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Research Article

Isolation and identification of bacteria associated with retailed and unsealed drugs sold in Aba, Nigeria

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Abstract

Bacteria associated with several retailed and unsealed drugs purchased from the open market, maternities and patent medicine stores in Aba, Nigeria were investigated between January and March, 2014. The examined drug samples were three different branded syrups (Chloroquine, Paracetamol and Benylin) and three different branded tablets (Metronidozole, Chloroquine and Paracetamol). The bacterial load was determined using standard methods. Results indicated that all the retailed and unsealed collected samples were contaminated. The total viable count of bacteria was in the range of 1.1×10^3 to 2.3×10^3 cfu/ml for the syrups and 1.4×10^3 to 2.9×10^3 cfu/g for the tablets which is beyond NAFDAC limits. The bacteria isolated and their percentage occurrences were *Lactobacillus* spp (66.7%), *Pseudomonas* spp (50.0%), *Staphylococcus* spp (33.3%), *Escherichia coli* (33.3%), and *Klebsiella* spp (16.7%). The presence of the isolates could possibly be through the production process, equipment used, sources of water and frequent opening of the containers containing the products during sales. This is an indication of improper handling and inadequate sanitary measures required for such important products. It is recommended that monitoring agencies should ensure that these drugs do not present health hazards to the public.

Keywords: Retailed unsealed drug, sealed drugs, bacteria isolates, open market, patent medicine stores, maternities

INTRODUCTION

Drugs are chemicals used in the treatment, cure, prevention or diagnosis of diseases or used otherwise, to enhance physical or mental well-being. Retailed drugs are those drugs in larger containers from where the vendors distribute to their customers. These drugs are administered orally which is the most common route of drug administration (Goodman and Gilman, 1996). It has been observed that pharmaceutical products of various forms are susceptible to contaminations by variety of microorganisms. Microorganisms are ubiquitous, and are found in food, beverages and even in pharmaceuticals (Ogbulie *et al*., 1998). Pharmaceutical products become ineffective when contaminated with pathogenic microorganisms (Eka *et al*., 1987). The administration of contaminated products to people poses real danger, even at low levels of contamination (Mendie *et al*., 1993; Betram, 2004; Itah *et al*., 2004). Administration of sub-standard pharmaceutical products also contributes to ineffectiveness of pharmaceutical products. Okeke and Lamikanra (2001) reported the sale of sub-standard ampicillin drugs in a small town in Nigeria. This would obviously have biological and clinical consequences. Allison and Gilbert (2004) and Busari *et al.* (2010) reported that microorganisms represent serious threat on those pharmaceutical products. Smith and Webb (2010) stressed that the metabolic versatility of the microorganisms is such that almost any formulation ingredient from simple sugars to complex metabolic molecules may undergo chemical modification by a suitable organism, thus leading to spoilage of the product. According to Hugo and Russell (1992) and Hodges *et al.* (2000), this spoilage may lead to deterioration of these products which may result in loss of potency of the drug or initiate infection on the user.

According to National Agency for Food and Drug Administration and Control (NAFDAC, 2000), the major health

concern is when such microbial contamination exceeds acceptable limits (1.0×10^3 cfu/ml). It has been observed that majority of pharmaceutical products contain more than the manageable limit. Hugo and Russell (1992) stressed that environment especially the air where the drugs are manufactured and retailed influence the microbiological quality of the drugs and materials used in their formulation. Nester *et al.* (2002) reported that microbial infections are not only the result of the physical presence of microorganisms, but also their metabolites/toxins that become harmful even when they are found in minute quantities. Some of these toxin-related illnesses include acute gastroenteritis, abdominal discomfort and diarrhea. In addition, contaminated drugs constitute wastage and may have serious economic implication not only to the consumers but also to the manufacturers. This study was designed to assess the quality of drugs sold in Aba, Abia State, Nigeria and determine the bacteria associated with the retailed drugs.

