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PREVALENCE OF CONTAMINATING MICROORGANISMS IN ANTI-MALARIAL DRUGS SOLD IN CALABAR, CROSS RIVER STATE, NIGERIA

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Prevalence, Anti-Malarial, Contaminating Microorganisms, Standard Microbiological Procedures And Total Aerobic Plate Count

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ABSTRACT: The challenges to ensure proper preparation, storage, preservation and distribution of drugs are enormous. Very often, this results in the production and distribution of contaminated drugs. The study, therefore aimed to evaluate the prevalence of contaminating microorganisms in anti-malarial drugs sold in Calabar, Cross River State, Nigeria. This was carried out using standard microbiological procedures which included total aerobic bacterial plate count, isolation and characterization of microbial contaminants and physical parameters analysis. It is evidence from the results that out of a total of 10 samples, only one sample was not contaminated by bacteria but for fungi, all the drugs sampled were contaminated. *Bacillus subtilis* was found to be the most prominent bacterial isolates with the frequency of occurrence of five (5) and 33.5%, followed by *P. aeruginosa* and *S. aureus with* the same frequency of three (3) and 20.0%, *Lactobacillus sp.* had frequency of two (2) and 13.3%, and *Sporolactobacillus* had frequency of one (1) and 6.6% while only one of the anti-malarial drugs had no contamination, that is 6.6% of the sample was not contaminated. Whereas *Penicillium sp*. was found to be the most predominant fungal isolates, with the highest frequency occurrence of six (6) and 46.2% followed by *Candida alblicans* with the frequency of three (3) and30.7% and *Aspergillus niger* had the least frequency of three (3) and 23.1%. This result reveals a high level of bacteria and fungi contaminants in the anti-malarial drugs sold in Calabar and makes need for intervention.

INTRODUCTION: Spoilage of pharmaceutical products or drugs involve basically, initial or early pioneer invaders of biodegrading or spoilage micro-organisms, which makes the environment (contaminated drugs) suitable for later invaders. These organisms are known to breakdown complex nutrients, altering the pH of the drugs and making more moisture available to subsequent invaders [17].

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The microbiological quality at the time of administration of non-sterile pharmaceutical products or dosage form such as tablets, had been shown to be on the bio-burden of the raw materials, both active ingredients and excipients [5, 22], thus making it necessary to observe strict good manufacturing procedure, reduction of contamination strategy at every stage of production [3]. Due to the observation made by Bos *et al.,* [5] and Skinner and Carr [22] that aqueous preparations were found highly contaminated, the observation showed that it can cause serious health implication to the consumers. It was suggested that a preservative should be included to inhibit proliferation of microorganisms in multi-dose pharmaceutical products [15].

Also development in packaging technology should be made to provide unit dose delivery without product exposure as this process will greatly reduce microbial contaminant risk. Microbial contamination is responsible for product spoilage and it potentially causes serious health hazards to the patient [5, 22].

According to Barer [3], only thirteen well documented microorganisms were responsible for pharmaceutical contamination. Microbial contamination most times, starts during the manufacturing process, rather than during use [7], and could be prevented by controlling people, environment, raw materials and formulation [2, 19]. It was further suggested that a preservative may be added or included to check proliferation of microorganisms [19]. This does not compensate for good manufacturing process [9]. Microbial contaminants are dynamic populations in which the organisms were actively multiplying or static populations in which no growth occurs [19].

A wide variety of non-sterile pharmaceutical products are susceptible to bacteria and fungi invasion, resulting in spoiled product that is unfit for use. Spoilage can occur in three different ways. It is often assumed that impurities in the active pharmaceutical ingredients are of greatest concern for the regulation and control. However, for many drug products and formulation type, this may not be the case [12]. According to Rose [19], Qui and Norwood [18] and later, Gorog [11] the physical and chemical status of pharmaceutical products considerably influence the type and extent of microbial spoilage from which it is at risk.

A drop in personal hygiene of the operator may cause bacteria other than normal flora of the skin to be transferred to pharmaceutical products at any stage. These includes faecal contamination organisms from the anal region or wound which support pathogenic bacteria and *Staphylococcus aureus* [24]. Other contaminating microorganisms includes gram negative intestinal bacteria, *Micrococci*, alpha-hemolytic and non-hemolytic *Streptococci, Clostridium spp., Bacillus spp.* and *Enterococci*. Hazardous transfer of microorganisms from human to products may be brought to lowest bearing limit by improved personal hygiene and regular medical checks [25]. There are other contaminants from the air, water, building, which may find their way to contaminating pharmaceutical products and thereby making it unfit for medications.

This work is therefore aimed at evaluating the prevalence of contaminating organisms in antimalarial drugs and also aimed at proffering the best possible solution to drugs contamination.

