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EVALUATION OF MICROBIAL QUALITY OF SELECTED BLISTER-PACKED PARACETAMOL TABLETS AND PARACETAMOL SYRUPS MARKETED IN NIGERIA.

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ABSTRACT

Ten brands of blister-packed paracetamol tablet and twenty brands of paracetamol syrup marketed in Nigeria were evaluated for their microbial quality. While no microbial contaminant was isolated from all blistered-packed paracetamol tablets, ten of syrups were contaminated with organisms such as *Escherichia coli, Klebsiellas spp, Pseudomonas aeruginosa* and *Staphylococcus aureus* at 14.3, 21.4, 21.4 and 42.9% occurrence respectively. *Penicillium spp* was isolated from two brands. Antibiotic susceptibility profile revealed all bacterial isolates to be multidrug resistant with *Escherichia coli* resistant to all antibiotics tested, while *Staphylococcus aureus* isolates were sensitive to Oxacillin, Cefuroxime and vancomycin. *Pseudomonas aeruginosa* isolates were sensitive to ofloxacin and gentamycin while *Klebsiella* isolates were sensitive to ofloxacin and nitrofurantoin. The study concluded that compliance with the provisions of good manufacturing practice as well as good quality control play role in determining the microbial bioburden of pharmaceutical products while isolation of multi-drug resistant organisms calls for establishment and adherence to antibiotics use policy in Nigeria.

Key Words: Blister-pack, multidrug resistance, good manufacturing practice, quality control, bioburden.

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Résumé:

Dix marques de comprimé de paracétamol boursouflée-emballés et vingt marques de sirop de paracétamol commercialisés au Nigeria étaient évaluées pour leurs qualités microbienne. Bien qu'aucun contaminant microbien était isolé à partir de tous les comprimés de paracétamol boursouflée-emballés, dix de sirops étaient contaminés par des organismes tels que : *Escherichiacoli*, *Klebsiellaspp*, *Pseudomonasaeruginosa*et *de Staphylococcusaureus*à14,3, 21,4, 21,4et42,9% respectivement**en l'occurrence.***Penicilliumspp*était isoléà partir de deuxmarques. Profilde sensibilité antibiotiquea révélé **qu'***Escherichia*coli**est**résistantes àtouslesantibiotiques examinés, tandis que**les**isolâtesde *Staphylococcusaureus*étaient sensibles àl'*oxacilline*, *céfuroximeet la vancomycine*. **Les** Isolâtesde*Pseudomonasaeruginosa*étaientsensiblesà l'ofloxacineetlagentamicinependant **que les** isolâtesde*Klebsiella*étaientsensiblesà l'ofloxacinela nitrofurantoïne**.** Alors, on peut concluque la conformitéauxdispositionsdebonnes pratiques de fabricationvis-à**-vis le** contrôlede bonne qualité prends part dansla détermination de lacharge-biologie microbiennedesproduits pharmaceutiques pendant que isolement des organismes résistants aux **nombreux** médicaments demandentpour l'établissementet l'adhésionau politique d'utilisation des antibiotiquesau Nigeria**.**

Mots clés: **Boursouflée-emballés,** activité de l'eau**, les bonnes pratiques**de fabrication**, le contrôle de qualité,**la charge-biologie microbienne**.**

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INTRODUCTION

Pharmaceutical preparations can be classified into sterile and non-sterile products. While sterile products are expected to be absolutely free of all microbes, non-sterile products are not to be free of all forms of microbes although there are limits as to the permissible levels of these contaminants in such pharmaceutical products(1).

Microorganisms possess diverse metabolic activities and are likely to present a variety of hazards (for examples, infections, toxicity, degradation of formulations) both to the user and to the stability of the products, if allowed to persist. Limits have therefore been set for the presence of microorganisms in medicines by commissions as European Pharmacopoeia commission, which vary depending on the product and its intended use. However, microbial contamination over and above these pharmacopoeia levels is a global problem which is still being reported in distributed medicines worldwide (2). The most commonly reported microbial hazards found in liquid medicines as syrups are pseudomonads and their related Gram-negative rods, with spores (bacterial and fungal) predominating in dry tablets, capsules and cosmetic powders. Occurrence of true pathogens as S*almonella* spp in syrups has been reported (3, 4).

