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Microbial Contamination of Pharmaceutical Products in a Tropical Environment

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Tablets, both coated and non-coated were examined for the microbiological contamination, growth and survival. Bacteria such as *Klebsiella aerogenes*, *Bacillus cereus*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* were dominated in all the samples screened. Fungi isolated were *Penicillium chrysogenum*, *Aspergillus flavus* and some yeasts as *Candida albicans* and *Saccharomyces spp*. Total bacterial counts ranged from 2.0×10^3 to 8×10^7 colony forming units (CFU) per millilitre while mould units ranged from 0.2×10^2 to 1.4×10^2 cfu/ml. The bacterial isolates were further found to be able to grow and utilize the drugs (tablets) as sole carbon and energy sources.

Key words: Tablets, Microbial contamination, Pharmaceuticals.

Introduction

The manufacture of any pharmaceutical products must necessarily begin with raw materials and the quality of these will ultimately determine the value of any finished product. The predominant factor is probably the bioburden of the raw materials used, both active drugs and excipient. It has been shown that microbial contaminants may effect the spoilage of pharmaceutical products through chemical, physical or aesthetic changes by rendering it unfit for use (Beveridge 1975; Ringertz and Ringertz 1982). Physical changes commonly seen are breakdown of emulsions, visible surface growth on solids and the formation of slimes, pellicle or sediments in liquids, sometimes accompanied by the production of unwanted flavors, thereby rendering the product unacceptable and possibly even dangerous to the patient.

Contaminants isolated from pharmaceutical products have been ranged from true pathogens such as *Clostridium tetani*, to opportunistic pathogens such as *Pseudomonas aeruginosa* and other free- living gram-negative organisms which are capable of causing disease under special circumstances (Hills 1946; Morse *et al* 1967). With their fastidious nature, contaminants potentially pathogenic to man are probably unable to replicate in most medicines but could remain viable and ineffective for an appreciable time. Although, infrequently reported as pharmaceutical contaminants, they attract considerable attention when they are present. For example, Salmonella infections have arisen from tablets and capsules of yeast, carmine, pancreatin, thyroid extract, and powdered vegetable drugs (Kallings *et al* 1966; Kormany *et al* 1967). The low levels of pathogens encountered in the finished medicines were traced to the raw ingredients used in the manufacture.

The microbial quality at the moment of administration of pharmaceutical preparations such as tablets is largely determined by three factors viz: i., the microbial contamination of the raw materials; ii., the effects of the manufacturing process on the microorganisms and iii. the fate of the contaminating microorganisms during storage (Bos *et al* 1989). During the manufacture of tablets, the viability of microbial cells can significantly be affected by the drying process of granulates (Parker 1984) and by actual compaction (Plumpton *et al* 1986; Fassihi and Parker 1987). Despite the fact that suitable substrates such as starch and lactose are abundantly present in tablets, microbial growth has rarely been observed. The availability of water probably plays an important role. As long as tablets are stored under dry conditions, spoilage due to growth of microorganisms is unlikely to occur (Blair *et al* 1988). However, in tropical regions with hot and humid climate, growth of contaminating microorganisms cannot be excluded. The microbiology of the atmosphere is a challenging one, particularly in areas where climatic and environmental conditions (warm climate and high relative humidity coupled with poor standard of hygiene) favour the survival and propagation of many microorganisms (Babalola *et al* 1987). In some regions

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such as the tropics, the average kinetic temperature is 31°C and the average relative humidity is 75% with maxima up to 100% during the rainy season (Grimm 1986). Moreover, in these countries, pharmaceutical preparations are frequently stored under controlled conditions and may be dispensed in non-protective packaging or even without any packaging at all. Few studies about the effects of storage on microbial stability of tablets have been reported (Waterman *et al* 1973; Fassihi and Parker 1977; Blair *et al* 1987).

In the present work, the microbial quality of some drugs namely; paracetamol, aspirin and multivitamin tablets was ascertained and attempts were made to determine the growth and survival of the isolates using the drugs as sole carbon sources.

