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Microbiological Assessment Of Oral Liquid Formulations Manufactured In Dar-Es-Salaam, Tanzania

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The aim of the study was to determine the microbiological quality of locally manufactured oral liquid pharmaceutical preparations in Dar es Salaam. Seven hundred and twenty drug formulations comprising of 320 (44.4%) syrups and 400 (55.6%) mixtures collected from retail pharmacies and drug stores were analyzed for the total viable aerobic count by the plate count method. Microorganisms isolated from the samples were identified with use of routine microbiological and biochemical identification tests.

Results obtained revealed microbial growth in 49.0% of the mixtures and 42.8% of the syrup preparations, but the difference was not statistically significant ($p>0.05$). Different organisms were identified from the two formulations and their distribution pattern was found to be significantly different. Gram-negative rods were isolated only from mixtures. Potential pathogens isolated, including Pseudomonas spp. and yeasts were found more in mixtures (10.5%) than in syrups (0.3%) and this difference was statistically significant ($p<0.005$). Mixtures of Magnesium trisilicate, Kaolin and Belladona, contained more potential pathogens compared to the rest of the mixtures. Water used during manufacturing of the formulations was considered to be the main source of contamination.

From these findings, strict criteria for issuing licenses for drug manufacturing in Tanzania are recommended, and adherence to Good Manufacturing Practice should be emphasized. The study findings 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.

INTRODUCTION

The microbial content of pharmaceutical preparations is an important parameter in assessing the quality of good manufacturing practice (GMP) of drugs. Drug formulations contaminated with microorganisms are easily spoilt thus shortening their shelf-life and pose infection risk to the consumer. Microbial contamination may arise from various sources, including the water used, equipment and personnel involved in the manufacturing process, as well as the environment in which production is conducted [1,2,3]. Some of the preservatives contain nutritious substances which favor bacterial and fungal growth. In addition, the raw materials used such as plant derivatives may harbor microorganisms [4]. Poor packaging and improperly sealed drug containers may predispose pharmaceutical products to microbial contamination.

In view of the inherent risk of microbial contamination of drugs, it is essential to institute a close monitoring of the microbial quality of pharmaceuticals, during preparation, storage and while in use. Reference levels of acceptable microbial content of different drug formulations have been established to provide guidance in quality control efforts [5,6]. Regular assessment of microbial quality of pharmaceutical preparations is therefore an essential component towards maintaining GMP. In developed countries, this exercise is usually part of the

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quality assurance routine, but in resource-poor settings, this is often done irregularly.

So far no studies have been documented in Tanzania on the occurrence and magnitude of contamination of locally manufactured medicinal preparations. There is also 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 lacking. This study, therefore, was undertaken to assess 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, with a view to establishing some baseline data for use in improving the quality of Tanzanian pharmaceutical products and in setting local standards. Such information may also enable planners and policy makers to establish acceptable and feasible strategies for the control of microbial contamination of locally produced medicinal preparations.

MATERIALS AND METHODS

Drug samples

The drug samples comprised of syrups and mixtures. The syrups were prepared using simple syrup (66.7% $^{w}/_{w}$ sucrose in water) as the vehicle. Mixture preparations were made using water as the vehicle.

Sample size

This was determined using recommended statistical methods [7,8] and preliminary data obtained in a pilot survey. Sample sizes of 320 for syrups and 400 for mixtures were derived.

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 [9,10]. 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. 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) in order to increase the possibility of buying new stocks from the manufacturers.

Total viable aerobic count

All analyses were performed in duplicate. Drug samples were first agitated to ensure an even distribution of microorganisms within the specimen [11]. Using sterile disposable syringes, 10 mls of the sample was drawn and placed into a bottle containing 90 mls of buffered Nacl-peptone solution [12]. Polysorbate 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 1 ml were drawn from the mixture and placed on 4 petri dishes; 2 containing Tryptone soy-agar, and the other 2 containing Sabourauds Dextrose Agar (SDA). Tryptone soy-agar plates were incubated at 37°C for 48 hours while SDA plates were incubated at 25°C for 7 days. The plates were examined for growth and the number of colonies counted were multiplied by 10 to get colony forming units per milliliter (cfu/ml) of original sample. Each colony formed was counted as one although it could have originated from more than one microorganism. Isolated microorganisms were identified by conventional microbiological methods [13-15]. Appropriate controls were included in the test procedure.

