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Original article

Multiple antibiotic resistance (MAR) index of some bacteria isolated from "JIKO" a herbal drink sold in some parts of Kaduna metropolis

Kereakede, E[1*]., Egbe, N. E[1]., Oaikhena, E. E[1]., Danjuma, A[3]., Onuh, K[1]., Hassan, A. U[1]., Ozojiofor, U. O[1]., Oyong, A. I[1]. and Omenesa, R. L [2].

1. Department of Biotechnology, Nigerian Defence Academy, Kaduna, Nigeria.
2. Department of Biological sciences, Nigerian Defence Academy, Kaduna, Nigeria.
3.Department of Basic Science and General Studies, Federal College of Forestry Mechanization, Afaka. Kaduna, Nigeria

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ABSTRACT

The presence of antibiotics resistance bacteria associated with herbal drinks commonly consumed has been a major public health concern. This study aims at determining Multiple Antibiotic Resistance (MAR) Index from "JIKO" a herbal drink consumed in some parts of Kaduna metropolis. Twenty-six bacteria were isolated from JIKO obtained from Kakuri and Ungwan-Tanko using spread plate method. The isolated bacteria were screened against fifteen (15) antibiotics belonging to five different classes, namely, quinolones (ciprofloxacin, perfloxacin, sparfloxacin, ofloxacin), penicillin (ampiclox, amoxicillin, augmentin), cephalosporin (**c**eftazidine, cefuroxime), aminoglycosides (streptomycin, gemtamycin, septrin), microlides and others (chloramphenicol, erythromycin, nalidixic acid). The antibiotic susceptibility testing of the bacteria revealed that 19 out of the 26 bacteria recorded varying degrees of resistance. The MARI values ranged between 0.1-0.9. The results identified some MAR bacteria of public health concern.

Keywords: Multiple antibiotics resistant index, susceptibility, herbal, jiko, health

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Corresponding author's email: ekereakede@nda.edu.ng

INTRODUCTION

Resistant to antibiotics and it's consequence of antibiotic treatment in persons is soaring, leading to serious public health issues of worldwide significance and having an immediate effect in combating infections caused by bacterial (1). Antibiotics intake is a great influence in development, discretion and spread antimicrobial resistant microbes in both animal and human therapeutics (2). This has impelled humans in taking costly second or third-generation antibiotics, persistent hospital admission which then results to expensive medical/or hospital cost, escalated death rate and birth rate and severe global economic meltdown (3,4).

Over dose of antibiotics in persons and animals for treatment has steered up antibiotics resistant strains dissemination into the environment (5,6). In Nigeria, various kinds of research have shown sudden rise in the occurrence of gastrointestinal bacterial resistance for the past fifteen years (7). Resistance bacterial Infections/or diseases are widespread and some of the pathogens causing these infections have developed resistance to different types of antibiotics used against them.

Antibiotic resistance can originate from gene mutations or by horizontal transfer of resistance markers between phylogenetically diverse bacteria (8,9). Bacteria that showed resistant to antibiotic incur their antibiotic desensitized conflict genes. For instance, these genes could code for "efflux" pumps that expel antibiotics from cells, these genes probably give way to enzymes that reduce antibiotics standard, or that synthetically modify and stimulate the antibiotics.

Multiple antibiotic resistance (MAR) index is designed to be a suitable means to evaluate danger/hazard. It evaluates the overall number of bacteria showing antibiotic resistance and the subsequent danger zone in constant susceptibility screening (10). Thenmozhi *et al*., (11) stated that MARI values higher than 0.2 indicates high risk sources of contamination with often use of antibiotics while values of less than or equal to 0.2 indicates sources from where antibiotics are seldomly used. Hence, this study was carried out to determining MARI from "JIKO" a herbal drink consumed in some parts of Kaduna metropolis by subjecting the isolates to antibiotic study.

MATERIALS AND METHODS

A total of 30 samples of JIKO was collected from both sites (Kakuri and Ungwan-Tanko market) into sterile bottles and transported to the laboratory of Biological Sciences Department, Nigerian Defence Academy in an ice-box container for bacterial isolation**.** The bacterial isolates were identified and characterized using conventional methods and the isolates were confirmed using 16S rRNA gene.

