ANTIBIOTICS SENSITIVITY PATTERN OF MICROBES ISOLATED FROM BLOOD TONICS

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Abstract: This study was carried out to ascertain the microbial contamination and antimicrobial susceptibility of forty-five (45) brands of blood tonics marketed in Idumota Market, Lagos State. The aim of this study is to evaluate the microbial quality of different brands of blood tonics from various manufacturers marketed in Lagos metropolis and determine the antimicrobial susceptibity pattern of the isolated microbes. The streaking method was used. The result revealed that 14 (31.1%) of the forty-five samples analyzed were contaminated with bacteria. Nine (20%) samples had isolates with gram positive reactions while 5(11.1%) had gram negative isolates. All the isolates were negative for spore formation test and motility tests. The biochemical and antimicrobial susceptibility testing of all the isolates obtained was carried out by the Vitek-2 automated system (Biomérieux, Marcy L'etoile, France) according to recommendations of the manufacturer. Computer kinetic analysis of growth readings was used to determine the mics of the antimicrobial agents included in various Vitek 2 test cards. The results were interpreted in accordance with the current guidelines of the clinical and laboratory standards institute. These findings indicate that some of the blood tonics in the market have bacterial contamination and effort should be made in ensuring production bacterial-free tonic product with a view to preventing the potential health implication of consuming contaminated tonics.

Keywords: Antibiotics, microbial contamination, blood tonics, susceptibility, resistance

Introduction

The environment, raw materials, atmosphere, equipment used in production, the personnel operating the process and or the final container in which they are packaged affects the microbiological quality of the pharmaceutical product. This can be eliminated by sterilization of the preparation in its final container though the mode of storage and usage can reintroduce contaminants. Some contaminants may grow even in the presence of preservatives and spoil the product while some destroyed by in-process heat treatment may still leave residues (cells) that could be toxic or pyrogenic because of the pyrogenic fraction, Lipid A present in the cell wall was not destroyed under same condition as the organism. Contamination of pharmaceutical preparations with microorganisms is a common problem which has been reported for many non-sterile drugs (Mugoyela and Mwambete, 2010). The use of such contaminated pharmaceutical preparations has been proven to be hazardous to the health of the users. There have been reports of drug-borne human infections worldwide (Coker, 2005). The extent of the health hazards varies from product to product and patient to patient, depending on the types and numbers of organisms present, route of administration, and the resistance of the patient to infections (Bloomfield, 2005). The contamination of these pharmaceutical preparations with microorganisms can bring about changes in their physical features such as the thinning of creams, fermentation of syrups, the breaking of emulsions, appearance of turbidity or deposit, changes in odour and colour (Shaik *et al.*, 1988). These changes make the products unacceptable and negatively affect their therapeutic potency and dosage delivery (Gamal *et al.*, 2011).

Pharmaceuticals are mostly contaminated with bacteria, yeasts and filamentous fungi. The incidence of microflora in non-sterile preparations is influenced by the nature of the ingredients used in their formulation and the attitude of the personnel involved in their handling (Ashour and Mansy, 2011). Non-sterile preparations are not required to harbour certain microorganisms such as *Escherichia coli*, *Salmonella* spp., *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Candida albicans* as stipulated by most Pharmacopeia (Stevic *et al.*, 2012). Non-sterile Pharmaceuticals are also required to pass microbial bioburden tests for total aerobic microbial count (Hossian, 2009). The presence of contaminating microflora, especially when exceeding the acceptable limit of $<10^2$ cfu/ml in oral drugs, brings a major threat in public health measures (USPC, 2009).

Iron deficiency is thought to be the most common cause of anaemia globally, but other nutritional deficiencies (including folate, vitamin B12 and vitamin A), acute and chronic inflammation, parasitic infections, and inherited or acquired disorders that affect haemoglobin synthesis, red blood cell production or red blood cell survival, can all cause anaemia (WHO, 2011). Iron deficiency anaemia, which contributes about 80% of all anaemia cases, affects majority of individuals in many developing countries due to their low consumption of foods that are high in iron (Yip and Dallman, 1996). Blood loss caused by heavy menstrual blood flow, intestinal and blood-borne parasite infections and malaria are common causes of anaemia among poor populations (Okoche *et al.*, 2003). The adult recommended daily intake (RDI) for iron is 10 mg/day for men and 15 mg/day for women. However, where dietary sources of iron are not enough for their management of iron deficiency anaemia, medicinal forms of iron such as ferrous sulphate caplets, blood tonics need to be ingested (Ivery and Elmen, 1986).

