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Microbiological Quality of some Expired Drugs in Sokoto Metropolis, Nigeria

[1]M.B. Yerima, [2]S.M. Jodi, [1]A.A. Farouq, [1]K. Oyinbo, [3]A.U. Junaidu, [4]M.N. Al-Mustapha, [5]J.M. Ahmed, and [6] A.L. Shinkafi

*[1]Department of Microbiology, Usmanu Danfodiyo University, Sokoto State, Nigeria
[2]Department of Pharmaceutical Microbiology, Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria
[3]Department of Parasitology and Public Health, Faculty of Veterinary Medicine
[4]Department. of Parasitology and Animal Health, Faculty of Veterinary Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria
[5]Department of Biological Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria
[6]Department of Science Laboratory Sciences, Abdu Gusau Polytechnic, Talata Mafara, Zamfara State, Nigeria.

ABSTRACT: Twenty eight samples of expired drugs were obtained from different medical stores including Sokoto State Medical Store in Sokoto Metropolis. The drugs included paracetamol, vitamin C (Ascorbic acid), Phenergan (promethazine), Chloroquine (A 4–aminoquinoline acid), flagyl (metronidazole), folic acid (Pteroylglutamic acid), tablets and vitamin A injection (Retinol). The tablets were ground into powder using sterile pestle and mortar. Powder of each tablet (0.2g) and 0.2ml vitamin A injection was dispensed into 9ml sterile nutrient broth. After incubation for 24 hours, a loopful from the growth was subcultured into nutrient agar and malt extract agar plates. The nutrient agar plates were incubated at 37^{o}C for 24 hours and the Malt Extract plates were incubated at 26^{o}C for 3-7 days After incubation, the organisms were identified using a combination of microscopy and biochemical tests. For each drug, the procedure was repeated four times. The frequency of occurrence of each organism was determined and found to be: *Aspergillus niger* 28(60%); *Aspergillus flavus* 28(32%); *Penicillium* spp. 28(14%); *Scopulariopsis* spp. 28(7%); *Neurospora* spp. 28(7%); *Mucor* sp. 28(4%); *Enterococcus avium* 28(35%); *Staphylococcus aureus* 28(29%); *Enteococcus gallinarum* 28(25%); *Staphylococcus epidermidi,* 28(17%); *Enterococcus durans* 28(14%) and *Staphylococcus saccharolyticus* 28(14%). The results demonstrated the involvement of pathogenic microorganisms; therefore, expired drugs should not be consumed no matter how neat they may appear.

Key words: Expired drugs, Microbiological quality, Sokoto

INTRODUCTION

The microbiological quality of pharmaceutical products is influenced by the environment in which they are manufactured and by the material used in their formulations. With the exception of liquid preparations, which are sterilized in their containers, the microflora of the final product may represent the contaminants from the raw materials, from the person operating the process or from the containers into which it was packed. Some of the contaminants may be pathogenic while others may grow even in the presence of preservatives and spoil the product (Hugo and Russell, 1984).

Most materials used in packaging pharmaceutical products support some form of microbial growth, depending on the nutritive properties and moisture content of the products (Ogbonna and Akueshi, 1984). Hence, dry powder or tablets are capable of undergoing some form of microbial spoilage or deterioration (Akerele and Ukoh, 2002).

Most pharmaceutical products are susceptible to microbial contamination, which may cause their spoilage by changing their chemical, physical or aesthetic nature thereby rendering them unfit for use. Contamination tends to arise during manufacture rather than during use and can be prevented by controlling personnel, environment, raw materials and formulations (Booth, 2002).

The risk of contamination and spoilage of a pharmaceutical product is generally significant, as such; each component of the drug must be pasteurized. But the sterilization of the components in aseptic processing is difficult because it contains non-sterile, non-sterilizable moveable objects, that is, human operators. Studies have shown that airborne contamination is the major route of microbial contamination of aseptic products during the filling operation (Akerele and Ukoh, 2002).

The aim of this study was to identify the types of microorganisms associated with expired drugs.

MATERIALS AND METHODS

Sample Collection

Drug samples used for this study were obtained from different medical stores located in Sokoto metropolis. These included Chloroquine, Folic acid, Vitamin C, Phenegan, Flagyl, Paracetamol and vitamin A injection. These drugs were considered because they are frequently being purchased and, therefore, most commonly used.

