 49% urea was prepared, sterilized and 100ml of this was added to 1000ml of sterilized urea broth base. This was mixed thoroughly and dispensed aseptically into sterile MacCartney bottles. The bottles were then inoculated from peptone water culture incubated for 48 hours earlier. Positive result gave a red colouration of the medium. This indicated urea-splitting or urea hydrolysis, control set up under the same condition didn't turn red, that is, the colour remained unchanged.

***2.9.0 Biochemical Tests on Yeasts***

***2.9.1 Urea Test***

Difcobacto broth 5: (w/v) was dispensed into tubes aseptically in aliquots of 0.5ml and stored in a deep freezer for about 5 weeks. A loopful of cells from a two old culture was suspended in the broth and incubated at $30^0$C for 4-5 days. The tubes were examined every half hour for a change of colour to red which showed urease activity. Control was set up in which tube was not inoculated.

***2.9.2 Acid Production***

Five grams of glucose, 0.5g, repreptitated calcium carbonate (chalk), 2g difco yeasts extract,
2g agar were dissolved in 100ml of distilled water. Heating was done for a few minutes to have a homogenous mixture and then autoclaved at $121^0$C at 15 Ibs for 15 minutes. Medium was cooled to $45^0$C, was poured into sterile Mccartney bottles and slanted. The cultures were streaked down the centre of the slant and incubated at $30^0$C for 3 days. Acid production was observed during the incubation by the dissolution of the chalk.

***2.9.3 Sporulation Test***

Presporulation medium considered of malt extract, 4g dissolved in 21m1 of Nort broth. Sporulation medium consisted of 0.01g glucose, 0.5g sodium, acetate, 1.5g bacto agar dissolved in 100ml of distilled water. Contents of the pre sporulation medium were thoroughly, mixed together in a chemical flask 10ml of this was poured into test tubes and sterilized at $121^0$C at 1.02kg/cm$^2$ for 15minutes. The isolates were inoculated aseptically and incubated at $30^0$C for 3 days. The yeasts cells were later harvested by centrifuging using MSE laboratory centrifuge. The weight constituents of the sporulation medium were dissolved in a conical flask, sterilized in an autoclave at $121^0$C for 15 minutes. 15ml were dispensed into Mccartney bottles in a slanting position and allowed to solidify.

By using a sterilized micro-spatula the yeast isolates from the preporulation medium were each inoculated in McCartney bottles containing the sporulation medium incubated at $30^0$C for 5-14 days. Production of ascopores was noted by making a smear of the isolates on glass slide, 1 drop of malachite green was added to it before observing under light microscope.

***2.9.4 Growth in Liquid Medium At Different Temperature***

The test organisms were grown in wort broth at different temperatures. The wort broth containing 25g glucose, 87.5 sucrose, 12.5ml wort was made up to 1 ltr. of distilled water in a conica flask. Fifteen ml of the wort broth was dispensed into test tubes before sterilization in an autoclave at $121^0$C for 15 minutes. Inoculation with yeast cells were done aseptically and the tubes were incubated at various temperatures for 3 days. Growth was assessed spectrophotomerically, with wavelength of 540nm. Uninoculated tubes served as control.

***2.9.5 Growth of yeast Isolates at Different Salt Solution***

Solution of sodium chloride at different concentration 10 and 15% were prepared 20ml of the appropriate salt and 80ml of modified bacto-yeast carbon base (Wickerham, 1951) were mixed together. 15ml of the mixture was dispensed into test tubes, sterilized by autoclaving and then aseptically inoculated and incubated at $30^0$C for 3days.Growth was assessed by turbidity. Uninoculated tubes served as control.

***2.9.6 Deamination of Amino Acids***

This test was carried out to ascertain if the isolates would split off amino acid to yield ammonia. 4% peptone broth was prepared and dispensed into screw tubes. After autoclaving and allowed to cool, they were inoculated with the test organisms. After incubating at $37^0$C for 2-5 days, a drop of the broth culture was added to a drop of Nessler's reagent on a white tile, using sterile wire loop. The presence of ammonia was indicated by a deep yellow colour. Control set up showed no change in colour.

***2.9.7 Identification of Isolates***

For bacterial isolates, identification was based on the basis of their Gram's reaction and biochemical tests with reference to Bergey's manual of determinative bacteriology. The yeast cells were stained with lactophenol in cotton blue and examined under the microscope. They were identified by their morphological characteristics.