Materials and Methods

A total of eight lots were sampled (comprising three coated and five non-coated tablets). Sampling was carried out at wholesale, retail, manufacture and import levels for easy representation and identification of lot structure. From each lot, a sample was analyzed. All sub-samples were examined for total bacterial count and also for the following microorganisms: *Staphylococcus aureus*, *Bacillus cereus*, *Pseduomonas aeruginosa,* coliforms, yeasts and moulds.

Ten tablets (each 100-500 mg) of each drug type were ground to fine powder by means of sterile mortar and pestle. Ten milliliters of sterile distilled water was added to form a paste. For inoculation, 0.2 milliliter of drug was plated on nutrient agar for isolation of bacteria and on potato dextrose agar for isolation of fungi. Nutrient agar plates were incubated at 37°C for 18-24h, while potato dextrose agar plates were left at room temperature for a minimum of seven days to ascertain fungal growth. In addition to the above method of isolation, tablets were also placed directly (without grinding into powder) on both nutrient and potato dextrose agar plates and incubated appropriately. Samples were inoculated in duplicates. Tablets treated as above were diluted serially in sterile distilled water for the enumeration of their microbial contents.

For enumeration, 1 g amount of each ground tablet samples was weighed and decimally diluted up to 10^{-6}. From each dilution, 0.2 milliliter was plated on plate count agar, mannitol salt agar, peptone agar, MacConkey agar, egg yolk polymycin agar and potato dextrose agar for the determination of total bacterial, staphylococcal, pseudomonad, coliform, bacilli and mould counts, respectively. They were either incubated for bacterial growth at 37°C or 44°C for 18-24 h or at room temperature for seven days for mould growth. About 0.2 milliliter of overnight cultures of isolated organisms were inoculated back on the tablets at different concentrations 5, 10, 15, 20 and 25% respectively this time using the drugs as the sole carbon sources in the mineral salts medium of composition g l^{-1}): K_2HPO_4, 0.5g; NH_4Cl, 1.0g; $MgSO_4.7H_2O$, 0.1g; Na_2SO_4, 2.0g; $FeSO_4.7H_2O$, 0.1g; final pH, 7.2.

Results and Discussion

Table 1 shows the occurrence of microorganisms in the tablets. The total bacterial count ranged from 2.0×10^3 in multivitamin tablets to 8.0×10^7 colony forming units (CFU) per gram in acetylsalicylic acid-paracetamol-codiene (APC) tablets. It was observed that the APC tablets, (non-coated) were the most contaminated. Bacteria isolated from the drugs included *S. aureus*, *B. cereus*, *P. aeruginosa* and *K. aerogenes*.

Table 1
Occurrence of microoganisms in different tablets

Type of drugs	Total bacterial count	Total coliform count	Total staphylo-coccal count	Total pseudom-onal count	Total bacillus count	Total yeast count	Total mould
	cfu/g						
Paracetamol	8.0×10^6	0.5×10^3	3.3×10^4	1.2×10^4	3.2×10^4	1.0×10^3	0.6×10^2
Panadol	3.2×10^4	0.1×10^3	1.1×10^3	2.0×10^3	2.0×10^3	2.0×10^3	0.2×10^2
Multivit	2.0×10^3	0.2×10^2	0.8×10^3	1.1×10^3	2.4×10^3	1.4×10^3	0.3×10^2
Phensic	1.5×10^4	0.2×10^2	0.5×10^3	0.6×10^3	1.8×10^4	0.4×10^3	0.4×10^2
Aspirin	2.2×10^3	0.3×10^3	0.7×10^3	1.0×10^3	0.6×10^3	1.2×10^3	0.3×10^2
Fersolate	1.8×10^4	0.4×10^3	2.3×10^3	0.8×10^3	1.2×10^4	0.8×10^3	0.3×10^2
APC	8.0×10^7	3.4×10^5	6.0×10^6	3.0×10^6	2.0×10^6	2.0×10^5	1.4×10^3
Aldomet	5.0×10^5	2.0×10^3	3.0×10^5	2.0×10^4	3.0×10^4	2.0×10^4	1.0×10^3

Figures 1-4 show the growth patterns of bacterial isolates obtained from the drugs using aldomet, APC, aspirin and paracetamol tablets respectively as sole carbon sources. Growth was highest with *P. aeruginosa* using aldomet tab- lets as sole carbon source (Fig 1) while it was least with *B. cereus* using aspirin as sole carbon source (Fig 2).

