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Full Length Research Paper

Bacteriological Assessment of Spoilt Pharmaceutical Products Sold in Yola Metropolis Adamawa State, Nigeria

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Abstract

Forty samples of spoilt pharmaceutical products (oral and ophthalmic) were collected from three different patent medicine stores and screened for potential bacteria contaminants using standard laboratory techniques. Of the 40 samples screened, 36 (90%) were seen to be contaminated by 5 different types of bacteria namely *Bacillus* species, *Staphylococcus aureus*, *Corynebacterium* species, *Micrococcus* species and *Pseudomonas aeruginosa* while 4 (10%) were free of contamination. The range of bacteria count for the oral and ophthalmic products ranges from 1.6 x 10^2 CFU/ml– 9.9 x 10^2 CFU/ml and 2.7 x 10^2 CFU/ml– 9.7 x 10^2 CFU/ml respectively. The presence of these pathogenic organisms may contribute to the spoilage of these products and also may be source of infection to the consumers. Therefore, good manufacturing practices, packaging, storage and distribution should be enforced.

Keywords: Isolation, Bacteria, Pharmaceutical Products, Spoilt, Oral and Ophthalmic.

INTRODUCTION

Drugs are chemical compounds that may be used on or administered to human or animals as an aid in diagnosis, prevention of diseases or other abnormal conditions for relieve of pain or suffering or to control any physiological or pathologic condition (Akerele and Godwin, 2002). Drugs are any chemical substances capable of affecting the body. They are used or intended to modify or explore physiological system or pathological state for the benefits of the recipient (Gervais *et al.,* 2008).

Pharmaceuticals of various forms and dosage are susceptible to contamination by a variety of microorganisms during manufacturing and use (Adeshina *et al*., 2009). Contaminated pharmaceuticals are considered microbiologically unsafe, if low level of pathogenic or higher level of opportunistic or toxic microbial metabolites persist even after death or removal of micro-organisms (Willey *et al*., 2008). The used contaminated products, even where the level of contamination is low may present potential health hazards to patients (Asaeda *et al*., 2012). In addition, such spoilt products constitute wastage and may have serious economic implication for the manufacturer. Pharmaceutical products with huge moisture content may be contaminated with microorganisms (Villà *et al*., 2009). The contaminating microorganisms may spoil the product due to loss of its therapeutic properties. If they are pathogenic, serious infections can arise (Denyer *et al.,* 2004). Modern research identified different types of microorganisms from the raw materials used during pharmaceutical productions. These organisms include *Aspergillus* species and *Penicillium* species (Obuekwe and Eichie, 2006). A study carried out by Mugoyela and Mwambete 2010, involved structured selection of representative tablets, syrups and capsules from the hospital's outpatient unit. They found the majority of microbial contaminants in non-sterile pharmaceuticals such as *Aspergillus* species*, Bacillus* species*, and Corynebacterium* species. Contamination of

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pharmaceuticals with microorganisms can also changes the physical characteristics of the product, including breaking of emulsions, thinning of creams, fermentation of syrups, turbidity or deposits, and changes in odor and color (Gad *et al*., 2011). The deteriorating effect on the products varies, ranging from introduction of toxic metabolites and cell fractions to chemical and physical modifications (Obuekwe *et al*., 2000). There have been reports of drug-borne human infections worldwide (Mwambete *et al*., 2009). Villà *et al*. (2009) reported that the contamination of thyroid tablets by *Salmonella muenchen* and *Pseudomonas aeruginosa*, the incidence of micro flora in non-sterile preparations generally is influenced by the nature of the ingredients (whether natural or synthetic), the quality of the vehicle, care and attitude of personnel handling the compounds (Parker, 2000). Gad *et al*. (2011) recommended stringent limits for objectionable microorganisms in drugs intended for use by immune compromised patients as smaller numbers of opportunistic pathogens become infectious when resistance mechanisms are impaired, either by severe underlying disease, or by use of immunosuppressive drugs (Sautour *et al*., 2008). Microbial limits cannot be formulated to cover every possibility of contamination that may occur. In assessing the results of microbiological testing, the number and types of organisms present should be considered in the context of the proposed use of the product. Thus, in manufacturing, packaging, storage, and distribution of pharmaceuticals, suitable measures must be taken to ensure product quality. The aim of this research work was to isolate and characterize potential spoilage bacteria of pharmaceutical products.