Sample Processing

Expired drugs used in this work were ground using sterile pestle and mortar. The powder from each table (0.2g) was weighed and 0.2ml vitamin A injection was dispersed in 9ml of sterile nutrient broth. This was followed by incubation at 37^0C for 24hours. A loopful of growth that developed in nutrient broth was streaked onto nutrient agar plates and malt extract agar plates. The malt extract agar plates were incubated at an average ambient temperature of 26^0C for 3 – 7 days. The nutrient agar plates were incubated at 37^0C for 24 hours before they were observed for growth. Similar procedure was repeated for second, third and fourth drug samples collected (Barrow and Feltham, 1993).

Cultural and Morphological Characterization of bacterial isolates

Gram staining technique was performed on growth from nutrient agar plates using standard procedures (Barrow and Feltham, 1993). The plates were further subcultured onto nutrient agar plates and incubated at 37^0C for 24 hours for the purpose of biochemical tests, which were performed for the purpose of identifying the bacterial isolates down to species level. The fungi were identified using a combination of colonial characteristics and microscopy (Robert and Ellen, 1988).

Biochemical Characterization of the isolates

The biochemical tests performed were coagulase, growth on mannitol salt agar, lactose fermentation, growth at 45^0C and haemolysis. The tests were performed in accordance with standard procedures described in Barrow and Feltham (1993).

Fungal Isolation and Identification

About 0.2g of each drug was used to make a suspension. A drop of these suspensions was mixed with the molten Malt Extract Agar and dispensed into plates. These plates were allowed on the bench for two weeks. The growth of individual fungus obtained was identified using standard procedure described in Robert and Ellen (1988).

RESULTS

The results shown in the Tables 1 and 2 indicate the occurrence of potentially pathogenic and saprophytic organisms in the drugs. More worrisome is the high frequency of occurrence observed particularly, for *Aspergillus niger*, *Aspergillus flavus* and *Staphylococcus aureus* that are potentially pathogenic.

Table 1: Identity of Bacteria Associated with the Drug samples

Bacterial isolates	Properties	Drugs Used	Remarks
Staphylococcus aureus	Coagulase, Lactose and Mannitol positive	Flagyl, Chloroquine, Folic acid and Vitamin A	Suspected pathogen
Staphylococcus epidermidis	Lactose positive	Flagyl, Chloroquine, Paracetamol	Saphrophytic Bacteria and may be pathogenic
Staphylococcus saccharolyticus		Paracetamol, Vitamin A	Saphrophytic bacteria
Enterococcus avium	α – haemolytic	Flagyl, Phenergan, Chloroquine, Vitamin C	Suspected pathogen
Enterococcus gallinarum	Grows at 45°C; β – haemolytic	Phenergan, Folic Acid, Vitamin C	Suspected pathogen
Enterococcus durans	Grows at 45°C	Folic Acid	

Table 2: Colonial Characteristics and Identity of Fungal Isolates from Selected Drugs

Drug Samples	Colonial Characteristics	Identity
	Light green powdery	*Aspergillus flavus*
	Light green powdery	*Aspergillus flavus*
Flagyl	Black powdery	*Aspergillus niger*
	Black powdery	*Aspergillus niger*
	Yellowish powdery	*Scopulariopsis* sp.
	Black powdery	*Aspergillus niger*
	Light green powdery	*Aspergillus flavus*
Phenegan	Grey powdery	*Mucor sp.*
	Orange powdery	*Neurospora* sp.
	Dark green powdery	*Penicillum* sp.
	Yellowish powdery	*Scopulariopsis* sp.
	Black powdery	*Aspergillus niger*
Chloroquine	Light green powdery	*Aspergillus flavus*
	Black powdery	*Aspergillus niger*
	Dark green powdery	*Penicillum* sp.
	Black powdery	*Aspergillus niger*
Folic acid	Dark green powdery	*Penicillum sp.*
	Black powdery	*Aspergillus niger*
	Black powdery	*Aspergillus niger*
	Black powdery	*Aspergillus niger*
	Black powdery	*Aspergillus niger*
Paracetamol	Black powdery	*Aspergillus niger*
	Light green powdery	*Aspergillus flavus*
	Light green powdery	*Aspergillus flavus*
	Light green powdery	*Aspergillus flavus*
Vitamin A	Black powdery	*Aspergillus niger*
	Black powdery	*Aspergillus niger*

DISCUSSION

The results from this study emphasize the importance of good manufacturing practice (GMP) related to the monitoring and setting of microbial limits for raw materials and the manufacturing environment, which will ensure that final products contain minimal number of contaminants, where preservatives if added could cope with. GMP is considered as that part of quality assurance, which ensures that products are consistently produced and controlled to the quality standard appropriate to their intended use and as required by the product specification. These guidelines are usually issued to eliminate or reduce the risk inherent in any product that cannot be prevented completely through testing of the final product (Orji, 2003).

