INTERNATIONAL RESEARCH JOURNAL OF PHARMACY **ISSN 2230 – 8407**
Available online http://www.irjponline.com
Research Article

MICROBIAL ASSESSMENT OF SOME SYRUP SOLD IN PATENT MEDICINE STORES IN MINNA METROPOLIS, NIGERIA

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Article Received on: 12/04/11 Revised on: 18/06/11 Approved for publication: 21/07/11

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ABSTRACT
The microbiological quality of eighteen different brands of syrups comprising of Paracetamol, Chloroquine phosphate and Vitamin C syrups purchased from different patent medicine stores in Minna metropolis was assessed. The microbial load was determined using the viable cell count method; the resulting contaminant microorganisms were isolated and characterized by standard methods. The results revealed the contamination in four of six; five of six and four of six, Vitamin C. Paracetamol and Chloroquine phosphate syrups respectively exceeding the tolerance limit of permissible microorganisms specified officially for syrups. The contaminant organisms isolated from analyzed syrups include bacteria*: Escherichia coli, Bacillus subtilis, Staphylococcus aureus,* and fungal isolates include: *Aspergillus niger, Aspergillus fumigatus, Penicillum notatum, Aspergillus flavus and Mucor specie. Bacilus subtilis* were found to be most predominant bacterial isolates while *Aspergillus niger* was the predominant fungal isolate. The pH values of the analyzed syrups ranged from 2.71-3.94 with the exception of Paracetamol syrups,brands of Vitamin C and Chloroquine phosphate syrups which had pH range of 5.28-7.11, 5.30-5.32 and 4.83-4.88 respectively. The susceptibility patterns of each bacterial isolates to antimicrobial agents showed resistance to Nalixidic acid,Ampicillin,Rocephin,Ampiclox and Amoxacillin, with high sensitivity to Pefloxacin,Ciprofloxacin, Streptomycin and Septri*n.*
KEYWORDS: Microbial, Syrup, Patent, Medicine store.

INTRODUCTION

Syrups are concentrated solutions of sugars formulated as an oral medicament used as therapeutic or prophylactic agents in disease conditions respectively. Syrups are used as sweetening, flavoring and coloring agents in oral pharmaceutical products[1]. Syrups are non-sterile liquid pharmaceutical dosage form, it is particularly popular in pediatric medicine for oral administration of drugs since tablets and capsules cannot be easily and conveniently administered to children. Contamination of these preparations with potentially pathogenic organisms constitutes serious threat to children because their immune system is poorly developed[2]. More dilute syrups are good media for microbial growth and require the addition of preservatives. Industrially formulated syrups often contain ingredients to improve solubility, stability, taste or appearance which also contribute to product preservation. The consumption of contaminated syrups result in serious health hazards through dissemination of pathogenic microorganisms such as *Escherichia coli*, *Staphylococcus aureus* and *Bacillus subtilis*[3] and *Aspergillus flavus* and *Penicillum notatum* among the fungi[4]. The microbiological quality of pharmaceutical products is influenced by the environment in which they are manufactured and by the materials used in their formulation. With the exception of preparations which are terminally sterilized in their final container, the microflora of the final products may represent the contaminants from the raw materials, from the equipments with which it was made, from the atmosphere, from the operator of the process or from the final containers into which it was packed[5].

In this study we wanted to examine some syrup sold in patent medicine stores in Minna metropolis, isolate and identify the microbial contaminants in the syrups examined and carry out susceptibility test on bacterial isolates.

MATERIALS AND METHODS

Collection of samples

Eighteen samples of different brands of syrups comprising of Pracetamol (TPS,MPS,PPS,APS,EPS and LPS), Chloroquine phosphate (PCS,ElCS,DCS,UCS,ECS and MCS) and Vitamin C (LVS,TVS,BVS,AVS,MVS and EVS) syrups were purchased from different patent medicine stores in Minna metropolis.

Determination of pH for syrups

The pH of different brands of syrups comprising of Paracetamol, Chloroquine phosphate and Vitamin C syrups was determined using a pH meter (Jenway 3305 pH meter, Grasmere Green Felsted Dunwow England,).

Enumeration of Microorganisms

The microorganisms were enumerated by methods described by Fawole and Oso[6]and compared with the standard of Microbiological specifications for certification of syrups[7].

Total microbial counts

Nine milliliters of distilled water was dispensed into 6 test tubes for each syrup sample (paracetamol,vitamin C and chloroquine syrups) and sterilized by autoclaving at 121°C for 15minutes. After sterilization and cooling, 1ml of each syrup sample was pipette aseptically into the first test tube labeled 10^{-1} with a sterile syringe and needle and mixed homogenously. 1ml of the diluents from the first test tube was transferred into the second test tube (10^{-2}) and mixed homogenously. The same serial dilution process was repeated for the third, fourth fifth and sixth tubes. One milliliter from the sixth tube was discarded to have equal volume in all the test tubes.