The risk (likelihood of harm actually occurring) associated with delivery of contaminated products is less clearly determined. It will depend upon the type of microorganisms present, the infective dose (dependent on the ability of the formulation to encourage microbial survival and level of preservative protection built with it), the route of administration of the product and the host's resistance to infection (including the immune status or the degree of tissue damage at the site of application).

The presence of the species as *Escherichia* spp, *Klebsiella* spp, *Pseudomonas* spp, *Proteus* spp, *Enterococcus* spp, *Micrococcus* spp, *Salmonella* spp, *Staphylococcal* spp, *Bacillus* spp, *Aerobacter* spp, *Aspergillus* spp as well as *Penicillium* spp in syrups, capsules and tablets within and outside Nigeria has been reported (3, 5-11).

The nature of the ingredients, the quality of the vehicle and the care and attitude of personnel involved in their handling will determine the incidence of microflora in non-sterile products (12). However, since raw materials, manufacturing environment, packaging materials and personnel have been implicated as potential sources of contaminants in pharmaceutical products, it implies that pharmaceutical preparations can be contaminated at the point of production and packaging by the manufacturer or use by the consumers.

This study aimed at comparing the microbial quality of some blister-packed paracetamol tablets to that of paracetamol syrups marketed in Nigeria as well as identifying the source(s) of such contaminants and the potential health hazard implication of such contaminants on the populace.

MATERIALS AND METHODS

Media

Nutrient broth, nutrient agar, MacConkey agar, Mannitol salt agar, Cetrimide agar, Mueller-Hinton agar, Salmonella-Shigella agar, Saboraud Dextrose agar; all ATER products from Topley House 52, Wash Lane, Bury, Lanchashire BL96AS, UK.

Preparation of tablet dispersion:

Two tablets of each brand of blister-packed paracetamol were aseptically removed directly into 10mL sterile normal saline and dispersed. Tablet dispersions were mixed in a vortex mixer for 5 minutes to dislodge possible microbial cells. The solid particles settled down and the supernatants were used.

Determination of microbiological quality of tablets:

One milliliter aliquot of each brand's dispersion was seeded in nutrient agar, poured and allowed to set in plates (in duplicates). The procedure was repeated using MacConkey agar medium, Mannitol salt agar medium, cetrimide agar medium, Salmonella-Shigella agar and Saboraud Dextrose agar medium. The Saboraud Dextrose agar plates were incubated at 25 ^{o}C for 72-96 hours while the other agar plates were incubated at 37 ^{o}C for 48 hours before they were observed for growth.

Determination of microbiological quality of syrups:

1 in 10, 1 in 100 and 1 in 1000 dilutions of each paracetamol syrup in sterile peptone water was made and 0.1mL of each dilution was seeded in nutrient agar medium, MacConkey agar medium, Mannitol salt agar, Cetrimide agar medium, Salmonella/Shigella agar medium as well as Saboraud Dextrose agar medium contained in sterile Petri dishes (in duplicates). All the plates were incubated at 37^{o}C for 24-48 hours except the Saboraud Dextrose agar plates that were incubated at 25^{o}C for 72-96 hours before they were observed for growth.

Bacterial isolates from the plates that showed growth were subjected to standard microbiologic identification tests based on colony morphology, and conventional biochemical tests to confirm their identity and/or purity.

Antibiotic susceptibility profiling:

Susceptibility of the Gram-negative and Gram-positive isolates to eight and three antimicrobial agents respectively was tested by the disc diffusion technique according to the guidelines by the Clinical and Laboratory Standards Institute (13). The Gram-negative antibiotic disc contained augmentin (30 µg); ofloxacin (5 µg); gentamycin (10 µg); nalidixic acid (30 µg); nitrofurantoin (200 µg); cotrimoxazole (25 µg); amoxycillin (25 µg) and tetracycline (25 µg) while the Gram-positive antibiotic single disc comprised of oxacillin (1µg); cefuroxime (30µg); and vancomycin (30 µg).