Antibiotic susceptibility testing

The bacterial isolates were subjected to antibiotic susceptibility screening using the method initiated by Bauer *et al.,*(12) after standardizing the bacterial isolates to 0.5 McFarland standards. The Antibiotic susceptibility testing of the isolates was carried out using Kirby – Bauer disk diffusion method and was evenly spread on the entire surface of Muller-Hinton agar and then allowed to dry for 5 minutes.

Antibiotics disc was now placed on the surface of the agar and it was incubated at 37°C for 24 hours. Any growth with less than 12 mm in diameter zone around the disc was considered indicative of drug resistance to the bacterial growth according to the guidelines of the Clinical and Laboratory Standard Institute (13). The following discs of antibiotics were used ciprofloxacin 10 μg, perfloxacin 10 μg, Sparfloxacin 10 μg, ofloxacin 10 μg, ampiclox 30 μg, augmentin 25 μg, streptomycin 30 μg, septrin 30 μg, gentamycin 10 μg, erythromycin 10 μg, amoxicillin 30 μg, chloranphenicol 10 μg, cefuroxime, ceftazidine 10 μg, nalidixic acid 30 μg.

Identification of Multdrug Resistance (MDR) bacteria

The number of antibiotic each bacterium was resistant to in the disc diffusion test was recorded as multidrug resistance bacteria. Multidrug resistance (MDR) was recorded as resistant to two or more antibiotics tested

Determination of Multiple Antibiotic Resistance (MAR) Index

Multiple antibiotic resistance (MARI) index was determined as a/b, “a” is the number of antibiotics to which the particular isolate was resistant and “b” is the total number of antibiotics to which the isolate was exposed (14).

RESULTS

Antibiotic susceptibility testing of the bacterial isolates from both sites (n=26)

Bacterial isolates were classified as being susceptible, intermediate resistant and resistant to the antibiotics depending on the inhibition zone diameters when compared to the guidelines of the Clinical and Laboratory Standard Institute. Table 1 and Table 2 reveals the Antibiotics susceptibility testing of gram negative and gram positive bacteria isolates. 19 amongs the 26 bacterial isolates that were isolated from the drink, 5 bacteria from Kakuri and 14 from Ungwan-Tanko reveals varying degrees of resistance to the antibiotics they were tested against as depicted below:

Table 1 Antibiotic susceptibility testing of bacteria isolates from Kakuri (n=11)

Bacterial isolates	ANTIBIOTICS														
	CPX	PEF	SP	OFX	APX	AM	AU	CFU	CEF	S	CN	SXT	CH	E	RD
B. cereus 1	I	I	R	I	R	I	I	S	S	I	I	R	S	S	I
B. cereus 1 a	S	S	S	S	S	R	S	I	I	S	S	S	S	S	S
B. cereus 1b	R	S	S	S	S	S	I	R	I	S	S	I	R	S	I
B. cereus 02	R	S	S	S	S	S	S	S	S	S	S	R	R	S	R
B. cereus 03	R	R	R	S	S	R	S	R	R	R	R	R	R	R	S
B. cereus 04	S	S	S	S	R	S	R	S	S	S	I	I	S	S	S
B. cereus 04a	S	S	S	S	I	S	I	S	I	S	S	S	S	S	S
B. cereus 04b	R	S	S	S	I	I	S	S	S	S	S	S	S	S	S
B. cereus 04c	S	S	R	I	S	S	S	S	S	S	S	S	S	S	S
B. cereus 04c2	S	S	S	I	R	S	S	S	S	S	S	S	S	I	S

*B. cereus*04c3	S	I	I	S	I	R	S	S	S	S	S	S	S	S	S

KEY: KT: Kakuri, CPX (Ciprofloxacin), PEF (Perfloxacin), SP (Sparfloxacin), OFX (Ofloxacin), APX (Ampiclox), AM (Amoxicillin), AU (Augmentin), CEF (Ceftazidine), CFU (Cefuroxime), S (Streptomycin), CN (Gemtamycin), SXT (Septrin), CH (Chloramphenicol), E (Erythromycin), RD (Nalidixic acid), S (Sensitive), R (Resistance), I (Intermediate).

