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MICROBIOLOGICAL PROFILE OF NON-STERILE PHARMACEUTICALS SOLD IN PATENT MEDICINE STORES IN NSUKKA, NIGERIA

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ABSTRACT

Twenty different samples each of ascorbic acid, paracetamol, multivite, vitamin B-complex, chloroquine phospate, ferrous sulphate and folic acid tablets; and five bottles each of calamine lotion (100ml each) and paracetamol syrup (60 ml each) were examined for bacterial and fungal contamination. Organisms isolated were members of the genera *Pseudomonas, Staphylococcus, Bacillus, Escherichia, Bacillus, Micrococcus, Aspergillus, Mucor, Geotrichum, Rhodotorula* and *Trichophyton.* Antibiotic resistance study of the bacterial isolates showed that twenty percent of the *Bacillus* sp isolates were resistant to ampicillin. The percentage resistance of *Staphylococcus aureus* isolates to lincomycin, ciproflox, and erythromycin were 28%, 39% and 12% respectively. *E. coli* showed high percentage of resistance to lincomycin (47%), ciproflox (60%), nalidixic acid (60%), ofloxacin (47%) and amoxicillin trihydrate (47%). *Pseudomonas* species also showed high resistance to lincomycin (44%), rifampicin (41%) and chloramphenicol (41%). None of the bacterial isolates showed complete resistance or susceptibility to all the antibiotics with which they were challenged.

KEY WORDS: Pharmaceuticals, bacteria, fungi, antibiotic susceptibility.

INTRODUCTION

Non-sterile pharmaceuticals can, ironically act as vehicles for the transmission of pathogenic and non pathogenic microorganisms. Several cases of infection caused by the administration of non-sterile medicaments contaminated with microorganisms have been reported (Ringertz and Ringertz, 1982; Spooner, 1988; De la Rosa, *et al.* 1993;).

Although it has been recognized that only sterile products are expected to be free from detectable microorganisms (Breach, 1975), the presence of microorganisms in pharmaceuticals is undesirable because they may cause spoilage of the product and present an infection hazard to the consumer or patient. In addition to this, many of these organisms become resistant to one or more of some antimicrobial agents used in therapy after exposure to these non-sterile pharmaceuticals (De La Rosa *et al*; 1993). The acquisition of resistance may be due to chromosomal mutation or plasmids that are often capable of transfer from one strain of bacteria to another even across the species barrier. The importance of resistant environmental strains which may be contaminants of the non-sterile medicaments is that in certain favourable situations they may transfer their resistance plasmids and/or transposons to pathogens. The problem is especially serious in hospitals (and other dispensaries) where the environment can be a factor in the selection of multidrug resistant strains (O' Brien and Acar, 1987; Bryan, 1989). If such bacteria are present in medicaments, they could behave as opportunistic pathogens and initiate an infection particularly in immunocompromised patents (De La Rosa *et al,* 1993).

In recent years, manufacturers of pharmaceuticals have improved the quality of the non-sterile products such that the majority contains only a minimal microbial population. Nevertheless a few rogue products with unacceptable levels and types of contamination do occasionally escape the quality control net and when used may contribute to the spread of diseases. This is even more problematic in tropical countries where pharmaceutical preparations are frequently stored under uncontrolled conditions and may be dispensed in non-protective packaging or even without any packaging at all (Akarele and Ukoh, 2002). Dispensing of most of these medicaments in patent medicine stores takes an average of 3-4 weeks under uncontrolled and therefore unhygienic conditions. In the light of this, the aim of this study is to investigate the microbiological load of non-sterile medicaments sold in patent medicine stores in Nsukka, Nigeria and to screen bacterial strains isolated from the test medicaments for *in vitro* susceptibility to antimicrobial agents.