MATERIALS AND METHODS

Study Area

The study was conducted in Aba, a commercial city of Abia State, Southeastern geopolitical zone of Nigeria. Aba lies within the forest zone, along the west bank of the Aba river, at the intersection of roads from Port Harcourt in River State, Owerri in Imo State, Umuahia (the State Capital of Abia State), Ikot Ekpene and Ikot Abasi in Akwa- Ibom State. The geographical co-ordinates for Aba are 5^o 07' N latitude and 7^o 22' E longitude and 205m (673ft) above sea level. Aba is a major settlement and commercial center in a region that is surrounded by small towns and villages.

Sample collection

Three brands of retailed syrups (Chloroquine, Paracetamol and Benylin) and three brands of unsealed tablets (Metronidozole, Chloroquine and Paracetamol) approved by NAFDAC, were randomly purchased from the open market, maternities and patent medicine stores in Aba. The selected drugs were common drugs used for treating malaria and cold. The sealed tablets and syrups served as control. The unsealed drugs were aseptically collected using sterilized bottles.

Serial Dilution

1ml of each drug sample was serially diluted into 9ml of diluents. After serial dilution, 0.1ml of the dilution was plated out using spread plate technique. The dilutions were plated out in duplicates on nutrient agar (NA), MacConkey, Salmonella Shigella agar, Manitol salt Agar (MSA), Eosine methylene blue agar. The plates were incubated at 37^0C for 24 to 48 hours. All isolated organisms were subjected to microscopic and biochemical analysis for characterization (Cheesbrough, 2004).

RESULTS

The result showed that all the sampled drugs (syrup and tablets) were contaminated by microorganisms (Table 1). The range of contamination is between 1.1×10^3 and 2.3×10^3 cfu/ml for syrups and 1.4×10^3 and 2.9×10^3 cfu/g for tablets. Metronidozole tablets were shown to have the highest bacterial count of 2.9×10^3 cfu/g while Benylin had the least contamination of 1.1×10^3 cfu/ml.

Table 1. Total viable count of bacteria isolated from the retailed drugs

Syrups	Total viable count (cfu/ml)	Tablets	Total viable count (cfu/g)
Chloroquine) (unsealed)	2.3×10^3	Chloroquine (unsealed)	1.4×10^3
Benylin (unsealed)	1.1×10^3	Paracetamol (unsealed)	2.3×10^3
Paracetamol (unsealed)	1.4×10^3	Metronidozole (unsealed)	2.9×10^3
Chloroquine (sealed)	0.1×10^2	Chloroquine (sealed)	0.3×10^2
Benylin (sealed)	0.5×10^2	Paracetamol (sealed)	1.0×10^2
Paracetamol (sealed)	0.8×10^2	Metronidozole(sealed)	0.2×10^2

Table 2 shows the morphological and biochemical characteristics of the isolated bacteria. Five bacteria genera were identified*: Pseudomonas* spp*, Staphylococcus* spp, *Lactobacillus* spp, *Escherichia coli and Klebsiella* spp.

Table 2. The morphological and biochemical characteristics for the identification of bacteria isolates from the sampled drugs

Morphol charact	Gram react	Shape	Catal	Oxid	Coag	Mot	Ind	Citr	Gluc	Lact	Probable organism
Circle, green colony on NA	-	Rods	+	+	-	+	-	-	A	A	*Pseudomonas* spp
Yellow to pink colony on MSA	+	Cocci in cluster	+		+	-		-	A	A	*Stapylococcus* spp
Circle, milky colony on NA	+	Rods	+		-	+	-	-	AG	AG	*Lactobacillus* spp
Green metallic colony on EMB	-	Rods	+	-	-	+	+	-	A	AG	*Escherichia coli*
Circle, milky colony on NA	-	Rods	+		-	-	-	+	AG	-	*Klebsiella* spp

Key: + = positive; - = negative; Morphol charact = morphological characteristics; Gram react= Gram reaction; Catal= catalase; Oxid = oxidase; Coag = coagulase; Mot = motility; Ind= indole Citr= citrate; Gluc= glucose; Lact= lactose; A= acid production; AG= acid and gas production NA= Nutrient Agar, MSA= Manitol salt Agar, EMB= Eosine Methylene Blue Agar.