Table 2 Antibiotic susceptibility testing of bacteria isolates from Unguwan -Tanko (n=15)

	ANTIBIOTICS														
Bacterial isolates	**CPX**	**PEF**	**SP**	**OFX**	**APX**	**AM**	**AU**	**CFU**	**CEF**	**S**	**CN**	**SXT**	**CH**	**E**	**RD**
B. cereus 01d	R	S	S	S	I	S	I	S	S	S	S	S	S	S	S
S. plymuthica	R	I	S	R	R	R	R	R	S	S	R	S	R	S	S
*B. cereus*2a	S	I	R	I	R	I	S	S	S	R	I	S	S	I	S
B. cereus 2b	R	R	R	R	R	R	R	R	I	R	R	R	R	R	R
B. cereus 2c	R	I	I	I	R	I	S	R	S	S	S	S	S	S	S
B. cereus 2d	R	R	S	I	R	S	S	S	S	S	S	S	S	S	S
B. cereus2e	R	S	R	I	R	S	S	S	S	S	S	S	S	I	S
B. cereus 2f	S	I	R	S	I	S	S	S	S	I	S	S	R	S	S
B. cereus 2g	R	I	R	I	S	I	S	S	S	R	S	S	S	S	S
B. cereus 2h	R	R	S	S	I	S	I	I	S	S	S	S	S	S	S
B. cereus 2i	R	S	S	S	R	I	S	S	S	S	S	S	S	S	S
B. cereus 3a	S	R	S	I	S	S	S	S	S	S	I	S	S	R	S
B. cereus 3c	S	R	S	I	R	S	S	S	S	I	S	S	S	R	S
B. cereus 3e	R	S	S	I	I	S	R	S	S	I	S	S	S	S	S
B. cereus 3g	S	S	S	I	R	S	S	S	S	R	S	S	S	I	S

KEY: UT: Uguwan-Tanko, CPX (Ciprofloxacin), PEF (Perfloxacin), SP (Sparfloxacin), OF(Ofloxacin), LEV (Levofloxacin), APX (Ampiclox), AM (Amoxicillin), AU (Augmentin), CEF (Ceftazidine), CFU (Cefuroxime), S (Streptomycin), CN (Gemtamycin), SXT (Septrin), CH(Chloramphenicol), E (Erythromycin), RD (Nalidixic acid), S (Sensitive), R (Resistance), I (Intermediate)

Multiple antibiotics resistant patterns of bacteria isolates from both sites (n=26)

Multiple antibiotics resistant patterns of the bacterial isolates obtained from JIKO from the two sampling sites and their percentage of resistance. The percentage of resistance of the bacterial isolates from Kakuri JIKO drink showed 1(13.3%), 2(20%), 1(26.7%) and 1(73.3%) values while the percentage of resistance of the bacterial isolates from Ungwan-Tanko JIKO drink showed 5(13.3%), 6(20%), 1(53.3%) and 1(93.3%) values as depicted in Table 3

Table 3: Multiple antibiotics resistant patterns of bacteria isolates and their percentage of resistance from both sites (n=5 and n=14)

Bacterial isolates	ANTIBIOTICS								
	2	3	4	5	6	7	8	9	10≥
KK (n=5)	1(13.3%)	2(20%)	1(26%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	1(73.3%)
UT (n=14)	5(13.3%)	6(20%)	0(0%)	0(0%)	0(0%)	0(0%)	1(53.3%)	0(0%)	1(93.3%)

KEY: 5 isolates from Kakuri and 14 from Ungwan-Tanko were tested to be Multiple antibiotic resistant. n : no of isolates, K: Kakuri, UT: Ungwan-Tanko

Multiple Antibiotic Resistant (MAR) Index from both sites

The bacterial isolates from Kakuri showed MARI values ranged between 0.1-0.7 while Ungwan-Tanko JIKO drink was 0.1-0.9. The MARI values 0.3-0.9 indicates existence from high risk contaminated sources with indiscriminate use of antibiotics while MARI values of less than or equal to 0.2 reflects bacteria from sources with less antibiotics usage

Table 4: Multiple Antibiotic Resistant (MAR) Index of bacterial isolates from Kakuri (n=5)

Bacteria isolates	No of antibiotic resistant	No of antibiotic exposed	MARI
*B. cereus*1	3	15	0.2
*B. cereus*1b	3	15	0.2
*B. cereus*02	4	15	0.3
*B. cereus*03	11	15	0.7
*B. cereus*04	2	15	0.1

Key: KK: Kakuri, MARI: Multiple Antibiotic Resistant (MAR) Index.