However, figures 1 and 2 showed similar growth patterns, reaching a maximum by the fourth day before a sharp decline at the seventh day. As for the growth of *B. cereus* using aspirin tablet as sole carbon source (Fig 3) and the growth of *S. aureus* using paracetamol tablets (Fig 4), there was a sharp increase in growth by the eight week before a decline by the tenth week. Thus, these organisms, not only survived in the milieu of the drugs, but actually used them as sole sources of carbon and energy.

All tablets samples examined in this study showed the presence of microorganisms. *P. aeruginosa*, *B. cereus*, *S. aureus* and *K. aerogenes* dominated amongst the bacteria. The most commonly isolated moulds were *Penicillin chrysogenum*, *Aspergillus niger* and *Aspergillus flavus* and the yeast *Saccharomyces cerevisiae*. The by-products of these contaminants may cause harmful and even lethal reactions in patients.

Bos *et al* (1989) studied the effects of storage under tropical conditions on the behaviour of microbial contamination of tablets. The investigation of the microbial quality of the starting materials showed that rice and tapioca starch had higher level of natural contamination than potato starch. The results demonstrated that after storage under tropical conditions a dramatic loss of microbiological quality occurred within a relatively short time. In the present work, it was observed (Figures 3 and 4) that the bacterial growth in the control tablets increased with the incubation time. The dramatic loss of the microbial quality of the control tablets here may be from contaminated raw materials used in processing.

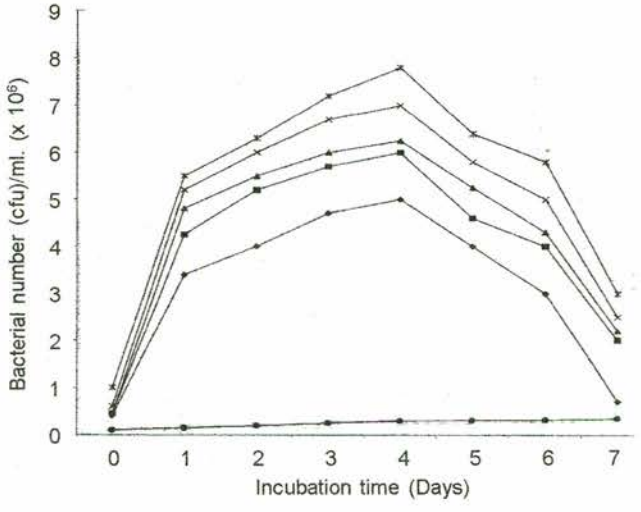

Fig 1. Survival of *Pseudomonas aeruginosa* in mineral salts medium using Aldomet tablet as sole carbon source.
Symbols: ● ..... ● control, ◆ ..... ◆ 5% concentration; ■ ..... ■, 10% conc; ▲ ..... ▲, 15% conc; x ..... x, 20% conc; * ..... * 25 % conc.

