MICROBIOLOGICAL ASSESSMENT OF ORAL LIQUID FORMULATIONS MANUFACTURED IN DAR-ES-SALAAM, TANZANIA.

MILDRED PHARES KINYAWA, B.Pharm(Dar)

A DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE DEGREE OF MASTER OF PHARMACY IN THE UNIVERSITY OF DAR-ES-SALAAM,

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SUPERVISOR

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ABSTRACT

Seven hundred and twenty drug formulations collected from retail pharmacies and drug stores were analysed. Three hundred and twenty (44.4%) were syrups and 400 (55.6%) were mixtures. Total viable aerobic count for the formulations were determined using the plate count method, and the organisms isolated were identified by conventional microbiological/biochemical methods.

The study revealed that microbial growth in mixtures was 49.0% and in syrups 42.8%; however, this difference was not significant. Different organisms were identified from the two formulations and their distribution pattern was found to be significantly different. It was shown that Gram-negative rods were found only in mixtures.

Potential pathogens isolated were found more in mixtures (10.5%) than in syrups (0.3%). Mixtures of Magnesium trisilicate, Kaolin and Belladona, contained more potential pathogens as compared to the rest of the mixtures.

Water used during manufacturing of the formulations was considered to be the main source of contamination.
Of concern was the presence of potential pathogenic

microbes in high numbers. The extreme degradative nature of the Pseudomonas and many yeast cells isolated should make one seriously consider the possible degree of drug destruction.

In view of the above findings, it is recommended that the authorities responsible for issuing of licences for drug manufacturing should be more strict, and adherence to Good Manufacturing Practice (GMP) should be emphasized. In addition, every manufacturing unit must have a functioning water purification system, and there should be frequent inspection of the microbial quality of locally manufactured drug formulations. Strong warning should be given for non-compliance and if necessary legal action be taken.

The findings of this study, provide baseline information which may be of use in an attempt to improve the quality of pharmaceutical products and in setting up local standards in Tanzania.

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As it is not possible to mention everybody who contributed in one way or another to the success of this work, I say, "thanks to all those who assisted".

DECLARATION

I hereby solemnly declare that this dissertation is my own original work and has never been submitted for a diploma or degree in any other University.

Signature:.....

Date: 28th June 1996

DEDICATION

To Mom and Dad, who made it all possible, and to my family, who gave me the inspiration.

1.0 INTRODUCTION

1.1 Background information

Dar es Salaam, a commercial city of Tanzania has a population of 1,360,865, which constitutes about 5.9% of the country's population[1]. It has the country's largest consultant and referral hospital, three district hospitals, thirteen private hospitals, six health centres, more than one hundred forty six dispensaries, more than one hundred and ten community pharmacies and several drug stores[2].

Within the city there are four pharmaceutical industries which supply drugs to the whole country. These industries engage mostly in the manufacture of liquid preparations rather than solid dosage forms because liquids are bulky to import. Only a few liquid preparations are imported as compared to solid dosage forms. The financial situation has slowed down the expansion of solid dosage formulations due to insufficient equipment and raw materials to cater for the needs of the whole country. The production of liquid formulations is also done in community pharmacies to alleviate shortages, since the industrial production is inadequate.

The Pharmacy Board regulations for pharmaceutical production in hospitals and community pharmacies state that the community pharmacies and hospitals are allowed to formulate liquid preparations only for their own use. However, due to shortage of facilities in most of the community pharmacies, some of these pharmacies formulate liquid preparations for whole-sale purposes to cater for those without facilities for local manufacturing of these drugs.

Dar es Salaam is a hot and humid city hence the shelf life of pharmaceutical products is shorter because increased temperatures and humidity accelerate the rate of deterioration of pharmaceutical preparation. The liquid products, which comprise oral and external formulations, are particularly susceptible to microbial contamination. The oral formulations are used mostly by children, whose immune system is not yet fully developed, so there is a need to ensure quality with acceptable low level of microbial contamination during their manufacture.

1.1.1 Sources of microbial contamination

The microbial bioburden of a product is derived from all its constituent materials, the processing equipment and the environment which it comes into contact with,

during its manufacture, packaging and storage. This provides scope for the introduction of a wide range of micro-organisms at differing levels. The major sources of contamination and types of organisms that may be introduced include:

a. Air

Air which enters the manufacturing area may contain airborne microbes. These include the Gram-positive bacteria, Staphylococci sp.; Micrococci sp.; Bacillus sp. and Clostridia sp., the yeasts like Rhodotolura sp.; and molds including Aspergillus; Cladosporium, and Penicillium. Some strains of these microorganisms are potentially pathogenic, even those with low virulence may cause opportunistic infection in immunosuppressed individuals.

b. Water

A pharmaceutical product will either contain water, utilize water during processing or come into contact with surfaces cleaned using aqueous solutions. Since microorganisms are essentially aquatic, water is the ideal vehicle for supporting and transferring contamination[3]. Gram-negative rods can grow rapidly in water from 10^5 to 10^6 colony forming units per millilitre

(cfu/ml) and this contamination level will not be visible to the naked eye.

The microbial flora of city water (potable) usually includes Gram-negative non-fermentative bacilli, including Pseudomonas sp., Alcaligenes sp., Acinetobacter sp., Flavobacterium sp., and Achromobacter sp. Other soil and sewage bacteria such as Bacillus sp., the Gram-negative rods e.g. Serratia sp., Klebsiella sp., Proteus sp, Enterobacter sp. and E.coli can be found in tap water used for manufacturing[4]. This water is adequate for some products but it must first be free from contamination with faecal coliforms. The disadvantage of using potable water is that its chemical purity may vary with time and location.

Purified water is obtained by distillation, ion-exchange, reverse osmosis, and it contains no added substances. The microbiological quality of purified water varies with the production method. Deionisation is the commonest treatment but has the highest potential for microbiological contamination[3]. Water softening units and filters in the system can become heavily contaminated with micro-organisms which will be released into the water. Species of Alcaligenes, Acinetobacter,

and Pseudomonas are the main contaminants but Gram-positive rods and cocci may also be present.