Blood tonics are liquid preparations that enhance the quality of blood, boost haemoglobin level and increase the number of erythrocytes in the body. Intake of blood tonics leads to increase serum erythropoietin in the body. Blood tonic is essential for the treatment of anaemia (Omiunu, 2015).

The aim of this study was to evaluate the microbial qualities of different brands of Blood tonics marketed in Idumota, Lagos State, Nigeria and to ascertain their antibiotic susceptibility pattern.

Materials and Method

Materials

Different blood tonic samples, Vitek 2 compact (Biomérieux Inc, U.S.A) and DensiChek Plus™ were used for the study. MacConkey agar, Cystine lactose electrolyte deficient (CLED) agar, Sabouraud dextrose agar (SDA), Nutrient agar (NA), Xylose lysine deoxycholate (XLD) agar and Müeller-Hinton agar used were manufactured by Sigma Aldrich® while Brain heart infusion broth was by Deben Diagnostics, UK. The culture media (agar and broth) were prepared according to the manufacturers instructions

Sample collection

Forty-five different brands of blood tonics marketed at Idumota market in Lagos Island, Lagos state, Nigeria were purchased from twenty different pharmacies and coded with numbers 1-45. Each had NAFDAC registration status, manufacturing and expiry dates.

Analysis of samples

Pour plate method was used for the estimation colony-forming bacteria in accordance with (Collin and Lyne, 1976). One millilitre was withdrawn aseptically from each blood tonic sample into the corresponding sterile labelled bottles, which contained of peptone water. The screw caps were tightly covered and shaken well to ensure complete dissolution of the drug samples. One millilitre from each bottle was transferred aseptically into triplicate agar medium plates and broths, and kept in an incubator set at 37ºC for 24 h. The SDA plates were incubated at 27ºC for 3 days. Bacterial colonies were counted and the number of colony forming units (cfu) per ml of each plate was calculated (Conn, 1975).

Identification of isolated microorganisms

The blood tonic sample were plated on various selective media such as MacConkey agar, SDA, CLED agar and XLD agar and then incubated at appropriate temperatures. The plates were observed after 24 h and the isolates were characterized based on their morphological appearance and Gram stain reaction. They were then sub-cultured for 24 h at 37ºC for distinct colony growth and preserved on Muller-Hinton agar slant at 4ºC prior to biochemical and antimicrobial sensitivity tests. The method used for the isolation of microorganisms was as described by Adeola *et al.* [2012).

Identification of microbial isolate using Vitek 2 compact system

The Vitek-2 is a fully automated system for rapid and accurate phenotypic identifications for most clinical microbes as well as for susceptibility testing. A sterile applicator stick was used to transfer a sufficient number of colonies of the pure culture of each isolate into a 12 x 75 mm clear plastic test tube containing 3.0 ml of sterile saline to make a suspension of each isolate. The turbidity of each suspension was adjusted to 0.5 Macfarland turbidity range using a turbidity meter called the Densichek.

The system had four reagent cards used for the identification of different microorganisms GN - Gram-negative fermenting and non-fermenting bacilli, GP - Gram-positive cocci and non-spore-forming bacilli, YST - yeasts and yeast-like organisms and BCL - Gram-positive spore-forming bacilli. Each reagent card had 64 wells that contained an individual test substrate which measured various metabolic activities such as acidification, alkalinization, enzyme hydrolysis, and growth in the presence of inhibitory substances. An optically clear film present on both sides of the card allowed for the appropriate level of oxygen transmission while maintaining a sealed vessel that prevented contact with the organism-substrate admixtures. Each card had a pre-inserted transfer tube used for inoculation. Identification cards are inoculated with microorganism suspensions using an integrated vacuum apparatus. A test tube containing the microorganism suspension was placed into a special rack (cassette) and the identification card was placed in the neighbouring slot while inserting the transfer tube into the corresponding suspension tube. The filled cassette was placed either manually into a vacuum chamber station. After the vacuum was applied and air was re-introduced into the station, the organism suspension was forced through the transfer tube into micro-channels that filled all the test wells. Inoculated cards were passed by a mechanism, which cut off the transfer tube and sealed the card prior to loading into the carousel incubator. All the cards were incubated on-line at 35.5 ± 1.0ºC. Each card was removed from the carousel incubator once every 15 min, transported to the optical system for reaction readings, and then returned to the incubator until the next read time. Data were collected at 15-minute intervals during the entire incubation period. A transmittance optical system allowed interpretation of test reactions using different wavelengths in the visible spectrum. During incubation, each test reaction was read every 15 min to measure either turbidity or coloured products of substrate metabolism. In addition, a special algorithm was used to eliminate false readings due to small bubbles that may be present. The results were interpreted by the ID- database after the incubation period of 3 h. All used cards were automatically discarded in a waste container.