MATERIALS AND METHODS: This features a summary of the basic processes involved in the collection of drugs samples and the necessary microbiological analysis and quality assurance. In the case of reagents, the manufacturer's instructions were adhered to strictly.

Sample collection: The ten different brands of drugs used were procured from pharmacies and patent medicine store in Calabar Cross River State, Nigeria, as enlisted symbolically below; *DIA* from location A, *HER* from location B, *XIN* from location C, *ORN* from location D, *INE* from location E, *UID* from location F, *MAX* from location G, *LUM* from location H, *SEN* from location I, and *AST* from location J.

Media / Reagents: All media and reagents used were prepared following standard microbiological procedures. Media employed include; Nutrient Agar, MacConkey Agar, Potato Dextrose Agar, Sheep blood Agar, and Sabouraud Dextrose Agar. Reagents used includes, Grams iodine, Crystal violet, Ethanol, Safranin, Kovac's reagent etc.

Physical examination of samples: Each sample was examined in turn for appearance, colour, odour, taste, turbidity, manufacturing date, expiration date, batch number, origin, pH and brand. Each data was recorded

Handling of powdered samples or suspensions: The powdered sample and suspensions were dissolved in sterile water according to the manufacturers' description. Using the method of Baird [7], the samples were diluted using sterile water. Two sterile disposable needles and syringes were used in this process. With the help of a cotton wool soaked in methylated spirit, the surface of rubber stop cover of injection vials were swabbed and allowed to dry.

A sterile needle was used to aseptically draw 5ml of the sterile water for injection from the container. The needle was discarded and another sterile needle was used to transfer the water into the powdered drug. The mixture was mixed well to form a solution with the syringe still in place. After this, 1.0ml of the mixture was drawn and inoculated on the solid Nutrient agar and MacConkey agar and then incubated at 37^{o}C for 24 hours for bacteria and Sabouraud Dextrose agar and then incubated at room temperature for 72 hours for Fungi [20].

Preparation of syrups: A ten-fold serial dilution was carried out to reduce the microbial load and 0.1ml was plated onto different agar medium (Nutrient, MacConkey and Sabouraud Dextrose agar) in sterile Petri dishes after 1ml of each sample was drawn aseptically with the aid of a sterile pipette into 9mls of sterile water and shaken for proper dilution. The inoculated plates for bacteria were incubated at 37^{o}C for 24 hours while Fungi plates were incubated at 28^{o}C for 72hours.

Microbiological examination of drug samples: Drug samples were examined using microbiological methods such as spread plate method and pour plate method.

Microbiological analysis of drug samples using plate count method: The plate count method provides a standard for determining the density of aerobic and facultative anaerobic heterotrophic bacteria in anti-malarial syrup samples. The samples in bottle were properly mixed by shaking gently. After which 1ml of each samples was aseptically transferred using a sterile micro-pipette into prepared sterile distilled water in a sterilized test tubes labeled to 10 from 1 using a tenfold serial dilution method (10^{0} to 10^{9}) for each sample. Then 1ml was transferred from the tube containing 9ml of distilled water with additional 1ml of sample, making a total of 10mls using sterile pipette.

Note that each of the serial dilution bottles, (tenfold) contains 9ml of sterile water. From 10^{-1}, 1ml of mixtures was transferred to 10^{-2} and was carried out till 10^{-9} where 1ml of the mixture is being discarded at the end. Viability was assessed using 0.1 ml volume of the final dilution for spread-plate method bacteria count and the plates were incubated at 37^{0}C for 24hrs for MacConkey agar and Nutrient agar. 1ml of the aliquot of each sample was spread on the surface of sabouraud dextrose agar, this was used for fungal count. Plates were incubated at room temperature, 28^{o}C for 72hrs. Each sample was inoculated in duplicates. The number of colony forming units were determined by the use of colony counter. The microbial counts were recorded as a mean of each sample calculated and expressed as mean colony forming units per milliliter (cfu/ml) [5, 16].

Isolation of microbial contaminants: After 24 hours of incubation, colonies were counted and identified. Different distinct colonies were sub-cultured to get a pure culture before characterization and identification. A loopful of a discrete colony was sub-cultured on nutrient agar and Sabouraud dextrose agar. Bacterial isolates from the MacConkey agar plates were sub-cultured into *Salmonella-Shigella* agar while those from the Nutrient agar plates were sub-cultured into Mannitol salt agar and incubated for 24 hours. Further identification and biochemical characterization of the bacterial isolates were carried out using standard microbiological procedures [20]. The microbial population of the isolates were assessed and the moulds identified using a well-established microbiological method by their morphological and biochemical characteristics [16].