Four or five colonies of each test organism taken from a nutrient agar culture plate was inoculated into 10 mL of sterile distilled water using a sterile loop. The suspension was thoroughly mixed with a spin mixer. The resulting suspension was adjusted to a turbidity of 0.5 McFarland standard. This was then applied to the surface of oven-dried Mueller Hinton agar and spread evenly with a sterile swab stick. The inoculated plates were incubated at 37^{o}C for 20 minutes for acclimatization and growth of the inocula. Antibiotic discs (Abtek, Liverpool, UK) were then lightly but firmly pressed onto the surface of the plates using a pair of sterile forceps. The plates were then refrigerated at 4^{o}C for thirty minutes to ensure adequate diffusion of antibiotics. *E. coli* ATCC 25922 was used as control strain. All plates were incubated at 37^{o}C for 18 hours. The diameters of inhibition zones were measured in millimetres and interpreted according to CLSI manual.

RESULTS

Of the ten selected brands of blister-packed paracetamol tablet used for the study, only one was imported from India while the remaining nine were manufactured in Nigeria. All the brands used have their identities revealed by indicating the date of manufacture, expiry date, NAFDAC number as well as the address of the manufacturer as shown in Table 1.

The container disclosures of all the twenty brands of paracetamol syrup, manufactured in Nigeria, used for the study are as shown in Table 2.However, of the twenty brands of paracetamol syrup used for the study, 50% were free of microbial contaminants while 50% were contaminated with microbial loads ranging from 2×10^1 in S_{13} to 1.05×10^4 cfu/mL in S_{18} as shown in Table 3.

A total of 14 bacterial isolates comprising of *Staphylococcus aureus,* 42.9%; *Klebsiella spp*, 21.4%; *Pseudomonas aeruginosa*, 21.4%; and *Escherichia coli*, 14.3% as shown in Table 4 were isolated from this study.

Antibiotic susceptibility profiles revealed all *Klebsiella* isolates to be sensitive to ofloxacin, and nitrofurantoin, with 2 strains sensitive to gentamycin, an aminoglycoside, and 1 strain with intermediate sensitivity. They were however resistant to other antibiotics tested namely: cotrimoxazole, amoxicillin, tetracycline, augmentin and nalidixic acid. All *Escherichia coli*, on the other hand, were resistant to all antibiotics tested while one strain of *Pseudomonas aeruginosa* was sensitive to ofloxacin and gentamycin while showing intermediate sensitivity to tetracycline. One strain was also sensitive to tetracycline with intermediate sensitivity to ofloxacin. One other strain showed intermediate sensitivity to ofloxacin and nitrofurantoin as shown in Table 5

All *Staphylococcus aureus* isolated were sensitive to oxacillin and cefuroxime, both ß-lactam antibiotics with varying sensitivity to vancomycin as shown in Table 6

TABLE 1: CONTAINER DISCLOSURE OF EACH BRAND OF BLISTER-PACKED PARACETAMOL TABLET USED FOR THE STUDY

SAMPLE	MANUFACTURING DATE	EXPIRY DATE	NAFDAC NUMBER	ADDRESS OF MANUFACURER
T_1	+	+	04-0633	+
T_2	+	+	04-0411	+
T_3	+	+	04-1957	+
T_4	+	+	04-0101	+
T_5	+	+	04-0975	+
T_6	+	+	04-1853	+
T_7	+	+	04-5762	+
T_8	+	+	04-1217	+
T_9	+	+	04-1207	+
T_{10}	+	+	04-4686	+