## III. RESULTS AND DISCUSSION

Table 1: List of Drugs and Preservatives Used

| | Drug Names | Manufactured | Expiry Date | Examination | Preservatives in formulation | Limit of preservatives |
|---|---|---|---|---|---|---|
| a) | Chloroquine syrup (A) (Antimalaria) | 10/2012 | 09/2014 | 4/2013 | Sorbic acid | 0.1% |
| b) | Chemiron Blood Syrup | 4/2012 | 3/2015 | 4/2013 | Sorbic acid | 0.1% |
| c) | Chloroquine Syrup (B) (Antimalaria) | 10/2012 | 09/2015 | 4/2013 | Sodium Benzoate | 0.1% |
| d) | Multivitamin Syrup (A) | 09/2012 | 08/2014 | 4/2013 | Sodium Benzoate | 0.1% |
| e) | Multivitamin Syrup (B) | 09/2012 | 08/2014 | 4/2013 | Sodium Benzoate | 0.1% |
| f) | Antacid Mixture | 07/2012 | 06/2015 | 4/2013 | Paraben A Sodium Methyl hydroxyl benzoate (B) | A) 0.2% |
| g) | Mist Kaolin | 07/2012 | 06/2015 | 4/2013 | Sodium propyl Hydroxybenzoate | B) 0.2% |

From the direct dilution made from the seven drugs manufactured locally, only 4 were identified to contain microbes. They were Mist Kaolin mixture; Multivite syrup (A), Chloroquine syrup (A) and Multivite syrup (B). Chloroquine Syrup B, Chemiron Blood syrup and Antacid Mixture were found to be free of microbial contaminations as no microbial isolates were found on them.

The results on Table 2 shows the isolates' morphological properties. Apart from the two yeasts (Mb and Ba) that were made to undergo staining with lactophenol blue, spore staining test was used to identify others. All strains of bacteria were rod shaped except $B_1$ and $0_1$ that were cocci. The spores were observed to be terminal for $M_2$, $M_6$, $B_1$, $B_2$, $0_1$ and $0_2$ while other have their spores centrally placed. The isolates from the corresponding drugs are as listed on the Table 2 above with Mist Kaolin having the highest isolates (six) while least isolate (one) was from the Chloroquine syrup. of *Bacilus pumilus* ($M_5$ and $M_6$) were identified and grouped as $M_5$ from Mist Kaolin being morphologically similar. $B_3$ and $B_4$ were also morphologically similar and were grouped as $B_4$.

Table 2: Microorganisms isolated from the drugs.

| **Organisms isolated** | **Designated as** | **No. of isolates** | **Where isolated** |
|---|---|---|---|
| *Bacillus subtilis* | $M_1$ | | |
| *Pseudomonas synringe* | $M_2$ | | |
| *Lactobacillus desidiosus* | $M_3$ | **6** | Mist Kaolin |
| *Hafnia alvei* | $M_4$ | | |
| *Bacillus pumilus* | $M_5$ | | |
| *Saccharoces rouxil* | $M_b$ | | |
| *Saccharomyces graminis* | $B_a$ | | |
| *Micrococcus varians* | $B_1$ | 4 | Multivitamin |
| *Pseudomonas mendocina* | $B_2$ | | Syrup (A) |
| *Bacillus licheniformis* | $B_4$ | | |
| *Pseudomonas putida* | $O_1$ | **2** | Multivitamin |
| *Pseudomonas stutzeri* | $O_2$ | | Syrup (B) |
| *Sporolactobacillus* | $C_2$ | 1 | Chloroquine Syrup (A) |
| - | - | - | Chloroquine syrup (B) |
| - | - | - | Chemiron Blood Syrup (B) |
| - | - | - | Antacid Mixture |

The biochemical characteristics of the isolates are as summarized on Table 3 for bacteria and Table 4 for the yeasts. The yeasts i.e. Mb and Ba, failed the acid production test. The yeast Mb was able to grow on both 10% and 15 % Nacl concentrations while Ba was only able to grow on 10% Nacl concentration but not on the 15% concentration. Also, both yeast strains tested positive to the urease test while they both failed the galactose and manitol sugar tests. However, Mb tested positive to inositol sugar test while Ba tested negative but both tested positive to all other sugars as indicated on Table 4. Table 5 shows the result of the sugar fermentation test conducted on the bacteria for their identification.