Distilled and reverse-osmosis water is sterile immediately after production but in practice, these production methods are too expensive for the manufacture of non-sterile products. This water can be contaminated with micro-organisms during storage or distribution system.

c. Raw materials

Pharmaceuticals derived from plant or animal sources carry a natural microbial flora[4]. Plant extracts (for example belladonna) may contain up to 10^4 colony forming units per gram (cfu/g) of fungi and 10^5 cfu of bacteria: The fungi would include species of Aspergillus, Penicillium, Mucor, Cladosporium, and Rhizopus. The bacteria would be mainly spore-forming Gram-positive Bacillus species, with Staphylococci and Micrococci occasionally present. The majority of natural materials are reported to be contaminated with Enterobacteriaceae, especially if no processing has been applied[3].

d. Equipment

The manufacturing equipment can add more contamination depending on how it is made. If it is not smooth, and

crevice free, not easily cleaned or dried, and if the microbiological quality of the cleaning fluid is not considered, this will inevitably lead to equipment contamination. Inadequate cleaning may leave product residues in the crevices. Equipment that consumes large volumes of air can contaminate products depending on the quality of air[3].

e. Personnel

Operators represent a significant risk of contamination since humans can transfer microbes shed from their skin, respiratory passages and in body secretions to non-sterile pharmaceutical products[4]. About 10^4 skin squamous cells per minutes and a high proportion of non-pathogenic Micrococci, Diphtheroid, Staphylococci, and occasionally Staphylococcus aureus are shed continually. Transient skin contamination with Enterobacteriaceae may occur if poor hygienic practices are employed. In addition some people are carriers of pathogenic microorganisms.

f. Environment

Non-sterile medicinal products are normally produced under hygienic conditions in an environment that contains micro-organisms derived from the personnel and other sources. The level of contamination will depend

on the operations and types of products handled in that area[3]. Products containing water are at a greater risk of microbial contamination from the production environment. The greatest risk is from Gram-negative bacteria (especially Pseudomonas), which are present in wet locations, introduced by personnel, equipment or cleaning fluids. The plant layout can also provide a source of contamination if there is no unidirectional flow of materials. A goods receiving area exposed to the outside can lead to airborne contamination mostly of spore-forming bacteria or fungi such as species of Bacillus, Penicillium, and Aspergillus. Moreover, non-sterile liquid products will always have micro-organisms even if very careful aseptic techniques are employed.

Contamination of pharmaceutical preparations can also occur at the user level. In multidose preparations where a container is opened several times to withdraw the medicine, contamination may occur since patients handling the medicine may also introduce micro-organisms. It is therefore necessary to inhibit growth of these microbes by including preservatives in such preparations.

1.1.2 Preservatives

These are agents used in non-sterile pharmaceutical products to inhibit or kill bacteria. The function of preservatives is to prevent product contamination and microbial colonisation after production.

There are two basic types of preservative system:

A) Chemical preservatives
- i) natural
- ii) Synthetic
- iii) Antoxidants.

These are antimicrobial agents which preserve the drug preparations against microbial colonisation.

B) Physical preservatives

Microorganisms require certain physical conditions to survive. Deliberate approaches based on altering the product's physical conditions can be done to limit microbial growth[3]. Combination of these systems can be used to maximize product protection.

A. Chemical preservatives

These can either be natural or synthetic. Natural substances such as essential oils or perfumes are among the first substances employed in preservation. These are however complicated mixtures and therefore difficult to control. Modern preservatives are normally synthetic,

which can be chemically defined. However, natural preservatives are being re-investigated in view of consumer pressure to eliminate synthetic additives.

i) Natural preservative Agents

a) Essential oils

The antimicrobial properties of essential oils and perfumes are due to their chemical constituents, which are blends of alcohols, aldehydes, ketones and terpenes. Many of these have been investigated or the constituents separated and tested individually. A major setback in the use of essential oils is the high concentration required for activity, which imparts unusual organoleptic properties to the product. Furthermore, essential oils may possess other biological activities, they are usually expensive and both features render them unsuitable for use.

b) Enzymes and proteins

Enzymes are mostly used in food industries and are not commonly used in pharmaceuticals. Due to their protein nature they are unsuitable for parenterals. For non-sterile products, enzymes and proteins are active against a range of microorganisms but

resistance has been demonstrated especially against Gram-positive bacteria.

ii) Synthetic preservatives

The most commonly used and the biggest group of preservatives for pharmaceuticals are of synthetic origin. These are normally classified on the basis of their chemical structure, which provides only limited information on their activity and applicability. The site of action and degree or extent of antimicrobial activity can be concentration-dependent. Within an individual chemical group several agents may be used as preservatives at low concentrations and as disinfectants or antiseptics at higher concentrations. The chemical classification is useful, however, as agents within a group will behave similarly when exposed to equivalent conditions. The various classes are acids and salts, alcohols, hydroxybenzoates, mercurials, phenols, quaternary ammonium compounds and biguanides.

iii) Antoxidants

These may be classified into three groups:

a) True antoxidants or 'anti-oxygens' which probably inhibit oxidation by reacting with free radicals. They are effective against autoxidation but not in reversible oxidation (redox reactions).

Examples are tocopherols and butylated hydroxyanisole[5].

b) Reducing agents: These agents have a lower redox potential than the drug or adjuvants which they are intended to protect and are therefore more readily oxidized than the drug. They may also act by reacting with free radicals. Examples are ascorbic acid and sodium formaldehyde.

c) Antoxidant synergists which usually have little antoxidant effect themselves but probably enhance the action of antoxidants in the first group i.e. the true antoxidants by reacting with heavy-metal ions which catalyse oxidation. Examples are citric acid, acetic acid and its salts, lecithin, and tartaric acid[5].

B. Physical preservative systems

These consist of deliberate adjustments of certain physical conditions which are required by micro-organisms to survive. The following requirements if adequately adjusted can suppress or kill micro-organisms, and this forms one of the oldest known forms of preservation. The following is an illustration of some of the examples:

i) **Available water**

Water is the single most important requirement for microbial growth[4]. Most organisms require over 70% water to grow. Products containing large amounts of water (like suspensions and mixtures) must be considered to be at risk from microbial colonisation.

The amount of water that is available to the organisms in a product is given by the following expression:

$$A_w = \frac{\text{vapour pressure of product}}{\text{vapour pressure of water}}$$

(at constant temperature) where A_w = water activity. This will always be less than one, owing to hydrogen bonding of the water in the product. Certain organisms will only survive and grow at specific A_w levels. If the A_w is lowered by adding solutes, microbial growth can be prevented. Variable level of preservation can be achieved by simple manipulation of the product's A_w value. Syrups with high concentrations of sugars increase osmotic pressure and decreases A_w to levels incompatible with most bacterial life.

ii) **pH value**

The pH can have a limiting effect on the survival of microorganisms but only at the extremes, such as 3.5 or

below and 10 or above[4]. Majority of microorganisms grow best at a pH of about 7 but survival is seen at pH values from 3.5-10. Lowering or raising the product's pH from neutrality provides a degree of preservation. However, the scope of pH variation in pharmaceuticals is limited by physiological acceptability and formulation stability.

iii) **Temperature**

Storage temperatures can greatly affect the survival and growth of microbes in pharmaceutical products. The majority of contaminants in pharmaceutical products are mesophilic organisms that grow best at ambient temperatures (15°C-45°C). A reduced storage temperature can be used as a means of inhibiting growth, although the antimicrobial activity of the preservative is severely decreased. Normally, pharmaceuticals are stored at a reduced temperature to improve chemical stability, with antimicrobial effect being secondary.