Inoculation by Pour plate method

Aseptically, 1ml of the diluents (10^{-2} and10^{-3}) dilution for each sample was plated into sterile petridishes for each media (Nutrient agar, Mac Conkey agar, Tryptone soy agar and Sabouraud dextrose agar) prepared respectively. The media was poured aseptically at 40-45°C, swirled and allowed to solidify for the enumeration of total, viable, coliform and fungal counts respectively. Nutrient, Mac Conkey and Tryptone soy agar plates were incubated for 24-48 hours in an incubator at 37°C while Sabouraud dextrose agar plates was incubated at 25±2°C for 3-5days in an inoculation hood. Typical colonies of microbial growth on plates were counted at the end of incubation period with the aid of Stuart colony counter[6]

NAFDAC Handbook (2000) states Standard Microbiological specifications for the certification of syrups.Typical viable and fungal counts for bacteria and yeast plate (cfu/ml) respectively must not exceed $1.0x10^{3}$cfu/ml and $1.0x10^{2}$ cfu/ml for bacterial and fungal growth respectively. The syrups (Paracetamol, Chloroquine phosphate and Vitamin C syrups) were assessed with this standard.

Isolation

The bacterial and fungal colonies developed on Nutrient agar, Mac Conkey agar, Tryptone soy agar and Sabraud's dextrose agar plates were subcultured on agar slants as pure culture and stored in the refrigerator at 4°C for further characterization and identification.

Identification of Microorganisms

In order to determine the specific type of microbial contaminants, the bacterial colonies were plated on various selective media such as Salmonella –Shigella agar (*Salmonella* and *Shigella* specie), Eosin methylene blue agar (*Escherichia coli*), Mannitol-salt agar (Staphylococci) and Sabouraud dextrose agar (for mould and yeasts). The colonies grown on these plates were identified on the basis of morphological and cultural characteristics[8].

Characterization of Isolates

The bacterial isolates were characterized according to the method described by Fawole and Oso and Chessbrough[9], in which the following reactions were examined:Gram staining reaction, spore test,motility test, starch hydrolysis test, catalase test,coagulase test,sugar fermentation test,methyl red test and indole test. The fungal isolates were identified based on their morphology and staining using the methods described by Fawole and Oso.

Antibiotic susceptibility test

The bacterial isolates were tested for their susceptibility to range of antibiotics by disc diffusion method as described by Bauer *et al*.[10].

RESULTS

Table 1 shows the range of bacterial counts of the analyzed syrups. The result revealed the contamination of four of six,five of six and four of six, of Vitamin C (AVS,BVS,TVS and LVS), Paracetamol (PPS,MPS,LPS,APS and EPS) and Chloroquine phosphate (PCS,ElCS,DCS and UC) respectively. Table 2 shows the total viable counts, the fungal counts and pH values of contaminated syrup samples. Table 3 shows the susceptibility pattern of isolated bacterial contaminants in the syrups analyzed. The study revealed the contamination of syrups by bacterial organisms: *Escherichia coli, Bacillus subtilis* and *Staphylococcus aureus* and the fungal isolates include: *Aspergillus flavus, A.niger, A.fumigatus, Penicillum notatum* and *Mucor specie.*

DISCUSSION

Two brands of Vitamin C and Chloroquine phosphate syrups (MVS, EVS and ECS, MCS), a brand of Paracetamol syrup (TPS) showed microbial growth within specified limit, NAFDAC Handbook reported that a total amount of 10^{3}cfu/ml and 10^{2} cfu/ml for bacterial and yeast/mould respectively are specification allowed for syrups. The pH range revealed that Chloroquine phosphate and Vitamin C syrups showed more acidic readings (2.82-4.88) and (2.71-5.32) while the pH readings for Paracetamol syrups is less acidic to neutral (5.28-7.11), in this study, the acidity and alkalinity of the syrups determine the effectiveness in administration.

The bacterial isolates identified include *Escherichia coli*, *Staphylococcus aureus* and *Bacillus subtilis*.*Bacillus subtilis*, reported to be the most frequent in syrups, and also found to be the number one contaminant of non-

sterile pharmaceutical in Spain associated with food poisoning[11]. The presence of Escherichia *coli* is good indication of feacal contamination resulting from water supply. Incidence of infant diarrhea associated with *Escherichia coli* (resulting from water supply) has been reported in some parts of Nigeria[12], the presence of *Staphylococcus aureus* in the analyzed syrups is of great importance, this organism secretes toxin which contributes to Gastrointestinal distress[13].

The isolated fungi growth include species of *Aspergillus*, *Pencillium* and *Mucor*, some of the Fungi isolated are possible toxin producers. *Aspergillus specie* causes Aspergillosis while *Aspergillus flavus* produces aflatoxin which is carcinogenic. Water, raw materials and lack of personal hygiene has consistently been shown to be the major sources of contamination of pharmaceuticals[14]. The susceptibility patterns of bacterial isolates showed high sensitivity observed in Ciprofloxacin, Pefloxacin, Streptomycin and Septrin, with resistance observed in Amoxacillin, Tarivid, Ampiclox, Rocephin and Nalixidic acid which may be due to change in permeability target to antibiotic, Production of enzymes capable of destroying antibiotics, condition of growth(acidity and alkaline environments of growth) and composition of medium[15] The microbial contamination can be minimized by practicing good manufacturing practices.