MATERIALS AND METHODS

Samples: Twenty different samples each of ascorbic acid (vitamin c), paracetamol, multivite, vitamin B-complex, chloroquine phosphate, ferrous sulphate, and folic acid were randomly collected from different patent medicine stores in Nsukka, Nigeria .Also randomly collected were 5 bottles each of calamine lotion (100ml each) and paracetamol syrup. (60ml each). All the medicaments used were registered trade mark specialties and were at least 6 months away from the expirations date written on the containers. The samples were prepared according to the methods of De La Rosa *et al.,* (1993), and Akerele and Ukoh (2002) after careful inspection of the product containers to detect leaks, irregularities in the appearance of solutions such as cloudiness, particulate and unexpected colour. Container lids were cleaned using cotton wool soaked in 70% ethanol before opening.

Five tablets each of ascorbic acid (1.528g), multivite (1.002g), vitamin B-complex (0.638g), chloroquine phosphate (1.923g), ferrous sulphate (1.924g) and folic acid (0.319g) were crushed and dispersed in 10ml sterile phosphate buffer with polysorbate 80 (0.3%) while five tablets of paracetamol (3.738g) were dispensed in 20ml of the same diluent. One ml each of calamine lotion and paracetamol syrup was added to 9ml of tryptone salt with polysorbate 80(10%). All the dispersions were mixed in a vortex mixer for five minutes.

Media: Media used were nutrient agar, Macconkey agar, Manitol salt agar, Sabauraud dextrose agar and Mueller Hinton agar (Oxoid, Basingstoke UK).

Isolation and Identification of contaminants: Surface plating method was used (Palmieri, 1983). An aliquot (0.1ml) of each dispersion was spread on nutrient agar, MacConkey agar, Mannitol salt agar and Sabauraud dextrose agar plates (in duplicates). Sabauraud agar plates were incubated at 25^{0}C for 72h while the other plates were incubated at 30^{0}C for 24h. Incubation of cultures at instead of 37^{0}C was to encourage the possible growth of bacteria such as *Achromobacter* which may not grow above 30^{0}C (Breach, 1975). After incubation, colonies were counted and the number of organisms per g or ml of sample

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was calculated. Bacteria were analyzed for their morphological, physiological and biochemical characteristics according to the methods of Cowan and Steel (1974), Krieg and Holt (1984) and Sneath *et al* (1986). The fungal colonies were identified based on cultural and microscopic characteristics (Evans and Richardson 1989; Beneke, 1974).

Antibiotic susceptibility tests: Susceptibility or resistance to the following antibiotics was assessed by the diffusion method of Bauer *et al.* (1966) using Muller Hinton agar and Optun laboratory paper disks: Lincomycin (2µg), rifampin (5µg), ampicillin (10µg), chloramphenicol (30µg), erythromycin (15µg), gentamicin (10µg), streptomycin (10µg), ofloxacin (10µg), ciproflox (10µg), amoxicillin trihydrate (30µg), nalidixic acid (30µg), co-trimoxazole (30µg), ampiclox (30µg) and norbactin (30µg). The plates were incubated at 35°C for 24h and results recorded by measuring the inhibition zones and scored as susceptibility ranges according to the standard table (Prescott *et al* 1999; De La Rosa *et al*, 1993; Anon, 1988).

RESULTS

The bacteria isolated belonged to the following genera *Pseudomonas, Staphylococus, Bacillus, Escherichia* and *Micrococcus* with *Bacillus* spp occurring more frequently in ascorbic acid, paracetamol tablets, Multivite, vitamin B complex and chloroquine phosphate than any other genus (Table 1). The most frequent species isolated from ferrous sulphate was *Staphylococcus aureus,* while *Pseudomonas* spp was isolated more from folic acid than any other bacterial genus. Fungi isolated were members of the genera *Aspergillus, Mucor, Geotrichum, Rhodotorula* and *Trichophyton* with *Aspergillus* spp isolated more than any other genus from most of the test medicaments (Table 2).

Twenty per cent of the *Bacillus* spp isolated were resistant to ampicillin while only 0.6% were resistant to chloramphenicol (Table 3). Table 3 also shows that 28% of *Staphylococcus aureus* isolates were resistant to lincomycin; 39% to ciproflox and 12% to erythromycin, gentamicin and ampiclox. A high percentage of *E. coli* isolates were resistant to lincomycin (47%), ciproflox (60%); nalidixic acid (60%); tarivid (47%), and augumentin (47%). *Pseudomonas* species also showed high resistance to lincomycin (44%), rifampicin (41%), and chloramphenicol (41%). None of the bacterial isolates showed complete resistance to all the antibiotics they were challenged with (Table 3).