RESULTS AND DISCUSSION: The under listed microorganisms in **Table 1** and **2** were confirmed according to Cowan and Steel [8] after analyzing the drug samples using standard microbiological procedures. Out of the ten (10) syrups/suspensions sampled, nine (9) were contaminated with bacteria, while one sample was free of bacterial contamination. Five (5) bacteria isolates were obtained from fifteen (15) culture plates.

The results of this study had shown that some antimalarial syrups and suspensions were highly contaminated. This suggests that the manufacturing practice and storage conditions were broken down. The level of contamination in four (4) anti-malarial preparations when far above the tolerable limit of permissible microorganisms specified for syrups and suspensions as stated by Booth [4].

Some of the anti-malarial drugs were contaminated with *Lactobacillus sp., Pseudomonas aeruginosa, Bacillus subtilis, Staphylococcus aureus, Penicillium sp., Sporolactobacillus, Candida alblicans* and *Aspergillus niger*. These organisms have been reported as drugs contaminants by Qiu and Norwood [18] in Britain and Okpo *et al.*,[16] in Nigeria. The results obtained in this work had implicated many microorganisms in contamination of antimalarial syrups and suspensions as shown in **Table 1** and **2**. These organisms are indicators of poor hygiene practices by the manufacturers and it's in accordance with the research carried out by Olaniyi [17].

TABLE 1: ANTI-MALARIAL DRUG SAMPLE AND ITS CONTAMINATING BACTERIAL ISOLATES

Sr. no.	Drug Sample Code	Bacteria Isolates
1	*ORN*	None
2	*HER*	*Bacillus subtilis*
3	*XIN*	*Bacillus subtilis* *Staphylococcus oureus*
4	*DIA*	*Pseudomonas aeroginosa*
5	*AST*	*Bacillus subtilis* *Sporolactobacillum*
6	*MAX*	*Lactobacillus*
7	*SEN*	*Staphylococcus oureus* *Pseudomonas aeroginosa*
8	*INE*	*Staphylococcus oureus*
9	*LUM*	*Bacillussubtilis* *Pseudomonasaeroginosa*
10	*UID*	*Bacillus subtilis* *Lactobacillus*

TABLE 2: ANTI-MALARIAL DRUG SAMPLE AND ITS CONTAMINATING FUNGAL ISOLATES

Sr. no.	Drug Sample Code	Fungi Isolates
1	*ORN*	*Candida albicans*
2	*HER*	*Penicillun Spp.*
3	*XIN*	*Penicillun Spp.*
4	*DIA*	*Aspergillus niger*
5	*AST*	*Penicillun Spp.* *Aspergillus niger*
6	*MAX*	*Penicillun Spp.*
7	*SEN*	*Penicillun Spp.* *Candidaalbicans*
8	*INE*	*Candida albicans*
9	*LUM*	*Aspergillus niger*
10	*UID*	*Candidaalbicans* *Penicillun Spp.*

Bacillus subtilis was found to be the most prominent bacterial isolates with the frequency of occurrence of five (5) and 33.5%, *S. aureus and P. aeruginosa* with the same frequency of three (3) and 20.0%, *Lactobacillus sp.* had frequency of two (2) and 13.3%, and *Sporolactobacillus* had frequency of one (1) and 6.6% while only one of the anti-malarial drugs had no contamination, that is 6.6% was not contaminated. *Penicillium sp*. was found to be the most predominant fungal isolates, with the highest frequency of occurrence six (6) and 46.2% followed by *Candida alblicans* with the frequency of three (3) and 30.7% and *Aspergillus niger* had the least frequency of three (3) and 23.1%.

The contaminating bacteria isolated suggested the route of contaminations possibly personnel, water and the environment. These bacterial species have previously been associated with drug contaminations [1, 13, 23]. There was high microbial count in *AST* with a mean microbial count of $8.03x10^4$cfu/ml on Nutrient agar, followed by *UID, MAX, HER, SEN, LUM* and *XIN*. *DIA* had the least bacterial count of $2.00x10^1$cfu/ml. Out of the ten (10) samples evaluated; nine (9) samples 90% were contaminated with both bacteria and fungi while one (1) sample 10% which is *ORN* suspension was free of bacterial contamination, but had a single yeast growth.

Result also showed that five samples had a microbial load of less than 10^3cfu/ml implying that the manufacturers complied with the official requirement for the microbiological quality of syrups and suspensions according to the FIP Working Committee (1975), the result of this research is in accordance with the work of Adeshina *et al.*,[1].