Table 3: Biochemical characteristics of the isolates from the pharmaceutical produce

| Drugs | Mist Kaolin | | | | | | Vitamin Syrups | | | | | Chloroquine phosphate $C_2$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Tests | $M_1$ | $M_2$ | $M_3$ | $M_4$ | $M_5$ | $M_6$ | $B_1$ | $B_2$ | $B_4$ | $O_1$ | $O_2$ | + |
| 1. Catalase | + | + | - | + | + | + | - | + | + | - | - | |
| 2. Nitrate Reduction | +g | +g | - | - | - | - | - | - | + | - | +g | - |
| 3. Starch hydrolysis | + | - | + | - | - | - | + | + | + | - | + | - - |
| 4. Methyl Red | - | - | + | - | - | - | - | - | - | - | - | + |
| 5. Voges Proskauer | + | + | + | + | - | - | + | + | + | + | + | - |
| 6. Case in hydrolysis | + | - | + | - | + | + | + | + | + | + | + | - |
| 7. Gelatin hydrolysis | - | - | - | - | + | + | - | + | + | - | + | SA |
| 8. Oxygen Relationship | SA | SA | SA | FA | FA | FA | SA | SA | FA | SA | SA | |
| 9. Anarobic growth | - | - | - | - | - | - | - | - | - | - | - | - - |
| 10. Indole Test | - | - | - | - | - | - | - | + | - | + | - | - |
| 11. $H_2S$ production | - | - | - | + | - | - | + | - | + | - | - | - |
| 12. Citrate reduction | + | - | + | - | + | + | - | + | - | + | - | |
| 13. Urea hydrolysis | + | - | - | - | - | - | - | - | - | + | - | - |
| 14. Deamination test production of acid | + | + | + | + | + | + | + | + | + g | + | + g | + + |
| 15. Oxidase Test | - | - | + | + | - | - | - | + | - | + | + | |

FA – Falcultative Aerobic: SA: Strictly Aerobes + = positive
-: negative. g = gas production.

Table 4: Biochemical Test on Yeast Stains

| Test | | Mb | Ba |
|---|---|---|---|
| Acid production Test | | - | - |
| Growth at different Salt concentration | | | |
| 10% | | + | + |
| 15% | | + | - |
| Urease Test | | + | + |
| Fermentation of Sugar | Glucose | + | + |
| | Raffinose | + | + |
| | Fructose | + | + |
| | Galactose | - | - |
| | Sucrose | + | + |
| | Mannitol | - | - |
| | Lactose | + | + |
| | Maltose | + | + |
| | Inositol | + | - |

Key: + positive - negative

Table 5: Fermentation of sugar pattern by the Isolates from the drugs

| | $M_1$ | $M_2$ | $M_3$ | $M_4$ | $M_5$ | $O_1$ | $O_2$ | $B_1$ | $B_2$ | $B_4$ | $C_2$ |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Glucose | + | - | - | + | + | +g | + | +g | + | + | + |
| Fructose | - | - | - | +g | - | - | + | - | + | +g | + |
| Galactose | + | - | - | +g | - | - | - | + | - | + | - |
| Raffinose | - | - | - | +g | - | - | - | - | - | + | + |
| Sucrose | + | - | - | +g | + | + | + | + | + | + | + |
| Mannitol | - | - | - | +g | - | - | - | +g | - | + | - |
| Lactose | + | - | - | +g | - | - | - | - | - | + | - |
| Maltose | - | - | - | - | + | - | - | - | + | +g | + |

+ : Acid produced but no gas
+g: Acid produced with gas - Neither acid nor gas was produced

The result of sugar fermentation tests carried out on isolates are shown on Table 3. All isolates except $M_2$ and $M_5$ were able to ferment glucose. $M_4$ fermented all the sugars used except Maltose while $B_4$ fermented all. $M_2$ and $M_3$ did not ferment any of the sugars. Gas was also produced by $M_4$ in all the sugars fermented.

The ability of the bacteria isolates to grow at different concentration of Nacl are shown on Table 6, all the isolates grew well up to 6% concentration of salt. Four of the isolates were able to grow at 8% salt concentration, and none could grow at 10% salt concentration. Table 7 shows that none of the isolates grew at $10^0$C except $M_3$ and $B_4$ that had partial growth. It can be also be seen that at $50^0$C $M_4$, $B_1$, $B_2$ and $B_4$ were able to grow well while none grew at $60^0$ C. all grew well at $30^0$, and $40^0$C.