MATERIALS AND METHODS

Media used include peptone broth, nutrient agar, chocolate agar and blood agar, the preparation of all media were according to the methods described by Chessbrough, (2000)

Collection and Processing of Samples

Unexpired Pharmaceutical products were collected from different locations in Pharmaceutical stores at Sabogari, Jimeta, and Sangere in Yola, Adamawa State. Products include syrups, tablets and eye drops produced by different manufacturers. All the samples were properly sealed, packaged and transported to the laboratory for microbiological analysis**.**

Bacteriological Analysis of Samples

The bacteriological analysis of samples was carried out by 10 fold serial dilutions whereby 1ml and/or 1g of samples were inoculated in test tubes containing 9mls of sterile peptone broth. The preparations were incubated at 37^{o}C for 24h (Beishir, 1987). Sub-culturing and isolation was done by the use of pour plate method. One Millilitre (1ml) of enriched samples after incubation was dispensed into sterile petri dishes and 20ml of freshly prepared bacteriological media, plated and mixed gently. After solidifying, the plates were incubated at 37^{0}c for 24 hours. Enumeration of microbial isolates and colony counting were done using Gallenkamp digital colony counter and total population expressed as colony forming units per ml (CFU/ml).

Microscopy and Morphological Characterisation

Pure cultures of bacteria were characterized based on colony, microscopic and biochemical tests. The identity of the isolates was confirmed by comparing their microscopic and morphological characteristic with known taxa (Chessbrough, 2000).

Gram Staining

The pure isolates were stained according to Gram staining technique (Chessbrough, 2000). The stained cells were examined under the microscope with oil immersion objective lens (x100). Gram-positive microorganisms were characterized by a deep purple or violet colour, while the Gram-negative organisms retained reddish pink colour. The shape of the cells was also noted (Chessbrough, 2000).

RESULTS

Total Colony Counts per Product

The result in Table 1 shows the total colony count by product. The highest bacteria count occur in ampliclox A with a total bacteria count of 9.9×10^{2}CFU/ml followed closely by gentamycin A (9.7×10^{2} CFU/ml) and Gentamycin capsule C (9.2×10^{2} CFU/ml) while cotrim had the lowest bacteria count of 1.6×10^{2}. Gelucid, ketras, ibuprofen and flagyl had no bacteria growth.

Range of colony count of bacteria by route of administration

The range of colony count of bacteria isolated by route of administration is shown in Table 2. Oral products had $1.6 \times 10^{2} – 9.9 \times 10^{2}$ CFU/ml while ophthalmic products had $2.7 \times 10^{2} – 9.7 \times 10^{2}$ CFU/ml Distribution of bacteria isolated**.** Table 3 shows the distribution of bacterial isolates from the screened pharmaceutical products. *Staphylococcus aureus*, *Bacillus* species, *Micrococcus*

Table 1. Total colony count by product

Product	Microbial count (CFU/ml)
Metronidazole tablet (A)	4.1×10^2
Metronidazole tablet (B)	3.8×10^2
Paracetamol tablet (A)	2.6×10^2
Paracetamol tablet (B)	4.1×10^2
Paracetamol tablet (C)	1.8×10^2
Paracetamol tablet (D)	5.6×10^2
Paracetamol tablet (E)	2.9×10^2
Chlopheniramine tablet(A)	4.0×10^2
Chlopheniramine tablet (B)	3.6×10^2
Chlopheniramine tablet (C)	2.3×10^2
Folic acid (A)	3.2×10^2
Folic acid (B)	8.5×10^2
Multivitamin syrup(A)	6.4×10^2
Vitamin A tablet (A)	4.7×10^2
Vitamin B tablet (B)	3.2×10^2
Cepain tablet	2.9×10^2
Cotrimoxazole tablet (A)	1.6×10^2
B.Complex tablet (A)	8.7×10^2
B.Complex tablet (B)	4.4×10^2
Gentamycin capsul (A)	9.7×10^2
Gentamycin capsul (B)	5.5×10^2
Cirofloxacin capsul	5.5×10^2
Fluconazole tablet	4.1×10^2
Gentamycin capsule (C)	9.2×10^2
Chloramphenicol capsul (A)	1.7×10^2
Chloramphenicol capsul (B)	3.8×10^2
Benzyline eye drop	2.9×10^2
Ampiclox capsul (A)	9.9×10^2
Ampiclox capsul (B)	6.4×10^2
Ampiclox capsul (C)	8.8×10^2
Ampiclox capsul (D)	5.8×10^2
Cotrimoxazole tablet (B)	3.2×10^2
Lincomycin capsul	4.9×10^2
Analgin tablet (A)	5.5×10^2
Analgin tablet (B)	4.4×10^2
Multivitamin syrup (B)	7.1×10^2
Flagyl tablet	0
Ketoconazole tablet	0
Magnesium trisilicate	0
Ibuprofen tablet	0