Table 1: Number and Percentage of Bacterial contaminants of test medicaments.

				Frequency of occurrence of isolated bacteria genera among sample packages (percentages)					
MEDICAMENTS	NO OF PACKAGES SAMPLED	No of Packages with bacteria contaminants	Total bacteria count (cfu/g or /ml	*Bacillus* spp.	*Micrococcus* spp	*Pseudomonas*spp	*E.coli*	*Staph. aureus*	*Staph. epidermidis*
A. Tablets:									
Ascorbic acid	20	14(70)	148	13(65)	4(20)	6(30)	1(5)	5(25)	5(25)
Paracetamol	20	9(45)	120	9(45)	5(25)	4(20)	2(10)	3(15)	1(5)
Multivite	20	4(20)	51	3(15)	-	-	-	2(10)	-
Vitamin B complex	20	7(35)	73	7(35)	1(5)	3(15)	-	5(25)	1(5)
Chloroquine phosphate	20	9(45)	26	8(40)	1(5)	3(15)	-	6(30)	-
Ferrous sulphate	20	8(40)	9	3(15)	2(10)	2(10)	-	7(35)	-
Folic acid	20	13(65)	37	2(10)	4(20)	5(25)	-	4(20	-
B. Liquids									
Calamine lotion	5	3(60)	30	3(60)	1(20)	-	-	1(20)	-
Paracetamol syrup	5	2(40)	23	1(20)	-	-	-	2(40)	-

(Values in parenthesis are corresponding percentages)

Table 2: Fungal content of test medicaments.

				Frequency of occurrence of different genera.				
MEDICAMENTS	No of packages sampled	No of packages with fungal contaminants	Total fungal plate count (cfu/g or /ml)	*Aspergillus* sp.	*Mucor* sp	*Geotrichum* sp	*Rhodotorula* sp	*Trichophyton* sp.
A. Tablets								
1. Ascorbic acid	20	8(40)	11	8(40)	6(30)	3(15)	1(5)	1(5)
2. Paracetamol	20	9(45)	8	5(25)	5(25)	-	-	-
3. Multivite	20	9(45)	5	7(35)	3(15)	-	-	-
4. Vitamin B complex	20	7(45)	7	3(15)	4(20)	2(10)	-	1(5)
5. Chloroquine phosphate	20	5(25)	3	3(15)	3(15)	-	-	-
6. Ferrous sulphate	20	9(45)	8	9(45)	5(25)	-	1(5)	-
7. Folic acid	20	7(35)	7	2(10)	4(20)	1(5)	-	-
B. Liquids								
1. Calamine lotion	5	3(60)	9	2(10)	2(10)	1(5)	-	-
2. Paracetamol syrup	5	1(20)	2	1(5)	1(5)	-	-	-

Table 3: Resistance to antimicrobials of bacteria isolated from medicaments (number of resistant isolates)

Genera	No of isolates (cfu)	L	E	RA	AM	C	GM	S	TA	CX	AU	NA	TXS	AX	NT
Bacillus spp	178	21	18	15	35	7	14	10	1	1	6	6	3	4	12
Micrococcus spp	54	13	13	10	14	5	15	11	9	7	10	7	7	5	9
Pseudomonas spp	27	12	5	11	7	11	3	4	3	1	2	1	2	1	1
Escherichia coli	15	7	4	2	6	6	4	2	7	9	7	9	3	2	10
Staphylococcus aureus	82	23	10	16	21	26	10	13	28	32	19	21	27	10	15
Staphylococcus epidermidis	19	20	6	7	5	9	9	3	2	3	2	2	1	5	6

L, lincomycin; E, Erythromycin; RA, Rifampicin; AM, Ampicillin; C, Chloramphenicol; GM, Gentamilcin; S, Streptomycin; TA, ofloxacin; CX, Ciproflox; AU, Amoxycillin trihydrate, NA, Nalidixic acid; TXS, co-trimoxazole; AX, Ampiclox; NT, Norbactin.