Table 6: The effect of Nacl concentration on the growth of Isolates

| Isolates | 4% | 6% | 8% | 10% |
|---|---|---|---|---|
| $M_1$ | + | + | - | - |
| $M_2$ | + | + | + | - |
| $M_3$ | + | + | + | - |
| $M_4$ | + | + | - | - |
| $M_5$ | + | + | - | - |
| $B_1$ | + | + | - | - |
| $B_2$ | + | + | + | - |
| $B_4$ | + | + | - | - |
| $C_2$ | + | + | + | - |
| $O_1$ | + | + | - | - |
| $O_2$ | + | + | - | - |

Table 7: Growth of Isolates at different temperature

Table 7: Growth of Isolates at different temperature

| Strain | $10^0$ C | 15$^0$C | 30$^0$C | 35$^0$C | 40$^0$C | 50$^0$ C | 60$^0$C |
|---|---|---|---|---|---|---|---|
| $M_1$ | - | | + | + | + | - | - |
| $M_2$ | - | - | + | + | + | - | - |
| $M_3$ | + (p) | + | + | + | + | - | - |
| $M_4$ | - | + | + | + | + | + | - |
| $M_5$ | - | + | + | + | + | - | - |
| $B_1$ | - | - | + | + | + | + | - |
| $B_2$ | - | + | + | + | + | + | - |
| $B_4$ | + (p) | + | + | + | + | + | - |
| $C_2$ | - | + | + | + | + | - | - |
| $O_1$ | - | - | + | + | + | + | - |
| $O_2$ | - | + | + | + | + | + | - |
| $M_b$ | - | + | + | + | - | - | - |
| $B_a$ | - | + | + | + | - | - | - |

Key +: positive +p: positive (partial) - negative

The effect of different concentration of preservatives were investigated on the growth on the growth of all the isolates and recorded on Table 8. The preservatives used were, Sorbic acid and sodium metabisulphite. Only strain $M_5$, $B_4$,O were able to grow on 0.1% sorbic acid preservative while strain $C_2$ was the only strain that could survive on 0.2% sorbic acid. None of the strains could survive on 0.3% concentration of the preservatives hence there were no optical density reading. There were no growth on sodium Metabisulphite beyond 0.2% concentration.

Table 8: The effect of sorbic acid on the growth of Isolates

| Strains | 0.05% | | | | 0.1% | | | | 0.2% | | | | 0.3% | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 6 hrs | 12 hrs | 18 hrs | 24 hrs | 6 hrs | 12 hrs | 18 hrs | 24 hrs | 6 hrs | 12 hrs | 18 hrs | 24 hrs | 6 hrs | 12 hrs | 18 hrs | 24 hrs |
| $M_1$ | 0 | 0 | 0.2 | 0.2 | - | - | - | - | - | - | - | - | - | - | - | - |
| $M_2$ | 0.1 | 0.1 | 0.1 | 0.1 | - | - | - | - | - | - | - | - | - | - | - | - |
| $M_3$ | - | - | - | 0.1 | - | - | - | - | - | - | - | - | - | - | - | - |
| $M_4$ | 0.2 | 0.1 | 0.1 | 0.1 | - | - | - | - | - | - | - | - | - | - | - | - |
| $M_5$ | 0.1 | 0.2 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | - | - | - | - | - | - | - | - |
| $B_1$ | 0.1 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| $B_2$ | 0.1 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| $B_4$ | 0.1 | 0.2 | 0.3 | 0.1 | 0.2 | 0.1 | 0.1 | 0.1 | - | - | - | - | - | - | - | - |
| $O_1$ | 0.1 | 0.1 | 0.1 | 0.2 | 0.1 | 0.1 | 0.1 | 0.1 | - | - | - | - | - | - | - | - |
| $O_2$ | 0.1 | 0.1 | 0.1 | 0.1 | - | - | - | - | - | - | - | - | - | - | - | - |
| $C_2$ | - | - | - | - | - | - | - | - | 0.1 | 0.1 | - | - | - | - | - | - |
| Mb | 0.2 | 0.1 | 0.1 | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Ba | 0.2 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |

Optical density readings at 550 nm  Key: - No readings or Nil.