The ideal preservative

Several workers have devised lists of properties of the ideal preservative, which include the following characteristics:

- broad spectrum of antimicrobial activity i.e. the agent should be active against all basic groups of

microorganisms such as Gram-positive and Gram-negative bacteria, yeasts and molds, and should be effective at low concentrations.

- soluble in the formulation at the required concentrations.
- non-toxic and non-sensitizing at the in-use concentrations i.e. the agent should possess a toxicity profile acceptable to regulatory authorities, safe to handle during manufacture, packaging and in usage.
- physically undetectable - that is the agent should not alter the physical properties of the product, such as colour, odour, taste or rheological properties of the formulation.
- chemically stable and microbiologically effective over wide ranges of pH and temperature, and there should be no loss of activity over the shelf-life.
- relatively inexpensive - the cost of the agent should not constitute a large portion of the product's cost.
- compatible with formulation components and packaging.

As with most ideals, this one is probably unattainable and a balance must be struck among these requirements.

1.1.3 Effects of microbial contamination on liquid preparations

The presence of micro-organisms leads to two possible problems: one is spoilage and the other is transmission of disease to user[3]. The contaminating micro-organisms use the product as a growth medium. Emulsifiers, thickeners, suspending agents and syrups are good carbon sources for fungi and bacteria.

Carbohydrates are metabolized to acids, a process which may be accompanied by gas formation. These metabolites may cause products to develop odours, change colour and shift pH. However, micro-organisms present in a pharmaceutical formulation may also induce disease without necessarily producing spoilage of the product. This could be due to infections if the organism is pathogenic, or through toxins excreted into the product[3]. The risk of microorganisms producing an infection is difficult to access and depends upon the species, dose administered (number of viable cells), route of administration and susceptibility of host.Even minor changes in physical properties are noticeable and can be disconcerting for the consumer. Since consumers cannot assess the active ingredients, they depend on their senses to determine the quality of the product. Changes in the products' gross physical properties are likely to

be interpreted as a loss of efficacy of the product. In addition to the risk to health, spoilage and disease transmission represent a financial loss to the consumer and producer, through the cost of replacement, lost sales or litigation[3].

Assessment of microbial contamination

For non-sterile products two features of contamination are recognized: the total number and types of micro-organisms present, in which case pathogenic or potentially pathogenic micro-organisms should be absent[3].

The European Pharmacopoeia and the United States Pharmacopoeia[6,7] include a test for the total viable count (colony forming unit, (cfu) per gram or millilitre), irrespective of species for finished products.

1.2 Literature Review

A study on microbial contamination of medicines dispensed in hospitals, reports that, the contamination of medicines with microorganisms has been a concern in many countries. They also indicated that the vehicles used in the preparations are often responsible for the high rate of contaminations[8]. The same study confirmed that the preparations containing water are those most likely to be contaminated with micro-organisms. Several products ranging from tablets, creams and solutions have been found to be contaminated.

Noble and Savin (1966)[9] reported the use of contaminated corticosteroid creams which resulted in an outbreak of clinical Pseudomonas infection in a dermatological institution. The same study included reports of severe eye infections among patients using hydrocortisone for eye treatment. These workers reported at the same time the contamination of thyroid tablets with Salmonella organisms that caused infection among many people. It has also been reported that contaminated dextrose infusion probably contributed to the deaths of three patients at the Devenport section of Plymouth General Hospital in U.K due to the presence of harmful microbes. In a study done in six Nigerian hospitals, a total of sixty samples of eighteen different pharmaceutical

preparations were examined and 57% were found to be contaminated with Alcaligenes, Aerobacter cloaca, Staphylococcus aureus, Staphylococcus albus, Streptococcus sp., Gram negative bacilli, yeasts and moulds[8]. The sources of contamination were the vehicles or raw materials used in the manufacture of these products.

A review of the nature and extent of microbial contamination of pharmaceuticals by Hooper (Poole General Hospital, U.K) revealed that 32% of products sampled were contaminated and contained 33 strains of Pseudomonas aeruginosa[10]. The studies of Lang *et al* (1967)[11] as well as those of Komarny, Oxyley and Brecher (1967)[12] showed that the use of contaminated carmine dye used for the investigation of intestinal abnormalities caused salmonellosis which occurred in hospitals in U.S.A.

In the United States Pharmacoepia-Food and Drug Administration (U.S.P. - F.D.A.) researchers reported that between 1968-1971 1550 isolations of Gram-negative bacteria were from raw materials and finished goods[3,13]. Recently cases of contamination of total parenteral nutrition (T.P.N), with Enterobacter cloaca from a band

in a sink, causing deaths of two children at Manchester Childrens' Hospital have been reported[14].

Of the oral dosage forms, liquids are the most preferred form for administration to children and the elderly. Children are more vulnerable to infections than adults because the immune system is not fully developed. For the elderly, however, the functioning of the immune system decreases. It is therefore important to ensure that this group of people is not exposed to microbial contamination during treatment with liquid preparations. Microbial contamination has been a problem in the pharmaceutical industry both at the stages of production, packaging and usage[15]. Micro-organisms present in a pharmaceutical formulation may cause infection and disease without necessarily producing spoilage of the product[4]. As far as clinical significance of contamination is concerned, little is known about the minimum dose of organisms needed to establish a human infection[8]. There are many examples of pathogenic micro-organisms contaminating medicinal products and inducing disease.

In Dar es Salaam many community pharmacies are engaged in small-scale manufacturing of liquid products. Liquid products which are formulated for sale on retail basis

at the premises are also sold on whole-sale basis to other pharmacies. The pharmaceutical formulators do not have quality control facilities, neither do they have an inspection procedure to check for the quality of these products[16].

Although non-sterile products are allowed to contain a specified level of micro-organisms, they should not pose a health hazard to the patient nor be susceptible to degradation because of this contamination. In the case of foodstuffs, there is evidence that contamination with microbial toxins constitutes a health hazard. It is possible that a similar risk exists with non-sterile pharmaceutical products manufactured in Dar es Salaam. The water used in some industries is not treated or pre-tested for total colony count which should be less than 40 colony forming units/millilitre (cfu/ml) for manufacturing pharmaceuticals[17].

During the literature search no study was found on microbial contamination of oral liquid preparations manufactured in Tanzania.