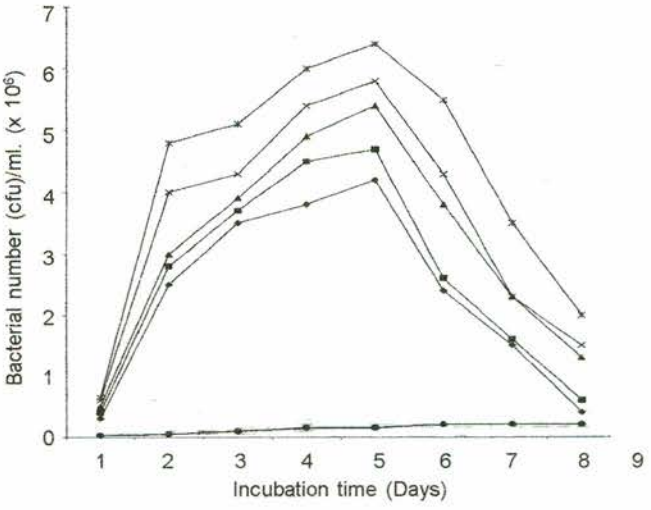

Fig 2. Survival and growth of *Staphylococcus aureus* in mineral salts medium using A.P.C. (Acetylsalicylic Acid paracetamol - Codeine) tablet as sole carbon source.
Symbols: ● ..... ● control, ◆ ..... ◆ 5% concentration; ■ ..... ■, 10% conc; ▲ ..... ▲, 15% conc; x ..... x, 20% conc; * ..... * 25 % conc.

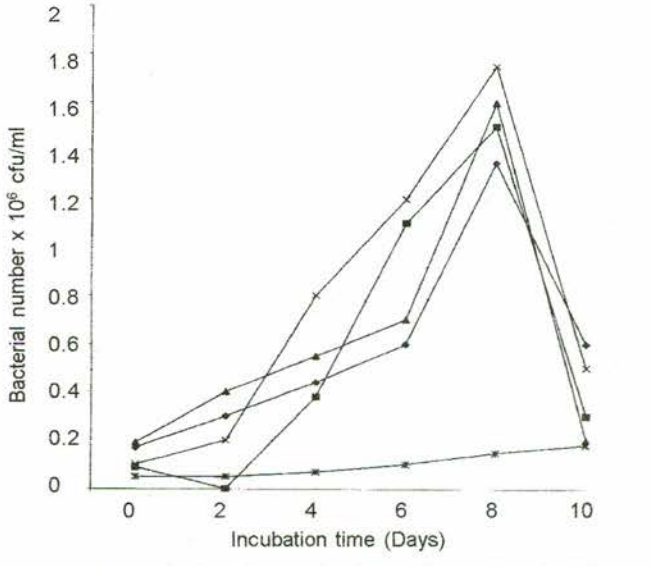

Fig 3. Survival and growth of *Bacillus cereus* in mineral salts medium using Acetysalicylic acid (Aspirin) as sole carbon source.
Symbols: * ..... * control, ◆ ..... ◆ 5% concentration; ■ ..... ■, 10% conc; ▲ ..... ▲, 15% conc; x ..... x, 20% conc; x ..... x 25 % conc.

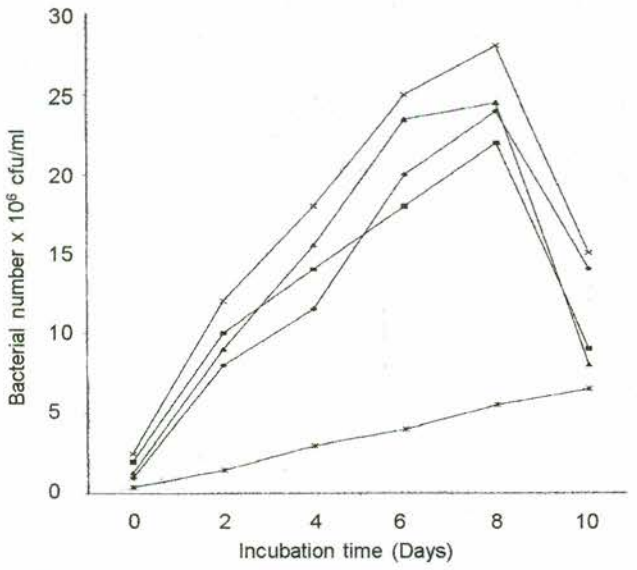

Fig 4. Survival and growth of *Staphylococcus aureus* in mineral salts medium using paracetamol as sole carbon source.
Symbols: * ..... * control, ◆ ..... ◆ 5% concentration; ■ ..... ■, 10% conc; ▲ ..... ▲, 15% conc; x ..... x, 20% conc; x ..... x 25 % conc.