TABLE 2: CONTAINER DISCLOSURE OF PARACETAMOL SYRUP SAMPLES USED FOR STUDY

Brand Name	Manufacturer's Address	Batch Number	Production Date	Expiry Date
S_1	+	LO66P	+	+
S_2	+	452	+	+
S_3	+	PL7108	+	+
S_4	+	TPS008	+	+
S_5	+	9169	+	+
S_6	+	L4909	+	+
S_7	+	P510001	+	+
S_8	+	242	+	+
S_9	+	08001	+	+
S_{10}	+	08760507	+	+
S_{11}	+	0254	+	+
S_{13}	+	PL 001	+	+
S_{14}	+	P05	+	+
S_{15}	+	S m 3325 P	+	+
S_{16}	+	014251	+	+
S_{17}	+	IZ1686	+	+
S_{18}	+	9011	+	+
S_{19}	+	PA0405	+	+
S_{20}	+	00111	+	+

TABLE 3: MICROBIAL QUALITY OF EACH PARACETAMOL SYRUP USED FOR STUDY

Paracetamol sample	Total viable count (cfu/mL	Organism(s) isolated
S_1	3×10^1	*Staphylococcus aureus*
S_2	2×10^2	*Penicillium spp*
S_3	Nil	
S_4	Nil	
S_5	6.75×10^2	*Staphylococcus aureus*
S_6	Nil	
S_7	Nil	
S_8	Nil	
S_9	Nil	
S_{10}	Nil	
S_{11}	1.9×10^2	*Staphylococcus aureus*
		Penicillium spp
S_{12}	Nil	

Paracetamol sample	Total viable count (cfu/mL	Organism(s) isolated
S_{14}	Nil	
S_{15}	1.6×10^2	*Pseudomonas aeruginosa* *Klebsiella spp* *Staphylococcus aureus*
S_{16}	2.45×10^2	*Pseudomonas aeruginosa* *Klebsiella spp* *Staphylococcus aureus*
S_{17}	2×10^1	*Escherichia coli*
S_{18}	1.48×10^4	*Pseudomonas aeruginosa* *Klebsiella spp* *Staphylococcus aureus*
S_{19}	1.3×10^2	*Escherichia coli*
S_{20}	Nil	

TABLE 4: PERCENTAGE OCCURRENCE OF ISOLATED BACTERIA IN PARACETAMOL SYRUP SAMPLES

ISOLATED ORGANISM	P PERCENTAGE PREVALENCE
Staphylococcus aureus	42.9%
Klebsiella spp	21.4%
Pseudomonas aeruginosa	21.4%
Escherichia coli	14.3%

TABLE 5: ANTIBIOTIC SENSITIVITY PATTERNS OF GRAM-NEGATIVE BACTERIAL ISOLATES OBTAINED FROM PARACETAMOL SYRUP SAMPLES

Samples	ANTIBIOTICS/ZONE OF INHIBITION IN MILLIMETERS							
	COT	AMX	TET	AUG	OFL	GEN	NAL	NIT
$S_{15(a)}$	0(R)	0 (R)	9 (R)	7 (R)	21 (S)	14 (I)	0 (R)	24 (S)
$S_{15(b)}$	0 (R)	6 (R)	20 (S)	0 (R)	13 (I)	0 (R)	0 (R)	0 (R)
$S_{16(a)}$	0 (R)	0 (R)	11 (R)	0 (R)	24 (S)	17 (S)	0 (R)	18 (S)
$S_{16(b)}$	0 (R)	0 (R)	10 (R)	0 (R)	14 (I)	0 (R)	0 (R)	16 (I)
$S_{17(c)}$	0 (R)	13 (R)	0 (R)	0 (R)	11 (R)	0 (R)	0 (R)	9 (R)
$S_{18(a)}$	0 (R)	0 (R)	14 (R)	0 (R)	16 (S)	20 (S)	0 (R)	17 (S)
$S_{18(b)}$	0 (R)	0 (R)	16 (I)	0 (R)	17 (S)	16 (S)	0 (R)	10 (R)
$S_{19(c)}$	0 (R)	0 (R)	0 (R)	0 (R)	12 (R)	0 (R)	0 (R)	10 (R)