DISCUSSION

The results presented here show that significant microbial contamination of non-sterile pharmaceuticals occurs. This is in consonance with earlier reports describing similar findings (De La Rosa *et al,* 1993; Akerele and Ukoh 2002). Spores of some of the fungi isolated are often found in air and can easily contaminate any available material. *Aspergillus* sp, for example, is ubiquitous in nature (Prescott *et al.,* 1999). Others such as *Geotrichum* species are found in soil and animal faeces (Evans and Richardson, 1989) from where they can contaminate any available substance. The undesirability of the presence of these fungi in non-sterile medicaments becomes more evident on the realisation that some of them are overt or opportunistic pathogens. *Trichophyton* species is an anthropophilic fungus and it is probable that they may have been introduced into these (ascorbic acid and vitamin B complex) medicaments from infected individuals. Their presence constitutes a health hazard to unsuspecting handlers of these pharmaceutical products. Although *Aspergillus* and Mucor spices are common environmental contaminants, their presence in reasonably large numbers in orally administered drugs is worrisome because they are known to cause diseases in immunocompromised patients. *A. fumigatus* for example is the known causative agent of aspergillosis and *Mucor* species is the infrequent aetiologic agent of mucormycosis (Prescott *et al.,* 1999; Evans and Richardson, 1989). *Geotrichum* sp. is a soil fungus known to cause opportunistic deep mycosis (Evans and Richardson, 1989). Its presence in over- the- counter drugs such as ascorbic acid, vitamin B complex, folic acid and calamine lotion posses a public health problem.

Bacillus spp were the bacteria most frequently isolated from the test medicaments (except ferrous sulphate, folic acid and paracetamol syrup) probably because they are widely distributed in the soil, dust, air, and water, and because they are resistant to environmental factors (De La Rosa *et al.,* 1993; Garcia Arribas, *et al.,* 1986). Members of this genus have frequently been regarded as non-pathogenic but a number of reports have described serious human infections including endocarditis, sepsis, meningitis and endophthalmitis caused by them mainly in immunosuppressed patients (Cotton *et al.,* 1987; Sliman *et al.,* 1987). *Bacillus* species also cause bacterial food poisoning (Kramer and Gilbert, 1989). Some species of *Bacillus* isolated in this work showed resistance to a wide range of antimicrobial agents – an undesirable phenomenon in medicine.

Also undesirable is the presence in ascorbic acid, paracetamol, Vitamin B complex etc (Table 1) of multidrug resistant strains of *Staph. aureus* and *Staph. epidermidis.* This is in line with earlier reports (Devleeschouwer and Dony, 1979; De La Rosa *et al.,* 1993) describing the occurrence of *Staphylococcus* species in pharmaceuticals and expressing concern over the public health implication of their findings. Isolation of *Micrococcus* species in this work is also in conformity with the results obtained by Garcia Arribas *et al.,* (1983) and Willense – Collinet *et al.,* (1981) with discrepancy only in percentage occurrence.

Escherichia coli, Pseudomonus sp and other Gram negative rods have been previously (Spooner, 1988; Baird, 1991) reported to be responsible for the majority of incidents involving non-sterile medicaments, an observation attributed to their resistance to antimicrobial agents and their metabolic versatility. This may explain, at least in part, the presence and antibiotic sensitivity pattern of these organisms in some of the analyte medicaments in this work. The concern here is that these Gram-negative organisms cause a wide range of infections including bacteremias, pneumonias and urinary tract infections (Cohen, 1992).

The results of this work show a worrisome level of microbial contamination of non-sterile over- the -counter medicaments sold in our locality and a high rate of resistance to antimicrobial agents of strains isolated. Such organisms may be clinically troublesome. More attention should therefore be paid to the sanitary quality of drug production process and dispensation.

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