Table 9: The effect of sodium Metabisulphite on the growth of Isolates

Table 9: The effect of sodium Metabisulphite on the growth of Isolates

| Strains | 0.05% | | | | 0.1% | | | | 0.15% | | | | 0.20% | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 6 hrs | 12 hrs | 18 hrs | 24 hrs | 6 hrs | 12 hrs | 18 hrs | 24 hrs | 6 hrs | 12 hrs | 18 hrs | 24 hrs | 6 hrs | 12 hrs | 18 hrs | 24 hrs |
| $M_1$ | - | - | - | - | 0.1 | 0.1 | 0.1 | 0.1 | - | - | - | - | - | - | - | - |
| $M_2$ | - | 0.1 | 0.1 | 0.1 | - | | | | - | - | - | - | - | - | - | - |
| $M_3$ | - | 0.1 | 0.1 | 0.1 | - | - | - | | 0.1 | 0.1 | 0.1 | - | - | - | - | - |
| $M_4$ | - | - | - | - | - | - | - | | - | - | - | - | - | - | - | - |
| $M_5$ | - | 0.1 | 0.1 | 0.1 | - | 0.1 | 0.1 | 0.1 | - | - | - | - | - | - | - | - |
| $B_1$ | - | - | - | - | - | - | - | | - | - | - | - | - | - | - | - |
| $B_2$ | - | 0.1 | 0.1 | 0.1 | - | - | - | | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | - | - | - |
| $B_4$ | 0.1 | 0.1 | 0.1 | | - | - | - | | 0.1 | 0.1 | 0.1 | 0.1 | - | 0.1 | 0.1 | - | - |
| $O_1$ | - | 0.1 | 0.1 | - | - | 0.1 | 0.1 | 0.1 | - | - | - | | - | - | - | - |
| $O_2$ | - | - | - | - | 0.1 | - | - | | - | - | - | - | - | - | - | - |
| $C_2$ | 0.1 | - | - | - | 0.1 | - | - | | - | - | - | - | - | - | - | - |
| $M_b$ | 0.1 | - | - | - | - | - | - | | - | - | - | - | - | - | - | - |
| $B_a$ | 0.1 | - | - | - | - | - | - | | - | - | - | - | - | - | - | - |

Optical density readings at 550 nm.

Key: - No readings or Nil.

The fact that microorganisms were able to grow in some of the selected drugs is of a great concern as no microbes is expected to grow considering the safe limit of the preservatives used which is suppose to prevent microbial growth. All the microorganisms isolated from the drug could pose a potential to the consumers, if they were allowed to stay on the shelves for a long period. If surveillance of these drugs are not done frequently, these contaminants could lead to drug spoilage, after they must have rendered the preservatives useless. This will be in agreement with the work of [25] that *Cladosporium resinae* under certain conditions could hydrolyse preservatives and render the medications useless. The fact that some of the isolates are sugar fermenters showed that they could ferment the sugars used in the drug formulations thereby changing the drug composition and invariably affect negatively the potency of the drugs or make the drugs unsafe for human usage. Though no pathogenic microorganism like *Pseudomonas aeruginosa* was isolated, it is essential to control all possible sources of microbial contamination in the drugs. The presence of isolates in the drugs is suspected to come from dirty areas or foci present where the drugs are manufactured. More of the suspected sources are through the raw materials. The raw materials from plants, soil and animal origin are very heavily contaminated [26]. Plant materials like gum tragacanth, Kaolin, carmine and gelatin have high microbial load that can contaminate

medicaments, if not properly screened before use. Water is another possible source of drug contamination, since water is the most used medium for dissolving the oral solutions. It has been identified as a good medium for Pseudomonas sp.[27] It is therefore possible that most of the *Pseudomonas sp*. Isolated were contracted from water used in the formulations of these drugs. [28] claimed that manufacturing environment could contaminate drugs especially with *Pseudomonas aeruginosa*. They stressed that routine good manufacturing practices should be adhered to and constant factory sanitation should be carried out so as to prevent the occurrence of cross contamination. The inclusion of preservative in medicaments is also primarily aimed at controlling the contaminants in drugs. Beveridge and Hope [29] and Block [30] claimed that preservatives are to protect consumers from microbial contamination and also include to maintain drug potency and stability.[31] described preservatives as being essential in formulations that are meant to be used more than once from the same containers. The preservatives are required to be bactericidal, at their recommended safe limits. Therefore for those preservatives (i.e. sodium benzoate and the parabens) to support growth of isolates at the safe limits limit render them unsafe.

The spoilage of a particular batch of medicaments can even lead to the closure of the manufacturers thereby creating a room for bad reputation, as the premise could be sealed up. If the parabens and sodium benzoate and any preservative are detected as not being effective, it may now be necessary to alert the authorities concern to enforce manufacturers to abstain from the use of these preservatives. Furthermore, manufacturers of drugs should be compelled to adhere strictly to the good sanitation practice that requires good clean environment. To complement these, there should be skilled workers with modern equipment especially in the quality control unit to test the drug effectively. If all these points are taken sacred, drugs especially non-sterile products like the oral mixtures and syrups meant for patients will remain very near sterile hence there would be less fear of drug contamination when they are consumed.

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