KEY:(a)-*Klebsiella*spp.; (b)- *Pseudomonas aeruginosa;* (c) - *E. coli;* S - Sensitive; I - Intermediate; R - Resistance

TABLE 6: ANTIBIOTIC SENSITIVITY PATTERNS OF GRAM-POSITIVE BACTERIAL ISOLATES OBTAINED FROM PARACETAMOL SYRUP SAMPLES

SAMPLES	ANTIBIOTICS/ZONE OF INHIBITION IN MILLIMETERS		
	OX	CXM	VA
S_1	13 (S)	25 (S)	15 (I)
S_5	14 S)	24 (S)	15 (I)
S_{15}	14 (S)	24 (S)	15 (S)
S_{16}	15(S)	23 (S)	15 (I)
S_{18}	21 (S)	34 (S)	17 (S)

KEY: S – Sensitive; OX = Oxacillin; I – Intermediate; CXM = Cefuroxime; R – Resistant; VA = Vancomycin

DISCUSSION

In the microbiological evaluation of non-sterile products, two tests are important namely: the extent of contamination test and the nature of the contaminant test. These tests are complementary and every non sterile product must pass the two tests before being released for customer use. While the extent of contamination test sets allowable limits for microbial contaminants in the product, the nature test addresses objectionable pathogenic organisms whose presence in the product will render the product unsuitable for use irrespective of their number in the product. These two tests were used in this study to determine the suitability for use or otherwise of some blister-packed paracetamol tablets and paracetamol syrups marketed in Nigeria.

Tablets are compacts drug delivery systems with low water content which usually afford them good protection against microbial contamination. Spoilage and clinical infections resulting from microbial contamination of tablets under hot and humid conditions of the tropics have been reported (7, 14). Tablets also undergo deleterious changes as discoloration, weakening of tablets matrixes and decreases in the potency of active ingredients when improperly stored (15). Potential contamination of tablets may arise from heavy microbiological burden in raw materials, though this is usually drastically reduced by lethal drying stage of wet granulation (16). However, the decreasing use of direct compression in manufacturing of tablets in pharmaceutical industries implies that some contaminants may survive up to the compression stage. The compression of formulation is known to effect some level of microbial destruction but this depends on the compression pressure applied, the properties of the contaminating organisms, and the formulation involved (17, 18). The effects of these variables in turn are believed to depend on the mechanism of microbial kill which has been proposed to include high localized heat shearing forces during compression (18, 19). The shear stresses manifested during compression depend largely on the principal mode of consolidation of the formulation which can be by fragmentation of plastic flow, with plastic having been shown to be a highly effective mechanism for microbial kill even at low compression pressures (17, 20). Binding agents employed in formulations are known to undergo a high degree of plastic deformation during compression and are forced into their inter-particulate spaces where they increase the area of contact between the particles and form strong solid bounds. All these factors played role in the result obtained for tablets used for this study as none of the tablets used for the study was contaminated by any form of microbial contaminant.

Syrups, on the other hand, usually consist of active ingredient, sugar, vehicle and/or preservative.

The ability of the bacteria isolated in this study to survive in syrup can be attributed to water activity of those brands of paracetamol syrup studied.

Reduced water activity will greatly assist in the prevention of microbial proliferation in pharmaceutical products. Because the water activity requirements for different Gram-reactive bacteria, bacterial spores, yeast and moulds have been described (12), the appropriate microbial limit testing program for products of differing water activities can be established. For instance, Gram-negative bacteria such as *Pseudomonas aeruginosa*, *Escherichia coli,* and *Salmonella* species will not proliferate or survive in products with water activities below 0.91 while Gram-positive bacteria like *Staphylococcus aureus* will not proliferate below 0.86, and *Aspergillus niger* will not proliferate below 0.77. Furthermore, most osmophilic yeast and xerophilic fungi will not proliferate below 0.60 (21).

Suffice it to say that the water activity of the product is a function of the organism to be excluded from the product. In this study in particular, it can be said that the water activity of all the paracetamol tablets and

some brands of paracetamol syrup, where neither bacteria nor fungi were isolated, was below 0.60.