1.3 Statement of the problem

It is evident from the forementioned that assessment of microbiological quality of oral liquid formulations

manufactured in Dar es Salaam is vital. Knowledge of the extent of microbial contamination of local medicinal preparations is important in ensuring safety to consumers, especially children and the elderly. The need to conduct this study is justified since this dosage form is used mostly by a fairly immunologically delicate group of persons.

While in industrialized countries modern drug production must follow Good Manufacturing Practice (GMP) which include personnel, premises, equipment and hygiene[18], most industries in developing countries, including Tanzania, do not adequately meet GMP requirements. In small-scale manufacturing units, quality control is not done, and this presents a potential risk of microbial contamination[19]. At present Tanzania has no policy on GMP, therefore manufacturers do not have national GMP guidelines. Unfortunately, standards have too often become "crutches rather than guides". It has become easier to enhance poor standards dogmatically rather than try to improve the standards to fit current practice[20]. Most developed countries have their own GMP guidelines, have microbial limit test and a standard for almost any test. Before adopting these standards, it is good to have our own standards for comparison purposes. This step will facilitate in tailoring the so

called international standards to suit our local requirements.

The pharmaceutical industries in Tanzania are undergoing expansion due to trade liberalization. As a result it is sometimes difficult to cope with standards since industrial expansion often takes place in a limited space which leads to congestion and overcrowding. This situation makes it difficult to maintain high standards of sanitation and hygiene, and may therefore predispose pharmaceutical preparations to microbial contamination.

So far no studies have been documented in Tanzania on the occurrence and magnitude of contamination of locally manufactured medicinal preparations. Also there is no national policy on the microbiological standards of the locally prepared drug formulations. Furthermore, regular surveillance to monitor the microbiological quality of these preparations is not done. This study, therefore, aims at checking the microbiological quality of a selected sample of locally prepared oral liquid pharmaceutical products in order to establish the nature and magnitude of microbial contamination. Findings obtained may provide baseline information which can be used to improve the quality of Tanzanian products and in setting local standards. It may also enable the

planners or policy makers to find out a way of establishing acceptable and feasible strategies for the implementation of microbial contamination control.

2.0 STUDY OBJECTIVES

Broad objective

To determine the microbiological quality of oral liquid formulations manufactured locally in Dar es Salaam, Tanzania.

Specific objectives

1. To determine the mesophile count of bacteria and fungi in liquid drug preparations.
2. To isolate, identify and categorise the microorganisms found in the preparations.
3. To determine the number and types of potentially and strictly pathogenic organisms found in these preparations.
4. To identify the types of drugs which have high level of potentially pathogenic organisms.

3.0 METHODOLOGY

3.1 Materials

3.1.1 Drug samples

The drug samples comprised of two groups:

a) **Syrups**

These were preparations made using simple syrup (66.7% $^{w}/_{w}$ sucrose in water) as the vehicle. The most commonly used syrup preparations were bought from various pharmacy shops and drug stores making sure that products from all the manufacturers were included. The syrups covered in this study are shown in Appendix I.

b) **Mixtures**

These were preparations made using water as the vehicle. The most commonly used mixtures were bought randomly in the same manner as syrups. The mixtures covered in this study are shown in Appendix II.

3.1.2 Positive control cultures (DIFCO) bacterial set A

The following organisms were used in the study for the preparation of positive control cultures:

1. Escherichia coli
2. Pseudomonas aeruginosa
3. Staphylococcus aureus

4. Staphylococcus epidermidis
5. Candida albicans

Freshly prepared culture of each organism was inoculated on the surface of Blood agar(BA) and incubated at 35°C for 18 hrs in case of bacteria and at 25°C for 48hrs for Candida albicans.

Negative controls were run using sterile peptone solution to check for the asceptic performance of the method. Routine controls containing tryptone soy agar, and Sabourauds Dextrose agar (SDA)} were run.

3.1.3 Media

Culture media used in the study included:

i. Buffered Sodium chloride-peptone solution pH 7.0
ii. Tryptone soya agar (Liquefied casein soya bean Digest Agar).
iii. Sabourauds Dextrose Agar (SDA) (Oxoid)
iv. Nutrient Broth (NB) (Oxoid)
v. Nutrient Agar (NA) (Oxoid)
vi. Blood Agar (BA) (Oxoid)
vii. MacConkey Broth (Oxoid)
viii. MacConkey Agar (MCA) (Oxoid)
ix. Selenite cysteine Broth (Oxoid)

x. Chocolate Agar (CA) (Oxoid)

xi. Lactose Broth (Oxoid)

3.1.4 Test reagents

i. Kovac's reagent for Indole test

ii. Ethyl alcohol(Analar)

iii. Glucose phosphate peptone water for Voges Proskauer

iv. Kliger Iron Agar (KIA)

v. API 20E (Analytab products)

vi. Human serum for germ tube test

vii. Hydrogen peroxide 3% for catalase test

viii. Human plasma for coagulase test

ix. Motility medium

x. Oxidation - fermentation (OF) medium

xi. Oxidase reagent

xii. Gelatin (Oxoid)

xiii. Koser's citrate medium

Sugars: Maltose

Lactose

Glucose

Arabinose

Mannitol

3.2. Methods

3.2.1 Sampling method:

Non-probability sampling ("Purposive selection") was used and the technique of choice was "quota sampling" whereby the general composition of the sample was decided well in advance[22,23]. Quotas or required numbers were drawn randomly from various retail pharmacies and drug stores in Dar es Salaam, and it was ensured that it was a representative sample of the commonly used oral liquid products manufactured locally and all manufacturers were represented (Table 1 and 2). Representation of various batches was also taken into consideration. This was achieved by taking samples from as many pharmacies as possible. In so doing various batches were covered during the collection. Samples were bought at intervals (June-September 1995) thus there was a possibility of buying new stocks from the manufacturers. The procedure was done in order to obtain a sample population as representative as possible of the target population.

3.2.2 Sample size (n)

The sample size was determined using the following formula

$$n = p\ (\frac{100 - p}{e^2})^{24,25}$$

where n = desired sample size

p = proportion from the target population estimated to have a particular characteristic. From a pilot study conducted on a selected sample, the prevalence of contamination was found to be 15% for syrups.

Hence P = 15

e = standard error of the mean. In this study it was set at 4.

Therefore the sample size for syrups was 320.

Mixtures: The prevalence of contamination was found to be 20% from a pilot study done on a selected sample of mixtures bought randomly from different shops, and the margin of error was set at 4. Using the same formula sample size for mixtures was estimated at 400.

Specimens

The study unit was 100 millilitre bottle.