CONCLUSION

The results of this study have shown that some pediatric preparations in Minna are heavily contaminated with microbial agents and therefore can serve as silent and unsuspected sources of infection to infants. Most of the microorganisms isolated can pose threat to the administration of drugs and the effective treatment of common ailments in children.

ACKNOWLEDGEMENT

We are grateful to the technologists of Microbiology Department, Federal University of Technology, Minna for their technical assistance.

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TABLE 1: Total viable bacterial counts of analyzed syrups

*Brands of syrups	colonial morphology *(cfu/ml)		
	MCA	NA	TSA
TPS	$1.0X10^3$	$1.0X10^3$	$1.0X10^3$
MPS	$3.0X10^3$	$2.0X10^3$	$1.0X10^3$
PPS	$3.0X10^3$	$1.0X10^3$	$3.0X10^3$
APS	$2.0X10^3$	$1.0X10^3$	$2.0X10^3$
EPS	$1.0X10^3$	$2.0X10^3$	$2.0X10^3$
LPS	$2.0X10^3$	$2.0X10^3$	$1.0X10^3$
PCS	$4.0X10^3$	$4.0X10^3$	$2.0X10^3$
ElCS	$3.0X10^3$	$2.0X10^3$	$2.0X10^3$
DCS	$2.0X10^3$	$3.0X10^3$	$3.0X10^3$
UCS	$1.0X10^3$	$2.0X10^3$	$3.0X10^3$
ECS	$1.0X10^3$	$1.0X10^3$	NG
MCS	$1.0X10^3$	NG	NG
LVS	$2.0X10^3$	$2.0X10^3$	$3.0X10^3$
TVS	$3.0X10^3$	NG	NG
BVS	$2.0X10^3$	$1.0X10^3$	$2.0X10^3$
AVS	NG	$3.0X10^3$	$3.0X10^3$
MVS	$1.0X10^3$	$1.0X10^3$	$1.0X10^3$
EVS	$1.0X10^3$	$1.0X10^3$	$1.0X10^3$

*Samples coded NG- No growth detected
*Cfu/ml:colony forming units per milliliter. CS-Chloroquine phosphate syrups
VS-Vitamin C syrups PS-Paracetamol syrups
MCA-Mac Conkey agar NA-Nutrient agar TSA-Tryptone soy agar

Table 2: Bacterial counts, fungal counts and pH values of contaminated analyzed syrup samples

Brands of syrups	bacterial counts [a] *(cfu/ml)	fungal counts *(cfu/ml)	pH values
MPS	$3.00X10^1$	$3.0X10^2$	5.28-5.29
PPS	$1.66X10^1$	$2.0X10^2$	6.21-6.23
APS	$1.66X10^1$	$2.0X10^2$	5.81-5.83
EPS	$1.66X10^1$	$2.0X10^2$	5.90-5.92
LPS	$1.66X10^1$	$1.0X10^2$	6.11-6.13
PCS	$3.33X10^1$	$3.0X10^2$	3.31-3.33
ElCS	$2.33X10^1$	$2.0X10^2$	4.82-4.83
DCS	$2.67X10^1$	$2.0X10^2$	4.87-4.88
UCS	$3.00X10^1$	$1.0X10^2$	3.84-3.86
LVS	$2.33X10^1$	$2.0x10^2$	3.62-3.63
TVS	$1.00X10^1$	$3.0X10^2$	3.47-3.48
BVS	$1.66X10^1$	$4.0X10^2$	5.30-5.32
AVS	$2.00X10^1$	$4.0X10^2$	3.35-3.36
EVS	NG	$2.0X10^2$	2.93-2.94

[a] -mean of three counts
*cfu/ml-colony forming units per milliliter.

Table 3: Susceptibility patterns of bacterial isolates in analyzed syrups

Antibiotic	**disc potency**	***E coli***	***Staph* aureus**	***Bacillus substilis***
Pefloxacin (PEF)	10µg	+	+	+
Gentamycin(CN)	10µg	-	+	+
Ampiclox(AMP)	30µg	-	-	-
Zinnacef(Z)	20µg	-	+	+
Amoxacillin(AM)	30µg	-	-	-
Rocephin(R)	30µg	-	-	-
Ciprofloxacin (CPX)	10µg	+	+	+
Streptomycin(S)	30µg	+	+	+
Septrin (SXT)	30µg	+	+	+
Erythromycin (E)	10µg	-	+	-
Ampicillin(PN)	30µg	-	-	-
Tarivid(OFX)	10µg	+	-	-
Ceporex(CEP)	10µg	+	-	-
Augumentin(AU)	30µg	+	-	-
Nalixidic acid(NA)	30µg	-	-	-

+ SENSITIVE, - RESISTANT