Antimicrobial susceptibility test (AST) using Vitek® 2 compact system

The VITEK® 2 Antimicrobial Susceptibility Test card is a miniaturized, abbreviated and automated version of the doubling dilution technique for determining the minimum inhibitory concentration (MIC). Each VITEK® 2 test card contains 64 microwells. A control well which contains only culture medium is included on all cards, while the remaining wells contains premeasured amounts of a specific antimicrobial agent in a culture medium base. A suspension of organism from a pure culture was prepared in a tube containing 0.5% sterile saline and standardized to a McFarland 0.5 using the DensiCHEK Plus™.

The card was automatically filled, sealed and placed into the incubator/reader by the VITEK 2 System. The growth of each well is monitored by the VITEK 2 Systems in the card for about 18 h. At the end of the incubation cycle, a report is generated that contained the MIC value along with the interpretive category result for each antimicrobial that was contained on the card.

Results and Discussion

Out of the 45 blood tonics examined, 14 (31.1%) were found to be contaminated with bacteria, of which 9 (20%) samples had isolates with Gram positive reactions while 5 (11.1%) had Gram negative isolates. All the isolates were negative for spore formation test and motility tests. The bacterial isolates were classified based on their Gram reaction, spore formation and motility characteristics. Gram's reaction, spore formation and motility tests of the isolates from the blood tonics used for this study are listed in Table 1.

Table 1: Gram reaction, spore formation and motility tests of the isolates

S/N	Grams reaction	Spore formation	Motility test
1	positive	negative	negative
5	positive	negative	negative
7	negative	negative	negative
8	positive	negative	negative
10	negative	negative	negative
13	positive	negative	negative
15	positive	negative	negative
16	negative	negative	negative
17	negative	negative	negative
21	positive	negative	negative
22	negative	negative	negative
23	positive	negative	negative
26	positive	negative	negative
42	positive	negative	negative

The biochemical identification of the isolates was carried out using Vitek 2 Compact system (BioMerieux USA). The Gram-positive isolates identified (*Staphylococcus hominis, Staphylococcus sciuri, Staphylococcus lugdunensis, Leuconostoc mesenteroides subsp. cremoris, Staphylococcus*

pseudintermedius, Enterococcus faecalis, Staphylococcus equorum and *Leuconostoc mesenteroides subsp. Dextranicum) ar*e shown in Tables 2a and 2b. All the isolates tested negative for D-amygdain, Ala-phe-proarylamidase, Novobiocin resistance, L-proline arylamidase, Tyrosine arylamidase, L-aspartate arylamidase, Beta glucoroidase, Urease, Alpha-mannosidase, D-galactose, Pullulan and Arginine dihydrolase 2.

Table 2a: Results of the Vitek biochemical identification test for Gram positive bacteria

S/N	ISOLATE	Biochemical parameters used in identification of the Isolates																						
		A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W
1	*S. hominis*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+
5	*S. sciuri*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	+	-
8	*S. lugdunensis*	-	-	-	-	+	-	-	+		-	-	-	+	-	+	-	-	-	-	-	-	+	+
13	*L. mesenteroides cremoris*	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
15	*S. pseudintermedius*	-	-	+	+	+	-	-	+	-	-	-	-	+	+	+	-	-	-	-	-	-	-	+
21	*E. feacalis*	-	-	-	+	-	-	-	-	+	+	-	-	-	-	+	-	-	-	+	-	-	-	-
23	*L. mesenteroides dextranicum*	-	-	+	-	+	-	+	+	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-
26	*S. equorum*	-	-	-	-	-	-	-	+	-	-	-	-	-	+	+	-	-	-	-	+	+	-	-
42	*L. mesenteroides dextranicum*	-	-	+	-	+	-	+	+	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-