Alphabets in parenthesis represents manufacturer

Table 2. Range of colony count of bacteria by route of administration

Route of Administration	Microbial Count Range (CFU/ml)	Specification BP (CFU/ml) British Pharmacopoeia, (2008)
Oral	1.6×10^2-9.9×10^2	1.0×10^3
Opthalmic	2.7×10^2-9.7×10^2	1.0×10^3

BP = British Pharmacopoeia

Table 3. Distribution of bacteria isolated from pharmaceutical products

Products (g/ml)	Bacteria isolate	Total No. Of isolates (per gram)
Metronidazole tablet (A)	*Pseudomonas aeruginosa, Staphylococcus aureus*	2
Metronidazole tablet (B)	*Bacillus species, Micrococcus species, Staphylococcus aureus*	3
Paracetamol tablet (A)	*Corynebacterium species, Bacillus species*	2
Paracetamol tablet (B)	*Staphylococcus aureus*	1
Paracetamol tablet (C)	*Micrococcus species, Bacillus species, Staphylococcus aureus*	3
Paracetamol tablet (D)	*Micrococcus species, Bacillus species, Staphylococcus aureus*	3
Paracetamol tablet (E)	*Micrococcus species, Bacillus species, Staphylococcus aureus*	3
Chlopheniramine tablet(A)	*Pseudomonas aeruginosa, Staphylococcus aureus*	2
Chlopheniramine tablet(B)	*Pseudomonas aeruginosa*	1
Chlopheniramine tablet(C)	*Bacillus species, Micrococcus species, Staphylococcus aureus*	3
Folic acid (A)	*Bacillus species, Corynebacterium species*	2
Folic acid (B)	*Bacillus species, Staphylococcus aureus*	2
Multivitamin syrup(A)	*Micrococcus species, Bacillus species*	2
Vitamin A tablet (A)	*Bacillus species, Pseudomonas aeruginosa*	2
Vitamin A tablet (B)	*Bacillus species, Staphylococcus aureus*	2
Cepain tablet	*Staphylococcus aureus*	1
Cotrimoxazole tablet (A)	*Bacillus species, Micrococcus species*	2
Ampiclox capsule (A)	*Pseudomonas aeruginosa, Staphylococcus aureus*	2
Ampiclox capsule (B)	*Pseudomonas aeruginosa, Micrococcus species*	2
Ampiclox capsule (C)	*Bacillus species, Micrococcus species*	2
Ampiclox capsule (D)	*Bacillus species, Staphylococcus aureus*	2
B.Complex tablet (A)	*Corynebacterium species, Bacillus species*	2
B.Complex tablet (B)	*Corynebacterium species, Bacillus species*	2
Benzyline eye drop(A)	*Pseudomonas aeruginosa, Staphylococcus aureus*	2
Benzyline eye drop (B)	*Pseudomonas aeruginosa, Micrococcus species, Staphylococcus aureus*	3
Cirofloxacin capsule	*Corynebacterium species, Bacillus species*	2
Fluconazole tablet	*Staphylococcus aureus, Bacillus species*	2
Benzyline eye drop (C)	*Micrococcus species, Corynebacterium species, Staphylococcus aureus*	3
Chloramphenicol capsule (A)	*Pseudomonas aeruginosa, Micrococcus species, Staphylococcus aureus*	3
Chloramphenicol capsule (B)	*Staphylococcus aureus, Corynebacterium species*	2
Benzyline eye drop (D)	*Staphylococcus aureus, Micrococcus species*	2
Lincomycin capsule	*Bacillus species, Staphylococcus aureus*	2
Cotrimoxazole tablet (B)	*Bacillus species, Staphylococcus aureus*	2
Analgin tablet (A)	*Pseudomonas aureus, Staphylococcus aureus*	2
Analgin tablet (B)	*Staphylococcus aureus, Micrococcus species*	2
Multivitamin syrup (B)	*Micrococcus species, Corynebacterium species*	2
Flagyl tablet	*NG*	0
Ketoconazole tablet	*NG*	0
Magnesium trisilicate	NG	0
Ibuprofen tablet	NG	0

NG = No Growth, Alphabets in parenthesis represents manufacturer

species, *Corynebacterium* species, and *Pseudomonas aeruginosa* were predominant.

Prevalence of bacterial isolated

The prevalence of bacterial isolated from the pharmaceutical products is shown in Figure 1. *Staphylococcus aureus* had the highest prevalence (32%) while *Corynebacterium* species were least prevalence.