The study covered syrup preparations and mixtures. These were formulated by different manufacturers. According to the number of industries which make a particular product, the proportion to come from each industry was obtained as follows:

Example

Cough syrups: Fortunately all the five manufacturers in Dar es Salaam make cough syrups. From the required number of 320 syrups, cough syrups form 1/5 of all types (Table 1) therefore 64 cough syrups were allocated equally to all the manufacturers. Thus 13 cough syrups from each factory were assessed and these were bought randomly from different shops in order to include as many batches as possible.

320 - five types of syrups from five industries
|
64 - cough syrups from five industries
|
13 - cough syrups from one industry.

The same procedure was used for selecting mixtures (Table 2).

Controls

Dilute specimens of the materials to be tested were inoculated with separate viable cultures of Staphylococcus aureus, E. coli, P.aeruginosa and Candida albicans, and the test procedure for total aerobic count was followed. Failure of a control to grow invalidated the method.

To test the sterility of the medium, the diluent, and the aseptic performance of the test, a total aerobic count method was carried out using sterile buffered sodium chloride - peptone solution (pH 7.0) as the test preparation. As a routine check for microbial contamination within the laminar flow hood and surrounding area, 2 plates were left open within the flow hood at the time of experiment, one for bacteria and the other for fungus. This was done in order to make sure that any colony isolated on the culture plates was from the test material and not from the air or other sources of contamination.

3.2.3 Experimental procedure

Total viable aerobic count (plate count)

All analyses were performed in duplicate. Drug samples were first agitated to ensure an even distribution of microorganisms within the specimen[26]. Using sterile disposable syringes, 10mls of the sample was drawn and placed into a bottle containing 90mls of buffered Nacl-peptone solution[27]. Polyserbate 80 was added at a concentration of 0.1% $^{w}/_{v}$ to assist the suspension of poorly wettable substances. The mixture was placed on a mechanical shaker for 15 minutes at room temperature. Using disposable syringes, aliquots of 1ml were drawn from the mixture and placed on 4 petri dishes (9-10cm in

diameter); 2 containing Tryptone soy-agar, and the other 2 containing Sabourauds Dextrose Agar (SDA). Tryptone soy-agar plates were incubated at 35°C for 48 hours while SDA plates were incubated at 25°C for 7 days [27,28]. The plates were examined for growth and the number of colonies counted were multiplied by 10 to get colony forming units per millilitre (cfu/ml) of original sample. Each colony formed was counted as one although it could have originated from more than one microorganism.

Microbiological analysis

The Gram smear morphology of colony types was determined and organisms were classified on the basis of Gram stain reaction. Cell type, morphology, colony appearance, motility, and chain formation were recorded[29,30].

Procedure for detection of fungi

a) Gram stain: A drop of sterile saline was placed on a clean glass slide. Using a sterile loop (by flaming) a colony of the culture was emulsified on the sterile saline to form a thin film and this was heat fixed and a Gram stain was performed. This was examined under oil immersion objective (100 x magnification) for the presence of fungi (yeast cells or pseudohyphae)[31].

b) Germ tube: This test was used for the presumptive identification of Candida albicans. Known C. albicans was included as a positive control and C. tropicalis was used as a negative control[32].

Biochemical tests

The methods of Buchanan *et al* (1974)[33] were used to determine the following characteristics:

Citrate utilization, production of acetoin (Voges-Proskaur), Methyl red test, acid production from glucose, arabinose, xylose, sucrose, mannitol and lactose, reduction of nitrate, hydrolysis of starch, gelatin, casein, lecithin, phenylalanine deamination, and production of urease, indole, catalase, coagulase and oxidase. The pigment production on tyrosine 0.1% agar was determined and test for fermentative or oxidative metabolism of glucose was done. Haemolysis was determined on plates containing Nutrient agar (NA) supplemented with 5% blood[31].

3.2.4 Statistical analysis

The results were analysed using a statistical analysis package "Epi Info (version 6) Computer System". The Chi-square (X^2) test was used for statistical analysis of two group sets of data and one group data where applicable. Significance was estimated at 5% level ($P < 0.05$).

3.3 Definition of terms used

Microbiological contamination

Microbiological contamination is defined as the presence of micro-organisms in a specified environment, in this case in a preparation, which may have developed accidentally during the processing procedures[15]. In general, these are bacteria, fungi, viruses, or rickettsiae.

Microbiological contamination control

Microbiological contamination control is the total procedure that achieves and maintains a desired microbiological state in/on, or around a stated environment, object, or preparation. Contamination control is achieved if the microbial load does not exceed the level established as the lowest acceptable limit.

Strictly pathogenic organisms

Strictly pathogenic organisms are organisms which are always disease producing.

Potentially pathogenic organisms

Potentially pathogenic organisms are those organisms which are potentially capable of producing a disease condition in a suitable host.

Coliforms

Is a term used to denote all the enteric bacilli. It is used more often, however, to describe Gram-negative inhabitants of the intestinal tract such as Escherichia coli, Klebsiella pneumoniae, and Enterobacter aerogenes. In the Enterobacteriaceae, other Gram-negative rods are included in the broad term because they are frequently found in the intestinal tract. Among the more important of these are Pseudomonas species[21].

4.0 RESULTS

4.1 Syrups and mixtures used in the study

The randomly sampled oral liquid products manufactured locally with their respective manufacturers appear in Tables 1 for syrups and Table 2 for mixtures.

Table 1: Sample of syrups used in numbers.

Product (Syrups)	MANUFACTURER					Total
	Elys	Shelys	Mansoor Daya	Inter-chem	Afya Lab.	
Paracetamol	13	13	13	13	12	64
Promethazine	13	13	13	12	13	64
Chloroquine	13	13	12	13	13	64
Cough syrup	13	13	13	13	12	64
B-complex	-	-	21	21	22	64
Total						320

Table 2: Sample of mixtures used in numbers.

Product (Mixtures)	MANUFACTURER					Total
	Elys	Shelys	Mansoor Daya	Inter-Chem	Afya Lab.	
Belladona	27	-	27	-	26	80
Kaolin	20	20	20	-	20	80
Mag.tris.	27	-	27	-	26	80
Cough mist.	27	-	27	-	26	80
Iron sulfate	-	-	40	-	40	80
Total						400

4.2 Determination of mesophile growth and contamination levels in the formulations.

Mesophile growth was identified in 137(42.8%) syrups and 196 (49%) mixtures as shown in Table 3.

Table 3: Mesophile growth in syrups and mixtures manufactured in Dar es Salaam.

Growth	Formulation		Total
	Syrup	Mixture	
	N(%)	N(%)	N(%)
Present	137 (42.8)	196 (49.0)	333 (46.3)
Absent	183 (57.2)	204 (51.0)	387 (53.8)
Total	320 (44.4)	400 (55.6)	720 (100)

$X^2 = 2.74$, $P > 0.05$

Higher levels of contamination were observed in mixtures compared to syrups as depicted in Table 4. Mean count was 1410 cfu/ml for syrups and 2201 cfu/ml for mixtures. It was also observed that the lowest count for both preparations was 10 cfu/ml and the highest count was 30,000 cfu/ml for both preparations.