Of the 50% contaminated paracetamol syrup samples used, S_{15}, S_{16} and S_{18} were heavily contaminated with 3 strains of bacteria namely: *Klebsiella spp, Pseudomonas aeruginosa* and *Staphylococcus aureus* while S_{19} was contaminated with only *Escherichia coli* and other samples with *Staphylococcus aureus* with the exception of S_2 and S_{13} that were contaminated with *Penicillium spp*.

Klebsiella spp are found in the respiratory, intestinal, and urinogenital tracts of animals and humans. However, when *Klebsiella* moves outside the gut, it can cause a serious infection. Thus, its presence in the assayed samples is also an indication of unhygienic conditions, and may have originated from pharmaceutical personnel. The presence of *Staphylococcus aureus* does not always mean that the consumption of medicines are potentially being hazardous to users as not all the strain of *Staphylococcus* sp. can necessarily produce enterotoxin where higher infectious dose (10^5-10^6CFU/mL) is required (6). *Staphylococcus aureus* may however cause a significant deterioration in the health status of patients, particularly those who are immunologically compromised and of infants with an immature immune system (22). *Staphylococcus* sp. might transmit from hands of handler during the preparation of drugs.

The presence of *Escherichia coli* is a good indicator of fecal contamination resulting from water supply. Incidence of infantile diarrhoea associated with *E. coli* as a result of poor water supply in Nigeria has been reported (23). However, the presence of all the isolated organisms in this study has rendered the product from which they were isolated unfit for human use, being indicator organisms according to the United States Pharmacopoeia (USP). Organisms as *Salmonella* sp., *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus*, and *Candida albicans* have been recommended as indicators of pathogenic microorganism contaminants of syrups (24).

Antibiotic sensitivity profiles revealed all isolates to be multidrug resistant as they were resistant to two or more antibiotics used for the study. Ofloxacin and Nitrofurantoin are the drugs of choice as far as this study is concerned as majority of the isolates displayed highest degree of susceptibility to them. However, all the Gram-positive isolates were sensitive to all the antibiotics against which they were tested in this study.

Quality control, as part of quality assurance, involves the sampling and testing of starting materials, intermediate, bulk and finished products and packaging materials to ensure compliance with appropriate standards and specifications. Quality Assurance is the totality of the process undertaken to ensure absolute quality of a product. Quality Assurance is Quality Control plus Good Manufacturing Practices (GMP), i.e. the product is manufactured through laid down procedures, contain necessary ingredients in correct proportions and of right purity, packed in proper containers and with proper labels. The pharmaceutical industries have an obligation to design, test and produce dosage forms that provide the consumer with products having the attributes of quality, purity, uniformity of content, stability, safety and physiological availability. These requirements necessitate the total involvement of corporate personnel with formal system of checks and balances. It is only through well organized, adequately staffed and accurately performed process and dosage form control before, during and after production that adequate quality assurance of the product can be achieved (25).

The presence of antibiotic-resistant organisms in paracetamol syrup as shown in this study is of concern as they pose danger to children especially the immunocompromised ones in whom they can aggravate illness as a result of secondary infection (26).

CONCLUSION

It can be concluded that since no organism was isolated from all paracetamol tablets studied while organisms were isolated from some paracetamol syrup samples studied, it follows that manufacturing processes to which both the drug substance and the excipients were subjected have effect on the microbial quality of the final dosage form. While none of the blistered-packed paracetamol tablets studied pose threat health-wise to the consumers, isolation of antibiotic-resistant contaminants from some samples of paracetamol syrup studied emphasize a need for rational use of antibiotics in the country.

Moreover, product of good microbial quality can be guaranteed through quality control and adherence to the provisions of good manufacturing practices. Also, low water activity can aid reduction in vulnerability of formulations to microbial contamination provided the pharmaceutical products are made from ingredients of good microbial quality, when manufacturing environments do not foster microbial contamination, when there are processes that inherently reduce the microbial content, to mention but a few.

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