Table 2b : Results of the Vitek biochemical identification test for Gram positive bacteria

S/N	ISOLATE	Biochemical parameters used in identification of the Isolates																		
		X	Y	Z	A1	B1	C1	D1	E1	F1	G1	H1	I1	J1	K1	L1	M1	N1	O1	P1	Q1
1	*S. hominis*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	*S. sciuri*	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
8	*S. lugdunensis*	-	-	-	+	+	+	-	-	+	+	+	-	+	-	-	-	-	+	-	-
13	*L. mesenteroides cremoris*	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
15	*S. pseudintermedius*	-	-	-	+	+	-	-	-	-	+	+	-	+	-	-	-	-	+	-	-
21	*E. feacalis*	-	+	-	-	-	-	-	-	-	-	-	+	-	+	+	-	-	-	-	-
23	*L. mesenteroides dextranicum*	-	-	-	+	+	+	-	-	-	+	+	-	+	-	-	-	-	+	-	-
26	*S. equorum*	-	-	-	-	+	-	-	-	+	-	-	-	-	-	-	+	-	-	-	-
42	*L. mesenteroides dextranicum*	-	-	-	+	+	+	-	-	-	+	+	-	+	-	-	-	-	+	-	-

Key for 2a and 2b:

A= D-amygdain; B=Ala-phe-pro-arylamidase; C=Leucine arylamidase; D=Alanine arylamidase; E=D-Ribose; F=Novobiocin resistance; G=D-raffinose; H=Optochin resistance; I= *phosphatidylinositol* phosphates; J=Cyclodextrin; K=L-proline arylamidase; L=Tyrosine arylamidase; M=L-lactate alkalinisation; N= Growth in 65%NaCl; O= O/129resistance (comp. vibrio); P=D- xylose; Q=L-aspartate arylamidase; R=Beta glucoronidase; S=D-Sorbitol; T= Lactose ;U=D- mannitol; V= Salicin; W=arginine dihydrolase; X=Beta galactopyranosidase; Y=Alpha- galactosidase; Z=Urease; A1=N-acetyl-D-glucosamine; B1=D-mannose; C1=Saccharose/ sucrose; D1=Beta galactosidase; E1=Alpha-mannosidase; F1=L-*pyrrolidonyl arylamidase;* G1=Polymixin B-resistance; H1=D-maltose; I1=Methyl-B-D-glucopyranoside ;J1=D-trehalose; K1=Alpha-glucosidase; L1= Alkaline phosphatase; M1=Beta-glucuronidase; N1= D-galactose; O1= Bacitracin resistance; P1= Pullulan; Q1=Arginine dihydrolase 2

The Gram negative bacteria (*Myroids spp., Chryseobacterium indologenes* and *Acinetobacter baumannii*) identified are shown in Tables 3a and 3b. The blood tonic samples with sample number 16, 17 and 22 had the same type of contaminant *Acinetobacter baumanni.*

Table 3a : Results of the Vitek biochemical identification test for Gram negative bacteria

S/N	Isolate	Biochemical parameters used in identification of the Isolates																						
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
7	*Myroids* spp.	+	-	-	-	-	-	-	-	-	-	-	+	-	+	-	+	+	+	-	-	-	-	-	-
10	*Chryseobacterium indologenes*	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
16	*Acinetobacter baumannii*	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-	+	-	-	-	+	-	-
17	*Acinetobacter baumannii*	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-	+	-	-	-	+	-	-
22	*Acinetobacter baumannii*	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-	+	-	-	-	+	-	-

Table 3b : Showing the results of the Vitek biochemical identification test for Gram negative bacteria