DISCUSSION

The result of this research showed that the samples

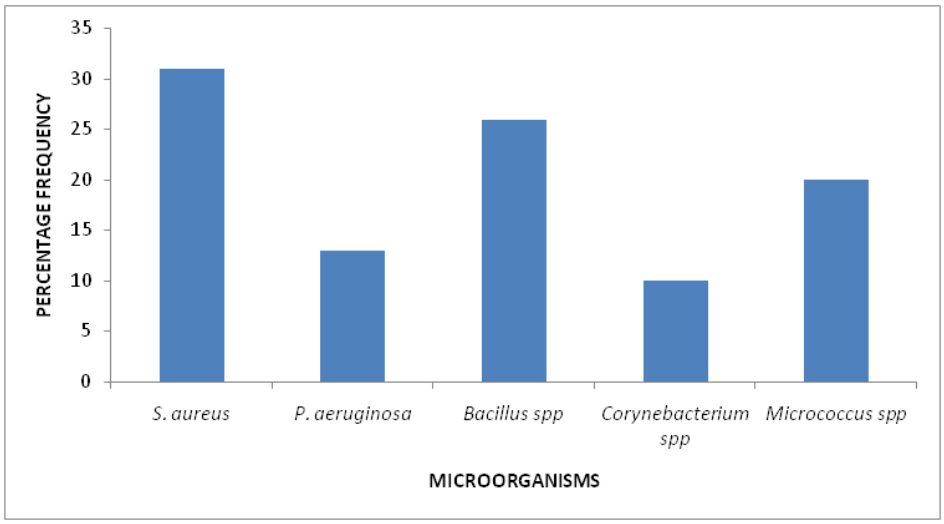

Figure 1. Frequency of bacteria occurrence.

tested had satisfactory bacteria levels compared to the British Pharmacopoeia specification of 10^3 CFU/ml. The organisms isolated were *Staphylococcus aureus (*32%), *Bacillus* species *(*27%), *Corynebacterium* species (10%), *Micrococcus* species (17%) and *Pseudomonas aeruginosa (*14%). Ampiclox syrup was most contaminated as it had 9.9 x 10^2 CFU/ml. The least contaminated sample was Cotrim Cough Syrup with a viable count of 1.6 x 10 CFU/ml. The low count recorded in Cotrim Cough Syrup may be attributed to the incorporation of trisodium citrate together with sugar content of the syrups which provide high osmotic pressure that is inhibitory to many microorganisms (Muhammed and Umoh, 2009). However the presence of *Staphylococcus aureus* reflects its nature of habitation i.e. human skin which could easily contaminate the products during processing. The organisms being a normal flora of the body can easily contaminate the products during handling and processing by personnel. Though, favorable growth environment for microorganisms is prevented by high sugar concentration, *Staphylococcus aureus* thrive well in fairly high concentration of sugar (Madigan and Martinko, 2006). The high number of *Staphylococcus aureus* in these preparations suggests that they are able to tolerate the presence of preservatives in such products as reported by (Cundell, 2009). The type of bacterial contaminants isolated suggests the root of contaminations possibly water, personnel, handling and the environment (Okunlola *et al*., 2007). These bacterial species have previously been associated with drug contaminations (Ibezim *et al*., 2002; Takon and Antai, 2006). The isolation of *Staphylococcus aureus* in these products indicates a possible health risk and the need to reduce the degree of contamination of such products by enforcing official guidelines such as good manufacturing practice (GMP) and ensuring compliance through regular monitoring of non-sterile products. The types of organisms isolated in this study suggest the main sources of contamination of these products were from the processing unit, handlings, and storage. *Bacillus* species which was isolated from the pharmaceutical products are ubiquitous organisms and considered harmless in this study, though undesirable because of their potential to spoil the products. The presence of this organism in the products can be traced back to the raw materials used in the production of pharmaceuticals since this organism is present in the water and un-sterile air in the manufacturing environment which need fumigation (Kulshrestha *et al*., 2008). The manufacturing equipment may be handicapped by a number of designed faults (Kulshrestha *et al*., 2008). The extent of microbial contamination depends on a number of factors such as availability of nutrients, presence of microorganisms; oxygen and the factors determining the outcome of medicament-borne infections include the type and degree of microbial contamination, root of administration and state of the patient's immune system. Gram positive isolates were mainly *Staphylococcus aureus, Corynebacterium* species, *Bacillus* species and *Micrococcus* species. *Pseudomonas aeruginosa* was the only gram negative rod. The presence of bacteria in the pharmaceutical products could be attributed to unhygienic practices and non-adherence to good manufacturing environment; unhygienic handling of the products and lack of microbiological in-house control may also have contributed to the microbial load in some samples. The strict compliance to this practice will consequently reduce the incidence of contamination and guarantee good quality products (Takon and Antai, 2006). In addition, some of the products without microbial contamination might be as a result of the

presence of preservatives.

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