Table 4: Microbial count of the two drug formulations.

Count cfu/ml	Formulation		Total
	Syrups	Mixture	
	N(%)	N(%)	
10 - 100	78(24.4)	56(14)	134(18.6)
101 - 10,000	57(17.8)	138(34.5)	195(27.0)
>10,000	2(0.62)	2(0.5)	4(0.55)
None	183(57.2)	204(51)	387(53.8)
TOTAL	320(44.4)	400(55.6)	720(100)

$X^2 = 25.6$, $P < 0.05$

4.3 Identification of micro-organisms isolated from the preparations

The most predominant organism isolated was Bacillus subtilis found in 87(27.2%) syrups and in 112(28%) mixtures, followed by Candida sp. found in 48(15%) syrups and in 38(9.5%) mixtures as shown in Table 5.

Table 5: Microorganisms isolated froms syrups and mixtures

Organism	Formulation		Total
	Syrups N(%)	Mixtures N(%)	N(%)
Alcaligenes	0	3(0.8)	3(0.4)
Aspergillus fumigatus	2(0.6)	3(0.8)	5(0.7)
Bacillus subtilis	87(27.2)	112(28)	199(27.6)
Candida	48(15)	38(9.5)	86(11.9)
Diphtheroids	0	4(1)	4(0.6)
Klebsiella	0	4(1)	4(0.6)
Micrococci	2(0.6)	4(1)	6(0.8)
Mucor	3(0.9)	1(0.3)	4(0.6)
Penicillium notatum	1(0.3)	1(0.3)	2(2.8)
Proteus	1(0.3)	1(0.3)	2(2.8)
Pseudomonas aeruginosa	0	36(9.6)	36(5)
Staphylococci albus	9(2.8)	7(1.8)	16(5)
Streptococci faecalis	3(0.9)	7(1.8)	16(2.2)
None	183	204	386(53.6)

$X^2 = 50.96, \quad P < 0.05$

Next in predominance were Pseudomonas aeruginosa, found in 36(9.0%) mixtures and Staphylococcus albus in 9(2.8%)

syrups and in 7(1.8%) mixtures, while Streptococci were found in 3(0.9%) syrups and in 4(1%) mixtures. Other organisms isolated included Aspergillus, Micrococci, Alcaligenss, Diphtheroids, Klebsiella, Mucor, Penicillium and Proteus in percentage ranges between 0.3% to 1%.

In some preparations more than one organism was isolated, and in most cases it was two organisms. Only one preparation was found to have three different organisms. The combinations encountered were Bacillus and Micrococci, Bacillus and Candida, Bacillus and Staphlococcus, Bacillus and Streptococcus, and Candida and Staphylococcus.

4.4 Identification of potentially pathogenic organisms

Table 6 represents potential pathogens isolated from 11.34% mixtures and 1.3% syrups. The distribution of potential pathogens in the two formulations was significantly different ($P<0.05$).

Table 6: Potential pathogens isolated from mixtures and syrups.

Organism	Formulation		Total
	Syrups	Mixtures	
	N(%)	N(%)	N(%)
Klebsiella	0	4(1)	4(0.6)
Proteus	1(0.3)	1(0.25)	2(0.3)
Pseudomonas aeruginosa	0	36(9.0)	30(5.0)
Streptococcus faecalis	3(0.9)	4(1)	7(1)
Total	4(1.3)	45(11.3)	49(12.3)

$P<0.05$

4.5 Identification of drugs with high levels of potential pathogens and high counts of non-pathogens

Twenty (5%) mixtures of Magnesium trisilicate were contaminated with Pseudomonas aeruginosa while 4(1%) of the same contained Klebsiella. Sixteen (4%) Kaolin mixtures contained Pseudomonas aeruginosa. One (0.3%) cofel cough syrup and 1(0.25%) mist expectorant sedative were contaminated with Proteus. Two (0.6%) chloroquine syrups, 1(0.3%) Broncholin cough syrup, 2(0.5%) Belladonna paediatric mixture, and 2(0.5%) mist expectorant sedative were contaminated with Streptococcus faecalis. Of the chloroquine syrup samples with growths, 2(0.6%) contained more than 30,000 cfu/ml of Candida sp, while 2(0.5%) mixtures of Belladona contained more than 10,000 cfu/ml of Candida sp.

5.0 DISCUSSION

Determination of mesophile growth, revealed that 137 (42.8%) of the 320 syrups and 196 (49%) out of the 400 mixtures were contaminated with microorganisms (Table 3). This proportion is high especially when one considers that these preparations are given or used by sick children or elderly patients. The possibility that contaminating microbes may cause infection is increased by the fact that these patients may have lowered resistance to these microbes. The study revealed no significant difference between contamination of syrups or mixtures by bacteria. (P = 0.05)

Table 4 is a summary of bacterial count done on all the samples used in the study. The order of the number of colonies for syrups was as follows: 78(17.8%) contained 10-100 colony forming units/millilitre (cfu/ml), 57(17.8%) between 101-10000 cfu/ml, and 2(0.6%) had counts greater than 10,000 cfu/ml. The order for mixtures was as follows; 56(14%) contained 10-100 cfu/ml, 138(34.5%) between 101-10,000 cfu/ml and 2(0.5%) contained more than 10,000 cfu/ml. It was also observed that the lowest count was 10 cfu/ml and the highest 30,000 cfu/ml for both preparations (Appendix III). The mean bacterial count was 1410 colonies for syrups and 2201 for mixtures. This shows that there was a higher

level of contamination in mixtures than in syrups, and this difference was statistically significant ($P < 0.05$). According to the limit for microbial contamination set in the United States Pharmacopoeia and European Pharmacopoeia, most of the counts observed in the present study are within this set limit (Appendix IV). However, the limits set in these monographs were for both solid and liquid dosage forms. The more than 10,000 colonies observed in 2(0.6%) syrups and 2(0.5%) mixtures was more or less the same for both formulations and that there was no significant difference ($P < 0.05$).