Table 3 shows prevalence of bacteria species isolated from the retailed drugs. *Lactobacteria* spp has percentage occurrence of 66.7%, followed by *Pseudomonas* spp with 50.0%, while the least was *Klebsiella* spp with 16.7%.

Table 3. Prevalence of Bacteria species in the sampled Drugs

Drug / Bacteria Isolates	Chloroquine syrup	Benylin syrup	Paracet syrup	Chloroquine tablets	Paracet tablets	Metr	Percentage (%)
Pseudomonas spp	+	-	+	-	-	+	50..0
Staphylococcus spp	-	+	+	-	-	-	33.3
Lactobacillus spp	+	+	-	-	+	+	66.7
Escherichia coli	-	+	-	+	-	-	33.3
Klebsiella spp	-	+	-	-	-	-	16.7

Key: + = positive; Paracet= Paracetamol; **-** = negative; Metr =Metronidozole

DISCUSSION

Drugs are supposed to maintain their original status during storage and use. In this research, it was revealed that all the unsealed and retailed tablets and syrups tested were microbiologically contaminated beyond acceptable limits (NAFDAC, 2000). However, the sealed syrups and tablets that served as control yielded results within the limits acceptable by NAFDAC safe for human consumption. The unsealed drugs are usually kept in bigger containers from where the drug vendors retail these drugs. Takon (2006) reported that most drugs are contaminated by microorganisms during handling and storage. The bacteria isolated include *Staphylococcus* spp, *Pseudomonas* spp*, Lactobacillus* spp*, Escherichia coli* and *Klebsiella* spp*.* Similar results were obtained by Daniyan *et al.* (2011) and Emejuru *et al.* (2013). The United States Pharmacopoeia recommends *Salmonella, Pseudomonas aeruginosa, Escherichia coli* and *Staphylococcus aureus* as indicators of microbial contamination of syrups (Center for Drug Evaluation and Research, 2000). The presence of these organisms in the drugs especially *Staphylococcus* spp is a cause for concern as it is a well known pathogen that exhibits multiple drug resistance. According to Prescott *et al.* (2004), the presence of *Staphylococcus aureus* is probably a result of the human body contact (hand, nose, skin and clothing). Denyer (1990) added that the presence of *Staphylococcus aureus* in drug is of great importance as this organism secretes toxin which contributes to gastrointestinal distress. The presence of *Escherichia coli* is a good indicator of fecal contamination resulting from water supply. Cheesbrough (2004) stressed that most enteric bacteria such as *Escherichia coli* are ubiquitous and that these organisms can be shed from the body. Itah and Ben (2004) also shared the same view. Mohammad *et al.* (2012) stated that the presence of certain microorganisms in non-sterile pharmaceutical products adversely affects the therapeutic activity of the products and even become detrimental to the health of the patients. The presence of an indicator organism like *E .coli* in a drug shows that the particular drug is unsafe for human consumption. Contamination of products may affect their stability, causing product degradation prior to expiration date, and this can lead to infections especially in children with poorly developed immune system. Therefore, there is a need for proper monitoring and quality control among producers, health workers and drug vendors, by educating them on personal and environmental hygiene to minimize microbial cross-contamination.

CONCLUSION

The microbiological quality of pharmaceutical products may represent contamination from raw materials, manufacturing equipment, environment, personnel and containers. There is a need for proper monitoring and quality control among

producers, health workers, and drug vendors to ensure that correct guidelines for pharmaceutical product are maintained. There is a need also to address storage problems in order to minimize the risk of drug borne infections. The government agencies such as Nigerian Drug Law Enforcement Agency (NDLEA) and National Agency for Food and Drug Administration and Control (NAFDAC) should subject the pharmaceutical industries and their products to routine monitoring and supervision to ensure strict compliance to good manufacturing practice.

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