Beveridge (1975) had ascertained that a product in addition to the drug can contain a wide variety of ingredients most of which serve as potential targets for microbial attack and support extensive microbial growth. Thus, many of the diluents, suspending and gelling agents, stabilizers and even the drugs and preservatives are readily attacked by various groups of microorganisms under suitable conditions. Many organic compounds, a large number of aromatic preservatives and other antimicrobial agents are attacked by microorganisms of the genera *Pseudomonas* and *Norcadia*. In the present work, all tablet samples served as sole carbon sources for the inoculated organisms. Growths were evident (Fig 1-4) before a decline.

The loss of microbial quality in these tablets must be viewed with concern especially when most of them are OTC drugs. These changes are of serious health importance. While it is not certain as to the point of contamination of these samples, it is most likely that the microbiological load would have been contributed to by the storage and handling conditions operating at the retail outlets, and enhanced by the prevailing humid tropical conditions.

References

Babalola G O, Desanya S O, Ogungbamila F O, Wirngo B F 1987 Biodegradation of panadol (4-aceta-midophenol) by microbial air-flora in Ile-Ife, Nigeria. *Nig J Biotechnol* **4** 61-63.

Beveridge E G 1975 The microbial spoilage of pharmaceutical products. In: *Microbial Aspect of Deterioration of Material.* eds Lovelock D W & Gilbert R J. Academic Press, London pp 213-235.

Blair T C, Buckton G, Bloomfiled A F 1987 Water available to *Enterobacter cloacae* contaminating tablets stored at high relative humidity. *J Pharm Pharmaco* **39** (Suppl) 125.

Blair T C, Buckton G, Bloomfiled A F 1988 Preservation of solid oral dosage forms. In: *Microbial Quality Assurance in Pharmaceuticals, Cosmetics and Toiletries.* eds Bloomfield S F, Baird R, Leak R E & Leech R. Ellis Horwood, Chichester. pp 104-118.

Bos C E, Van-Doone H, Lerk C F 1989 Microbial stability of drugs stored under tropical conditions. *Int J Pharm* **55** 175-183.

Fassihi A R, Parker M S 1987 Inimical effects of compaction speed on microorganisms in powder system with dissimilar compaction mechanisms. *J Pharm Sci* **76** 466-470.

Grimm W 1986 Stability testing in industry for world-wide marketing. *Drug Dev Ind Pharm* **12** 1259-1292.

Hills S 1946 The isolation of *Clostridium tetani* infected talcum. *N Z Med J* **45** 419-423.

Kallings L O, Ringertz O, Silverstople L, Ernerfeldt F 1966 Microbial contaminations of medical preparations. *Acta Pharm Suec* **3** 219-228.

Kormany L E, Oxley M E, Brecher G 1967 Hospital acquired salmonellosis traced to carmine dye capsules. *New Engl J Med* **276** 850-852.

Morse I J, Williams H I, Grenn F P, Elderidge E F, Rotta J R 1967 Septicemia due to *Klebsiella pneumonia* originating from a hand cream dispenser. *New Engl J Med* **277** 472-473.

Parker M S 1986 The preservation of oral dosage forms. *Int J Pharm Technol Prod Manuf* **5** 20-24.

Plumpton E J, Gilbert P, Fell J T 1986 Effect of spatial distributions of contaminant microorganisms within tablet formulations on subsequent inactivation through compaction. *Int J Pharm* **30** 237-240.

Ringertz O, Ringertz S 1982 The clinical significance of microbial contamination in pharmaceutical and allied products. In: *Advances in Pharmaceutical Sciences,* eds Beans, H S, Beckett A H & Carless J E. Academic Press, London. Vol. 5 pp 201-226.

Watermn R F, Summer E D, Baldwin J N, Warren F W 1973 Survival of *Staphylococcus aureus* on pharmaceutical oral dosage forms. *J Pharm Sci* **62** 1317-1320.