Statistical analysis

The results were analyzed using the Epi Info version 6 software [16]. 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.

RESULTS

The study investigated 64 each of paracetamol, promethazine, chloroquine, cough syrup and vitamin B complex syrup preparations and 80 each of Belladona, kaolin, magnesium trisilicate, cough and iron sulphate mixture formulations from five manufacturers in Dar es Salaam.

Mesophile growth was identified in high proportions of the samples, 137 (42.8%) syrups and 196 (49%) mixtures. The difference in the microbial isolation rate between the two drug formulations was not statistically significant ($p>0.05$). Microbial growth was not seen in 183 (57.2%) syrups and in 204 (51.0%) mixtures. As shown in Table 1, the overall mean counts were 1410 cfu/ml for syrups and 2201 cfu/ml for mixtures. Significantly higher levels of contamination were observed in mixtures compared to syrups ($p<0.05$). For both preparations the lowest count for both preparations was 10 cfu/ml and the highest count was 30,000 cfu/ml.

Table 1: Microbial count of the two drug formulations

Count cfu/ml	Syrups n (%)	Mixture n (%)	p value
10 - 100	78(24.4)	56(14)	.0004
101 - 10,000	57(17.8)	138(34.5)	.0000
>10,000	2(0.62)	2(0.5)	.7794
None	183(57.2)	204(51)	.0030
TOTAL	**320(44.4)**	**400(55.6)**	

Table 2 summarizes the spectrum of microorganisms isolated from the two drug formulations. *Bacillus subtilis* and Candida spp. were the most commonly isolated contaminants in both syrup and mixture drug formulations. Similar rates of contamination of the two drug formulations by *Bacillus subtilis* were observed, 87/320 (27.2%) for syrups and 112/400 (28%) for mixtures. Contamination rates by Candida spp. were 48/320 (15.0%) and 38/400 (9.5%) for syrups and mixtures respectively. *Pseudomonas aeruginosa*, Diphtheroids, and Klebsiella were found only in mixtures. Of the samples which

Table 2: Microorganisms isolated from syrups and mixtures

Organism	Syrups n (%)	Mixtures n (%)	p value
Alcaligenes	0 (0.0)	3 (0.8)	
Aspergillus fumigatus	2 (0.6)	3 (0.8)	.8021
Bacillus subtilis	87 (27.2)	112(28.0)	.8086
Candida	48 (15.0)	38 (9.5)	.0237
Diphtheroids	0 (0.0)	4 (1.0)	
Klebsiella	0 (0.0)	4 (1.0)	
Micrococci	2 (0.6)	4 (1.0)	.8840
Mucor	3 (0.9)	1 (0.3)	.4638
Penicillium notatum	1 (0.3)	1 (0.3)	
Proteus	1 (0.3)	1 (0.3)	
Pseudomonas aeruginosa	0 (0.0)	36 (9.6)	
Staphylococcus albus	9 (2.8)	7 (1.8)	.3365
Streptococcus faecalis	3 (0.9)	7 (1.8)	.4431
None	183 (57.2)	204 (51.0)	

were contaminated with *Pseudomonas aeruginosa* 20/400 (5%) were magnesium trisilicate and 16/400 (4%) were kaolin mixtures. Mixed growth, usually comprising of two different micro-organisms, was encountered in some preparations. Only one preparation was found to have three different organisms. The combinations encountered were Bacillus and Micrococci, Bacillus and Candida, Bacillus and Staphylococcus, Bacillus and Streptococcus, and Candida and Staphylococcus.