S/N	Isolate	Biochemical parameters used in identification of the Isolates																					
		25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47
7	*Myroids spp.*	-	-	-	+	-	-	-	-	-	+	-	-	-	-	+	-	-	-	-	-	-	+	-
10	*Chryseobacterium indologenes*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
16	*Acinetobacter baumannii*	-	+	+	+	+	-	-	-	+	-	-	-	+	-	+	-	-	-	-	-	-	-	-
17	*Acinetobacter baumannii*	-	+	+	+	+	-	-	-	+	-	-	-	+	-	+	-	-	-	-	-	-	-	-
22	*Acinetobacter baumannii*	-	+	+	+	+	-	-	-	+	-	-	-	+	-	+	-	-	-	-	-	-	-	-

Key for tables 3a and 3b

1=Ala-phe-pro-arylamidase	2=H_2S production	3=Beta-glucosidase	4=L-proline arylamidase
5=Saccharose/ sucrose	6=L-lactate alkalinisation	7=Glycine arylamidase	8=O/129 resistance
9=Adonitol	10=B-N- acetylglcosamidase	11=D-maltose	12=Lipase
13=D-tagatose	14=41-alpha glucosidase	15=Ornithine decarboxylase	16=Glu-gly-arg- arylamidase
17=Pyrrolyclonyl-arylamidase	18=Glutamyl arylamidase pNA	19=D- mannitol	20= Palatinnose
21=D-trehalose	22=Succinate alkalinisation	23=Lysine decarboxylase	24=L-malate assimilation
25= L-arabitol	26=D-glucose	27=D-mannose	28=Tyrosine arylamidase
29= Sodium Citrate	30=B-N-acetylgalatoseaminidase	31=L-histidine assimilation	32= Ellman
33=D-cellobiose	34=	35=Beta-xylosidase	36=Urease
37= Malonate	38=Alpha-galactosidase	39= Coumarate	40=L-lactate assimilation
41=Beta-galactosidase	42=Glucose Fermentation	43=Beta-alanine	44= D-sorbitol
45=5-keto-D-gluconate-5KG	46= Phosphatase	47= Beta-glucoronidase	

Antibiotic susceptibility profile of the isolates was carried out using the VITEK®2 compact Antimicrobial Susceptibility Test systems. The Gram positive and Gram negative cards were used accordingly for the different isolates.
The antibiotic susceptibility profile of Gram positive isolates is shown in Tables 4a and 4b. *E. faecalis* was not resistant to any of the antibiotics used while *S. sciuri* and *S. lugdunensis* were resistant to Clindamycin and Trimethropin/Sulfamethoxazole respectively. The organisms (*L. Mesenteroides dextranicum*) isolated from samples 23 and 42 though the same type of organism demonstrated different antibiotic susceptibility patterns.

Table 4a: Antibiotic profile of the Gram positive isolates

S/N	CEF		BEN		OXA		GEH		GEN		CIP		LEV		ICR		ERY		CLIN	
	M	I	M	I	M	I	M	I	M	I	M	I	M	I	M	I	M	I	M	I
1	+	Ø	>0.5	Ø	>4	Ø	-	-	>0.5	S	>0.5	S	0.5	S	-	-	0.5	S	>4	R
5	+	Ø	>0.25	Ø	>4	Ø	-	-	>0.5	S	>0.5	S	1	S	-	-	0.5	S	>4	R
8	-	Ø	>0.5	Ø	>4	Ø	-	-	>0.5	S	>0.5	S	>0.2	S	-	-	>0.25	S	0.5	S
13	+	Ø	>0.5	Ø	>4	Ø	-	-	>0.5	S	>0.5	S	>0.2	S	-	-	>0.25	S	>4	R
15	+	Ø	>0.5	Ø	>4	Ø	-	-	1	S	>0.5	S	>0.2	S	-	-	>8	R	>4	R
21	-	Ø	1	Ø	>4		-	--	-	-	2	1	2	S	--	-	0.5	S	-	-
23	+	Ø	>0.5	Ø	>4	Ø	-	-	>0.5	S	>0.5	S	>0.2	S	-	-	>0.25	S	0.5	R
26	+	Ø	>0.5	Ø	>4	Ø	-	-	>0.5	S	>0.5	1	4	1	-	-	>8	R	>4	R
42	-	Ø	>0.5	Ø	1	Ø	-	--	>0.5	S	>0.5	S	>0.2	S	-	-	>8	R	>4	R