Table

5: Multiple Antibiotic Resistant Index (MAR) Index of bacteria isolates from Unguwan-Tanko (n=14)

Bacterial isolates	NO of antibiotic resistant	NO of antibiotic exposed	MARI
S. plymuthica	8	15	0.5
*B. cereus*2a	3	15	0.2
*B. cereus*2b	14	15	0.9
*B. cereus*2c	3	15	0.2
*B. cereus*2d	3	15	0.2
*B. cereus*2e	3	15	0.2
*B. cereus*2f	2	15	0.1
*B. cereus*2g	3	15	0.2
*B. cereus*2h	2	15	0.1
*B. cereus*2i	2	15	0.1
*B. cereus*3a	2	15	0.1
*B. cereus*3c	3	15	0.2
*B. cereus*3e	2	15	0.1
*B. cereus*3g	2	15	0.1

Key: UT: Ungwan-Tanko, MARI: Multiple Antibiotic Resistant (MAR) Index.

DISCUSSION

Antibiotics are powerful drugs that are generally harmless and very helpful in combating disease, but when abused antibiotics can actually cause harm. The pathogenic bacteria isolated from these herbal drinks showed wide resistance among the isolates. The high level of *Bacillus cereus* resistance to tested antibiotic is in conformity with the report of (15). *Bacillus cereus* recorded highest multidrug resistance with three (3) isolates showing varying degrees of MAR pattern. In particular one *Bacillus cereus* isolate recorded the highest MAR pattern showing resistant to 14 antibiotics namely: ampiclox, amoxicillin, augmentin, cefuroxime, ciprofloxacin, chloramphenicle ofloxacin, perfloxacin, sparfloxacin, streptomycin, septrin, erythromycin, nalidixic acid and gentimycin having 93.3% resistant. This result corresponds with the work of (16) whose antibiotic susceptibility resistance patterns of *Bacillus spp* isolated from herbal medicinal products showed resistance to ciprofloxacin, tetracycline, norfloxacin, augmentin, ceftriaxone, gentamicin, and cotrimoxazole.

Bacillus cereus was the most dominant in the herbal preparation because they are widely distributed in the soil, dust, air and because they are resistant to environmental harmful factors.

Serratia plymuthica recorded with MAR pattern showing resistant to 8 antibiotics (53.3%): ciprofloxacin, perfloxacin, ofloxacin, ampiclox, amoxicillin, augmentin, cefuroxime and gentamycin. This report is in union with the report of (17) in which *S. plymuthica* from human infection showed sensitive to ciprofloxacin, amoxicillin- clavulanic acid, gentamicin, and cefotaxime.

Multiple Antibiotic Resistant (MARI) Index of isolates from both sites was determined. *Bacillus cereus* recorded MARI values between 0.3-0.9 while *Serratia plymuthica* was 0.5. Hence, reflecting that the isolate *Bacillus cereus* and *Serratia plymuthica* might have originated from contaminated sources that have been obtained from multiple harvest sites, soil and environment or reflecting indiscriminate use of antibiotics. The result is in agreement with the work of (18) whose MARI result of *Bacillus spp* from liquid herbal products ranged between 0.4 – 0.6. Thus, all isolates from this study having MARI values greater than 0.2 and above perhaps be misuse of antibiotics or could be due to expression of resistance genes present in the bacteria. Also, it may be as a result of genes channelled from other bacteria species through horizontal gene transfer. High resistance to multiple antibiotics may present high mortality and can create an opportunity for epidemic outbreaks which are tough to contain. Conclusively, this study shows that the herbal drinks are contaminated with pathogenic bacteria. Therefore, it is mandatory both the consumers and producers of herbal drinks adhere to quality and safety rules.

Recommendation

From our findings, it is recommended that further study should be done to identify the genes responsible for resistance or virulence in these organisms to ensure curative and preventive measures are been adhere to.

Conflict of Interests

Authors declare that there is no conflict of interest regarding the publication of this paper.

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