The differences in the levels of contamination between the two formulations can be attributed to the fact that microoganisms do not survive well in high sugar concentrations (syrups contain 66% sugar). The high sugar concentration gives the product a high water activity (A_w) which hinders microbial proliferation (microorganisms need, among other things, water, for survival[15]). However, there are osmophilic organisms which are capable of surviving in high sugar concentrations and among them are Candida[35]. The contamination of syrups could also have been due to change in temperature especially during transport or storage. Syrups must remain at constant temperature since any variation may result in evaporation of some of

the water content followed by condensation and dilution of the surface layers. This will give A_w values which may permit the growth of osmophiles and spoil the syrups. Another explanation of the presence of microorganisms in syrups could also have been caused by inadequate closure of some of the bottles, which led to leakage through the caps. During sample collection some bottles were leaking through the caps. This was due to loose caps to fit the inner lining and/or absence of the inner cap which was observed. Sugar crystals were observed around the mouth, thus confirming the leakage. Evaporation of the syrups would lead to formation of a thin water film within the loose caps and create a favourable environment for bacterial growth and eventual contamination of the syrups[35,36].

The mixtures were found to have higher microbial count than the syrups. Mixtures have usually high percentage of water, thus forming a good medium for microbial proliferation[8]. Water can carry a high level of contamination[10]. The use of plastic tube from still to container could have increased the risk of contamination especially if it was not frequently checked and cleaned. The number of microorganisms proliferates rapidly when stored at ordinary temperatures, and this was the case in most of the manufacturing plants. During visits to

the manufacturers of the formulations, it was observed that production was done in very wet conditions. Wet environment is one of the factors contributing to microbial contamination of pharmaceutical preparations[4].

The various methods used for identification of microorganisms are well standardized and widely used. The colonies were identified on the basis of Gram staining. This classified the isolated organisms into four major groups according to cell type and morphology. It included Gram positive rods, Gram negative rods, Gram positive cocci and Gram positive yeast cells.

In this classification it was observed that Gram-positive rods were numerically the most predominant in both formulations due to their wide distribution in water, soil and air. The second group in dominance was yeast cells seen in both formulations. The Gram-negative rods were present in mixtures only since they are able to multiply rapidly in water and there was none in syrups. The difference in classification of the isolated organisms in the two drug formulations was statistically significant (Chi-square = 50.96, $P<0.05$) (Table 5). This difference could be due to differences in the physico-chemical properties of the two formulations. Some of the contaminated formulations had

more than one type of cells. Gram-positive cocci and Gram-positive yeasts were found in combination with Gram-positive rods, and Gram-positive yeast was found in combination with Gram-positive cocci. Similar findings were obtained by Akinmoji *et al*[8] who observed that most of the medicine samples contaminated yielded one or two types of microorganisms, but a few samples yielded three different types of organism.

After classifying the organisms according to Gram stain characteristics, further identification was done to get the specific organism. This included a series of biochemical and serological tests. The frequency of isolation of organisms as shown in Table 5 splits the various morphological classifications into individual organisms.

The Gram-positive cocci were identified to consist of Micrococci, Staphylcocci and Streptococci. The Gram-positive rods consisted of Bacillus and Diphtheroids, while the Gram-negative rods were identified to be Pseudomonas, Proteus, Klebsiella and Alcaligenes, and the Gram-positive yeast cells were identified to be Candida sp. These organisms are normally found in water, air and the environment, therefore their level of

occurence in manufacturing premises can be lowered if proper control is instituted.

Baccilus sp were the most predominant organisms isolated in both formulations (27.2% in syrups and 28% in mixtures). Members of this genus are the most common microorganisms found in non sterile drugs used for oral administration[34], and so the results are in agreement with those previously documented. They can grow at temperatures as low as -5°C to 25°C (psychrophiles) and at temperatures, as high as 45°C to 75°C (thermophiles)[37]. They are widely distributed in soil, air and water[38]. Their presence in the preparations could have been due to inadequate filtering of the air in the manufacturing area, or inadequate treatment of water used in manufacturing. Studies done by Gaurcia *et al*[29] also revealed the isolation of 118 strains of Bacillus from non-sterile oral pharmaceutical preparations. Due to their nature of occurence and abundance in air, Bacillus is not usually included in the routine work of laboratory control because of the difficulties involved[39,40].

Candida sp. was identified in 15% of the syrups and in 9.5% of the mixtures. Candida are known to survive in high sugar concentrations and are capable of fermenting

various carbohydrate sources. Although the identified species were non-pathogenic, a high count can lead to spoilage of drug through fermentation. The source of contamination of these organisms in the products could be the personnel, poor hygienic practices (like working without masks) and the surrounding atmosphere. It is important to note that some of the airborne microorganisms change with the time of the day, ventilation, temperature, humidity, and activities of the people during manufacturing[38].

Pseudomonas was isolated in 9.3% of the mixtures. This was differentiated from Enterobacteriaceae by its being oxidase positive, non-fermentative properties and its ability to oxidize glucose[34,35]. This organism has a common habitat in wet locales; its natural habitats are soil and water. In this study, it was isolated in counts of more than 3,000 colony forming units per millilitre (cfu/ml). Most likely it was introduced as a result of poor manufacturing practices, and the presence of wet locales in the manufacturing premises[41,42]. Most of the premises for liquid preparations are left wet for a long time. A study on quantitative microbiological monitoring of haemodialysis fluids revealed that most of the organisms in excess of 200 colony forming units per millilitre (cfu/ml)

isolated were Pseudomonas sp.[26] In another study on microbial contamination of medicines dispensed in hospitals[8], about a third of the medicines observed contained Gram negative rods (Pseudomonas inclusive). A study conducted in Spain on isolation of aerobic heterotrophic bacteria from natural spring water also revealed presence of Pseudomonas[34]. This shows that this organism is a problem in many areas and that strict measures have to be taken to control its presence in oral formulations. This also indicates that water for manufacturing was probably not pre-tested, since Pseudomonas is an objectionable organism in water for pharmaceutical production.

Bacillus in combination with Candida was isolated in 1.8% of syrups and mixtures. However, it is known that Candida do not survive well on an artificial media in mixed population[35]. These two organisms were the most predominant in the formulations.

Three different cocci were identified in the preparations, including Micrococci, Staphylococci and Streptococcus faecalis. Micrococci were isolated in both preparations. These are commensals normally found in the nose and are widely spread in nature[35]. They are usually not parasitic. These were introduced into the

formulations probably by using stored industrial water or through airborne contamination as previously reported by other workers[43]. The use of stored water is practised in most manufacturing plants due to the frequent water shortages which occur sometimes and may therefore affect production schedules.

Staphylococci were identified by colonial and microscopic morphology, catalase production, coagulase, mannitol and glucose fermentation, and the identified organism was Staphylococcus albus which does not ferment mannitol and is coagulase negative[33]. Staphylococcus albus was found in 2.8% of the syrups and in 1.8% of the mixtures. It is found as a normal flora on skin. It is usually not pathogenic although it may cause diseases such as sub-acute bacterial endocarditis (SBE) and meningitis after accidental introduction into the body. Members of the genus Streptococci form the dominant bacterial flora of the mouth and pharynx of humans[35]. Some species of Streptococci are found in the intestine. Streptococcus faecalis was identified in 0.9% of both syrups and mixtures. Introduction of this organism into the preparations could be through, among other sources, the personnel, water and air as most of the workers did not have appropriate clothing, and in some places air filtering facilities were not available[41].