The results obtained show a gross contamination of some of the samples with potential pathogens such as *A. flavus*, *A. niger* and also with opportunistic pathogens such as *S. aureus* and *S. epidermidis*. The highest frequency of occurrence was observed for *A. niger* and *S. aureus* (Figure 1 and 2). This can be serious as the two organisms are highly pathogenic to man. The presence of these contaminants could be attributed to the raw materials, operators (personnel) and non-adherence to GMP. The findings of this research agree with that of Obuekwe and Ogbimi (1998) who also reported the incidence of similar contaminants is some drugs. People are generally considered the major source of contamination of drugs. This is because no environmental programme is complete unless there are provisions for personnel monitoring. Operators shed both viable and non-viable particles at the rate of 10 cells/day. There are approximately 2,300 microorganisms per cm^2 of the skin. The skin flakes that make up most of the dust act as rafts for these organisms. Since these rafts are very light, they are carried by the air current surrounding the operator, thus, bringing about contamination of the drugs. The occurrence of bacteria and moulds in the drugs analyzed poses a great danger to the consumers since these organisms are incriminated in human disease causation.

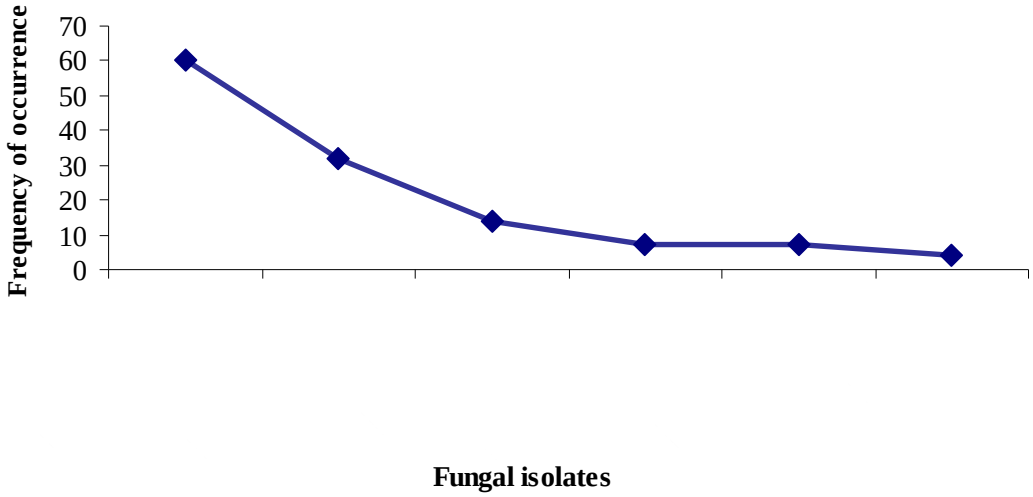

Fig 1: Frequency of Occurrence of Fungal isolates from the Drug samples

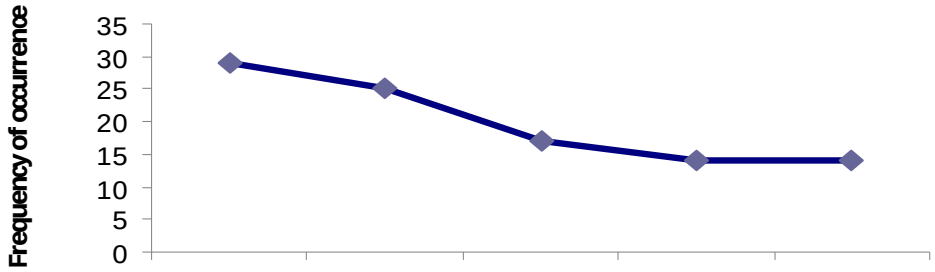

Fig. 2: Frequency of occurrence of bacterial isolates from the drug samples

CONCLUSION

In conclusion, the expired drugs in Sokoto have poor microbial quality as they are associated with bacteria and fungi which are potentially pathogenic.

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