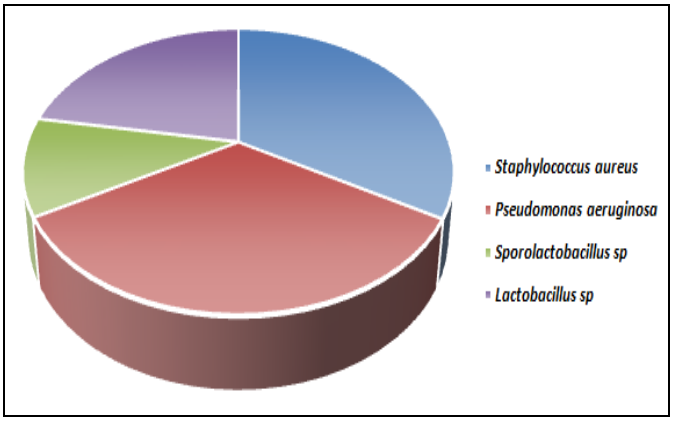

FIG. 1: PERCENTAGE DISTRIBUTION OF BACTERIA ISOLATES OBTAINED FROM ANTI-MALARIAL SYRUPS / SUSPENSIONS

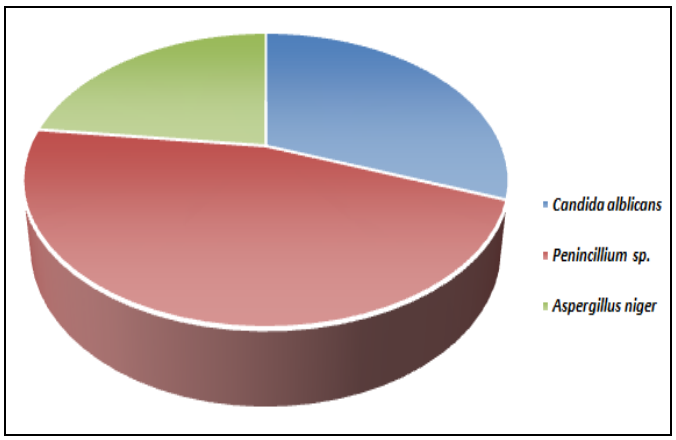

FIG. 2: PERCENTAGE DISTRIBUTION OF FUNGI (YEAST AND MOLDS) ISOLATES OBTAINED FROM ANTI-MALARIAL SYRUPS/SUSPENSION.

The results of this work showed more syrup samples were contaminated than the suspensions and powder which is in line with the results of Brittain [6], Takon and Antai [23] and Sharp [21]. Most of the suspensions were also contaminated. Contamination of suspension had been reported by Olaniyi [17] and Takon and Antai [23]. Suspensions are expected to be free from contamination because of absence of moisture. But in this study, suspensions were highly contaminated and this unusual occurrence could have been enhanced by improper sterilization procedure during manufacture, packaging and poor storage facilities in the pharmacy or patent medicine store as reported by Bloomfield *et al.*, [26].

CONCLUSION: This study reveals that about 40% of the sampled antimalarial syrups were highly contaminated above the official permissible limits of microbial load of non-sterile pharmaceutical preparations (syrups and suspensions) and as such are not in conformity with the regulated requirements. It is generally accepted that a high assurance of overall product quality can only come from a detailed specification, control and monitoring of all the stages that contribute to the manufacturer of pharmaceutical products.

Thus a more realistic estimate of the microbial quality of some brands of antimalarial preparations would be achieved from a knowledge of specific parameters (such as bio-burdens of starting materials, compaction data etc.), than contaminant content of the finished products. Although sterility is not a requirement in official compendia for non-sterile pharmaceutical products, bio-burdens need to be within acceptable limits. The major sources of contamination of pharmaceutical products have always been water, the production environment, the personnel and packaging material. Therefore proper attention should be given to the prior treatment of these factors to ensure reduction in the level of microbial contaminants. Strict precaution should be followed by manufacturers of this product during production and the incorporation of sufficient concentration of appropriate preservatives can also be employed to reduce the microbial load of these preparations, though most preservations turns to contaminate the products.

Since many microorganisms may be hazardous to patients or cause spoilage of formulations under suitable conditions, it is necessary to perform a risk assessment of contamination of each product, and strategies should be developed to reduce the overall risk to acceptably low levels.

RECOMMENDATIONS: In order to reduce or avoid contamination of pharmaceutical products, it is therefore pertinent to make the following recommendation. All raw materials used particularly water and other materials used as suspending agents and ingredients should be of high microbiological safety standard. All apparatus or processing equipment used should be subject to planned preventive maintenance and should be properly cleaned and sterilized after use to prevent cross-contamination between batches. During packaging of the end-product, proper care should be taken to prevent contamination and reduction of its shelf-life. Pharmaceutical products should be stored in a cool and dry place, with syrups stored at 4^0C and suspensions 25^0C to avoid spoilage and degradation.

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