Potential pathogens (Klebsiella, Proteus, *Pseudomonas aeruginosa* and *Streptococcus fecalis*) were isolated at a significantly higher rate from mixtures 45/400 (11.3%) compared to syrups 4/320 (1.3%), $p<0.05$. The drug samples from which potential pathogens were isolated were magnesium trisilicate (25% *Pseudomonas aeruginosa* and 5% Klebsiella), kaolin (20% *Pseudomonas aeruginosa*), Cofel cough syrup (1.6% Proteus), expectorant sedative (1.6% Proteus and 3.1% *Streptococcus fecalis*), chloroquine syrup (3.1% Candida and 3.1% *Streptococcus fecalis*), broncholin syrup (1.6% *Streptococcus fecalis*) and Belladona paediatric mixture (2.5% *Streptococcus fecalis* and 2.5% Candida).

Of the chloroquine syrup samples with growths, 2 contained more than 30,000 cfu/ml of Candida spp, while 2 mixtures of Belladona contained more than 10,000 cfu/ml of Candida spp.

DISCUSSION

The present study evaluated the microbiological quality of locally produced medicinal preparations in Dar es Salaam. The proportion of drugs found to be contaminated was significantly high in both formulations. This observation has a serious implication when one considers that these preparations are given to sick people, including children and elderly patients.

Generally there was a significantly higher level of contamination in mixtures than in syrups. 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 [17,18]. However, the limits set in these monographs were for both solid and liquid dosage forms.

The differences in the levels of contamination between the two formulations could be attributed to the fact that microorganisms do not survive well in high sugar concentrations (syrups contain 66% sugar). High sugar concentration gives the product a high water activity (A_w) which hinders microbial proliferation. However, there are osmophilic organisms like Candida spp. which are capable of surviving in high sugar concentrations. 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. 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 noted to have loose caps and leaking. Sugar crystals were also observed around the mouth of containers, thus confirming the leakage. Evaporation of the syrups would lead to formation of a thin water film within the loose caps and create a favorable environment for bacterial growth.

The mixtures were found to have higher microbial count than the syrups. Mixtures usually have high percentage of water, thus forming a good medium for microbial proliferation. During visits to the manufacturers, it was observed that production was done in wet conditions. Wet environment is one of the factors contributing to microbial contamination of pharmaceutical preparations [19, 20]. Gram-positive rods were numerically the most predominant organisms in both formulations, possibly due to their wide distribution in water, soil and air. The Gram-negative rods were present in mixtures only since they are able to multiply rapidly in water. The pattern of mixed contamination found in the present study compares with that reported by Akinmoji *et al* [21], who observed that most of the contaminated medicine samples yielded one or two types of microorganisms, but only a few grew more than two different types of microorganisms.

Most of the organisms isolated are normally found in water, air and the environment, suggesting that their level of occurrence in manufacturing premises could be lowered if proper control measures are instituted. Bacillus spp, the most predominant organisms isolated in both formulations in the present study, has previously been reported to be the most common contaminant in non sterile drugs used for oral administration [22]. 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. Candida spp., which was the other commonly isolated contaminant, is known to survive in high sugar concentrations. 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.

Pseudomonas aeroginosa was isolated in counts of more than 3,000 cfu/ml. This organism has also been reported to be a common contaminant of drug preparations by others [21] and it is likely that it was introduced as a result of poor manufacturing practices. The recovery of opportunistic pathogens and yeasts in high counts

may cause product degradation and constitutes a 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.

The potential pathogens isolated were present in more than 3000 cfu/ml. Contamination rate of 0.3% is usually accepted, but this should not include the pathogens. 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 [23]. The high level of contamination observed for potential pathogens could therefore pose a problem to users depending on dose and physical state of the patient, nutrition and age.

Drugs with potentially pathogenic organisms were Magnesium trisilicate mixture, Kaolin mixture, Belladonna pediatric 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.

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

In conclusion, the study findings revealed that most of the locally manufactured oral liquid formulations assessed, especially mixtures, were contaminated. Non pathogenic and potentially pathogens organisms were isolated from the products. Most of these potential pathogens are common 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 makes the quality of the formulations assessed questionable. It is important to promote strategies geared towards GMP and to enforce strict criteria in the issuing of licenses to pharmaceutical manufacturers in order to prevent microbial contamination of locally produced oral medicinal preparations.

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