Table 4b: Antibiotic profile of the Gram positive isolates

S/N	LIN		DAPT		TEIC		VAN		TET		TIG		NIT		RIF		TRI	
	M	I	M	I	M	I	M	I	M	I	M	I	M	I	M	I	M	I
1	>8	-	4	-	>32	R	>32	R	>16	R	>2	-	>16	S	>4	R	>320	R
5	2	S	>0.12	S	2	S	>0.5	S	>1	S	>0.12	S	>16	S	>0.03	S	>10	S
8	1	S	>8	-	>0.5	S	>0.5	S	>1	S	>0.12	S	>16	S	0.25	S	80	R
13	>4	R	>8	-	>0.5	S	>0.5	S	>1	S	>16	S	64	I	>4	R	80	R
15	2	S	>8	-	>32	R	>32	R	4	S	>16	S	>16	S	0.25	S	160	R
21	2	S	2	S	>0.5	S	2	S	>1	S	>0.12	S	>16	S	-	-	-	-
23	1	S	>8	-	>0.5	S	>0.5	S	>1	S	0.25	S	>16	S	1	S	80	R
26	>8	-	2	-	16	I	>32	R	>1	S	0.5	S	>16	S	>4	R	>4	S
42	2	S	>8	S	8	S	>0.5	I	>1	S	>0.12	S	>16	S	>0.3	S	>10	S

Key for 4a and 4b:

CEF=Cefoxitin
BEN=Benzylpenicillin
OXA=Oxacillin
GEH=Gentamicine High level (synergy)
GEN=Gentamicine
CIP=Ciprofloxacin
LEV=Levofloxacin
ICR=Inducible Clindamycin Resistance
ERY=Erythromycin
CLI=Clindamycin
LIN=Linezolid
DAPT=Daptomycin
TEICO=Teicoplanin
VAN=Vancomycin
TET=Tetracycline
TRI/SUL=Trimethropin/Sulfamethoxazole
NIT=Nitrofurantoin
RIF=Rifampicin
TIG=Tigecycline

Table 5a: Antibiotic profile of the Gram negative isolates

S/N	% prob	AMP		AMO		PIP		CEF		CEF-A		CEFT		CEFO		CEFE		ERTA		IMIP		MERO	
		M	I	M	I	M	I	M	I	M	I	M	I	M	I	M	I	M	I	M	I	M	I
7	96	>2	S	>2	S	>4	S	4	S	4	S	16	I	-	-	-	-	-	-	>0.25	S	1	S
10	94	>32	R	>32	R	32	I	>64	R	>64	R	>64	R	-	-	-	-	-	-	>0.25	S	2	S
16	99	>32	R	4	R	>4	S	32	R	32	R	16	I	-	-	-	-	-	-	>0.25	S	>0.25	S
17	99	>32	R	4	R	16	S	>64	R	>64	R	32	I	-	-	-	-	-	-	>0.25	S	0.5	S
22	99	>32	R	4	R	>4	S	32	R	32	R	16	I	-	-	-	-	-	-	>0.25	S	>0.25	S

Table 5b: Antibiotic profile of the Gram negative isolates

S/N	% prob	AMIK			GEN			NAL			CIP			TIG			NIT			COL			TRIM		
		M	I		M	I		M	I		M	I		M	I		M	I		M	I		M	I	
7	96	>64	R		>16	R		>32	R		0.5	S		>0.5	S		>16	S		-	-		160	R	
10	94	>64	R		>16	R		16	S		>4	R		4	I		>512	R		-	-		>320	R	
16	99	-	-		>16	R		8	S		>0.25	S		>0.5	S		256	R		-	-		>320	R	
17	99	-	-		>1	S		4	S		>0.25	S		>0.5	S		>512	R		-	-		>20	S	
22	99	-	-		>16	R		8	S		0.5	S		>0.5	S		>512	R		-	-		>320	R	

Key for Tables 5a and 5b:

Ampicillin = AMP; Meropenem= MERO; Piperacillin/Tazobactam= PIP/TAZ; Cefuroxime = CEF; Ciprofloxacin= CIP;Ceftriaxone= CEFT; Cefoperazone/Sulbactam= CEFO/SUL; Cefepime= CEFE; Ertapenem= ERTA; Imipenem= IMI; Amoxicillin/Clavulanic Acid= AMOX; Amikacin= AMIK; Gentamicin= GEN; Nalidixic Acid= NAL; Cefuroxime Axetil = CEF A; Tigecycline= TIG; Nitrofurantoin= NIT; Colistin= COL; Trimthoprim/Sulfamethoxazole= TRI/SUL