Alcaligenes faecalis is a strict aerobe which fails to oxidize and ferment glucose. It is grouped as a miscellaneous Gram negative rod[35]. It is found in water, soil, alimentary tract and this could be one of the ways through which it was introduced into the preparations. It is a normal flora in the intestine of man and may cause opportunistic infection in immunocompromised patients. It was isolated in 0.8% of the mixtures indicative of water contamination.

Bacillus in combination with Streptococci was found in 0.6% of syrups only. The most likely sources of contamination was probably the air and personel. Both Micrococci and Staphylococci were found in combination with Bacillus in 0.6% of syrups. Diphtheroids were another Gram positive rods isolated in 1% of the mixtures. They are considered to be contaminants. Other organisms isolated and identified were Aspergillus fumigatus, Penicillium notatum and Mucor, but these were considered as contaminants as they were encountered in those plates which were incubated for more than 48 hours on (SDA) and in the open plates left as control on 3rd day.

The contaminating microbes can be broadly classified as pathogens capable of causing infection in heathy

individuals, the opportunists capable of causing infection in immunocompromised individuals and the non-pathogens. Many of the microbes generally considered to be non-pathogenic have been found to be opportunists[42].

Of the potential pathogens isolated (Table 6) Pseudomonas were most predominant and were isolated in 9.3% of the mixtures. Once the organism is removed from its natural habitat and established elsewhere somewhat removed from their natural habitat, it can produce a wide variety of infections[44,45]. Pseudomonas sp are not particularly invasive, but once they are established as infective agents, they are very difficult to eradicate. Their presence in more than 3000 colony forming units per millilitre (cfu/ml) could pose a problem to users depending on dose and physical state of the patient, nutrition and age[35]. It is indigenous to fresh water contamination and is nutritionally undemanding.

Klebsiella was isolated in 4 (1%) of the mixtures. This Gram-negative rod is widely distributed in nature[43] and has been recovered from every part of the human body, as well as from soaps, water and animal sources. Its introduction into the preparations could be through inadequate rinsing after using contaminated soap, or from the environment. Klebsiella is potentially

pathogenic and is part of the normal flora in approximately 10% of population in the throat and intestinal tract. It is known to cause pneumonia in individuals with lowered resistance depending on the infectious dose. However, the count encountered was 1000 cfu/ml and this frequency of isolation is >0.3% which is the accepted level of contamination[40,41]. The source of this pathogen in the preparations could be due to untreated water resulting from soil erosion, heavy rainfall and decaying plant matter or unhygienic practices of the personnel. It has been found that the more a project is split and delegated within the manufacturing premises, the more people of less specialized training become involved[40]. It is known that no training is given to these people, and this magnifies the problem of microbial contamination. Contamination rate of 0.3% is usually accepted, but this should not include the pathogens. As a matter of fact, it can be said that almost any microbe can cause infection if the dose is sufficient, the route of transmission is optimal and the resistance of the patient is low enough[42].

Proteus was another potential pathogen isolated in 0.3% of both formulations. This Gram-negative rod, which does not ferment lactose, liquefies gelatin, is urease positive is also found in the intestinal tract of man.

In nature the organism is found in locales that are faecally contaminated[43]. It may cause pyogenic infections in other parts of the body when it is accidentally introduced. The source of this organism in the preparations could be water.

Drugs with potentially pathogenic organisms were Magnesium trisilicate mixture, Kaolin mixture, Belladonna paediatric mixture and Mist expectorant sedative. Both mixtures of Magnesium trisilicate and Kaolin had more than 3000 cfu/ml of the potential pathogens. Components of Kaolin and Magnesium mixture may inactivate a preservative by adsorption, and this could have been the cause of the high count encountered in both drugs. Also it is known that efficiency of a preservative greatly depends on nature and number of contaminating microorganisms, environment in which they are manufactured and the materials used in the formulation. Belladonna is a natural product and therefore the raw material may have a high count of micro-organisms.

Certainly, the notion that disease production in any host resides solely in the pathogenic and virulent properties of microbes can't be accepted any longer[35,47]. The recovery of opportunistic pathogens and high counts

of yeast which may cause product degradation, constitutes a steady challenge for manufacturers and policy enforcers. Extreme susceptibility of pharmaceutical preparations to Pseudomonas and Candida should make one seriously consider the possible degree of destruction of the ingredients of heavily contaminated medicines.

Most of the contaminants may be due to the environment of preparation, level of hygiene of the staff and raw materials used, and other contaminants grow in the presence of a preservative but it is to be noted that the packaging material may be contributory. The hazard of transfer of microorganisms from humans to pharmaceutical preparations may be reduced by comprehensive training in personal hygiene, coupled with regular medical checks[36].

Knowledge on the distribution, survival, lifestyle of microorganisms in the factory environment, should enable process designers, controllers and quality control personel to comprehend, trace and eradicate the sources of failure due to extraneous bacterial contaminants in the finished product.

It is envisaged that with necessary information some control of contamination may be facilitated and adequate preservation and packaging may be carried out, in addition to the reflections on the design of our manufacturing plants.

6.0 CONCLUSION

The results of the study revealed that most of the locally manufactured oral liquid formulations assessed were contaminated. Mixtures had a higher level of contamination compared to syrups. Contamination levels between 10 to 30,000 colony forming units per millilitre (cfu/ml) were encountered in both mixtures and syrups but the mean count was lower for syrups. Non pathogenic and potential pathogens were isolated from the products. Most of these potential pathogens were indegenious in water, indicating that water may be the most likely source of contamination. Although, there was no strictly pathogenic organisms isolated, the presence of Pseudomonas and yeasts which have extremely degradative ability indicate some doubts on the quality of the formulations assessed.

7.0 RECOMMENDATIONS

a) The law governing the licensing of manufacturers of pharmaceuticals should be more strict than it is now. Among other things, every manufacturing unit must have a functioning water purification system. The water purification system should also be regularly inspected and preventive maintenance conducted as often as possible since water has been considered to be the main source of contamination.

b) There should be frequent inspections and strong warnings to those who fail to comply with the regulations, and legal action should be taken against non-compliers.

c) Since the study is the first of its kind in the country, it should be extended to manufacturing plants in other regions and products for external use should also be assessed.

d) Regulatory authorities are urged to make use of the findings of this study to design strategies for the control of microbial contamination.

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