The antibiotic susceptibility profile of Gram negative isolates is shown in Tables 5a and 5b. The organisms isolated from sample 7 (*Myroids spp.*) and 10 (*Chryseobacterium indologenes*) were resistant to Nalidixic Acid and Ceftriaxone, respectively. The same type of organism (*Acinetobacter baumannii*) was isolated from samples 16, 17 and 22 but they showed diverse antibiotic susceptibility patterns. *Chryseobacterium indologenes* showed the highest number of resistance to the antibiotics used.

The issue of microbial contamination of essential ingestible products such as blood tonics is a serious concern since the primary aim of blood tonics is to replenish and nourish the body and help to build up more blood in the body. The discovery that most times people taking some different kinds of drugs had come down with some form of bacterial infections that most of the times are resistant to antibiotics and the fact that some bacteria toxins had been found to alter or damage cellular membranes and causes some genetic modification in body prompted this research work.

The presence of microorganisms in contamination of blood tonics can occur at any stage of the production, processing, marketing and administration. Ingestion of contaminated blood tonics is harmful to health. Consumption of contaminated product can cause ailments and or diseases thereby compromising the health status of the individual leading to cellular damage or even death. From this study, some of the blood tonics examined were indeed contaminated with bacteria; this is in line with the work of Tukur *et al*., (2012) who also isolated some bacteria from multivitamin drugs. Although most of the microbial isolates are opportunistic bacteria, their ingestion especially in high quantity by immune compromised individuals could lead to adverse health effect. Survival and growth of these microbes can deteriorate the quality of the product.

The presence of potentially pathogenic bacteria, like *Acinetobacter baumanii* and *Enterococcus faecalis* is not desirable. This calls for more stringent measures to prevent the possible detrimental effects. The presence of these microbes suggests the route of contamination to be possibly through water, personnel and environmental factors. *A. baumannii* is a water organism that preferentially colonizes aquatic environments while *Enterococcus faecalis* is the dominant aerobic gram-positive coccus that occurs in high quantities in faeces.

Distribution of staphylococcus species to other isolated microbes were significant (8.9%). Though high sugar concentration prevents the growth of microorganisms, staphylococcus species thrive well in fairly high concentration of sugar (Frazier and Westhoff, 1994). The high resistance to antimicrobials of *Staphylococcus* spp. may contribute to their survival in processed products (Oghule *et al.*, 2009).

Myroides species and *Chryseobacterium* species are uncommon clinical isolates, though more frequently reported to cause infections than other pigmented non-fermentors (Deepa *et al*., 2014) soil and water. Environmental studies have revealed that these organisms can survive in chlorine-treated municipal water supplies, often colonizing sink basins and taps and creating potential reservoirs for infections inside hospital environments (Hoque *et al.*, 2001). *Chryseobacterium indologenes* is the only isolated organism that was resistant to Ceftriaxone and it also demonstrated very high resistance to most of the antibiotics used.

The susceptibility patterns of the bacterial isolates showed high sensitivity to Ciprofloxacin, Levofloxacin, Gentamicin, and Tetracycline for the Gram positive isolates and Piperacillin, Imipenem, Nalidixic acid and Tigecycline for the Gram negative isolates. The high resistance observed in Erythromycin, Clindamycin, Rifampicin, Trimethroprim and Vancomycin could be due to the prevailing environmental conditions e.g. pH, viscosity, and composition of the drug.

The result of this study has shown that some blood tonics are contaminated with microbial agent and therefore can serve as silent and unsuspected source of infection on patients. It is therefore suggested that Good Manufacturing and Packaging Practice, proper treatment of water and air, personal hygiene improvement of the production personnel and pre-treatments of natural raw materials be enforced and maintained. Also, proper handling and storage of these products be carried out to eliminate or reduce microbial factors to ensure reduction in the level of microbial contamination. The microbial contamination can be minimized by ensuring that all blood tonics undergo standard quality control process before and after production.

Conflict of Interest

Authors declare no conflict of interests in the conduct and reporting of this study.

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