ORIGINAL RESEARCH ARTICLE

Antibiogram of bacterial flora of public health significance associated with postharvest *Irvingia gabonensis* seeds in Lagos State, Nigeria

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Abstract

The spread of resistant bacteria within the community has continually posed obvious additional problems for infectious control. Efforts to identifying sources of resistant bacteria have not been channeled towards medicinal food condiments. This study investigated the antibiogram of bacterial flora of public health significance associated with postharvest *Irvingia gabonensis* seeds in Lagos State, Nigeria. The blended, homogenized and serially diluted samples of *I. gabonensis* seeds were plated using the spread plate technique on selective and differential agar media. API 20E and API 20NE were used for identification of members of *Enterobacter*iaceae and non-*Enterobacter*i*aceae*, respectively. The agar disc-diffusion method was employed to determine the antibiotic resistance profiles of the isolates. A total of 263 bacterial isolates (129 Gram-positive and 134 Gram-negative) were encountered. Eighty-five (66%) of Gram-positive isolates exhibited resistance to penicillin, gentamicin 65 (50.4%), erythromycin 69 (53.5%), cloxacillin 63 (43.8%), chloramphenicol 73 (56.6%), amoxicillin 75 (58.1%), tetracycline 58 (45%) while 69 (53.5%) showed resistance to streptomycin. However, 87 (65%) of Gram-negative bacterial strains exhibited resistance to cloxacillin, ceftazidime 82 (61.2%), ciprofloxacin 67 (50%), gentamicin 77 (57.5%), cefotaxime 74 (55.2%), augmentin 84 (62.7%), nitrofuratoin 61 (45.5%) and 24 (17.9%) to ofloxacin. This study showed that *I. gabonensis* seeds could be a source of antibiotic-resistant bacterial strains, despite its enormous medicinal properties, which is a threat to public health. The antibiotic resistance patterns of isolated bacterial strains are of medical importance as there are chances of transferring resistant traits.

Keywords: *Antibiogram, antibiotic resistance, bacteria, Irvingia gabonensis, public health.*

INTRODUCTION

Irvingia is a genus of African and Southeast Asian trees in the family Irvingiaceae. It is considered as one of the most domestically consumed wild fruit tree as it is a dominant tropical forest tree of Central and West Africa. In Nigeria, particularly in the southern and Eastern regions, *Irvingia gabonensis*, also known as bush mango, African mango seed, dika nut or apon, is grown and consumed basically because of its kernels which serve as a major condiment in the preparation of Nigeria's famous "*ogbono*" or "*apon*" soup.[1]

They bear edible mango-like fruits (Plate 1), and are especially valued for their fat and protein-rich nuts.[2] The subtly aromatic nuts (Plate 2) are usually sun-dried in order to preserve, and could be sold in the form of powder or as whole. Dika bread or Gabon chocolate is produced from the paste form of *Irvingia* seeds. Their high content of mucilage enables them to be used as thickening agents for dishes such as ogbono or *apon* soup. Vegetable oil could also be extracted from these seeds. The fruit is a large drupe, with fibrous flesh.

Adegbehingbe *et al*.[2] reported that the seeds of legumes account for up to 80% of dietary protein and could be the only source of protein for certain group. The cooked forms of the seeds could be eaten as meals while the fermented forms as condiments that enhance the flavors and texture of foods. The condiments could serve as a delicious complement to sauces or soups to substitute for meat or fish. Fermented legumes have characteristic organoleptic properties, which probably are the most important factors attracting consumers.[3]

Studies have shown that seed extract of *I. gabonensis* caused a significant reduction in body weight among obese people.[4] Earlier studies had attributed a reduction in the incidence of diseases such as cancer, cardiovascular disease, cataracts, and brain and immune dysfunction to consumption of fruits and vegetables which in turn was credited to natural antioxidant phytochemicals inherent in them.[5]Ekpe *et al*.[6] also showed that *Irvingia* seeds had high antioxidant capacity.

The soluble fibre of the seed of *I. gabonensis* improves gradual absorption of dietary sugar as it has the potentials to delay stomach emptying. This helps reduce the elevation of blood sugar levels that is typical after a meal. The fibre of *I. gabonensis* seed has the potentials to bind to the gut bile acids and remove them from the body, so that the body could convert more cholesterol into bile acids. Several grams per day of *I. gabonensis* may help reduce total blood cholesterol, low-density lipoprotein (LDL) cholesterol, and triglycerides and in some cases raise high-density lipoprotein (HDL) cholesterol.[7] *I. gabonensis*, however, has the tendency to convey potential pathogens from farm to kitchen.

Plate 1: *Irvingia gabonensis* whole fruit

Plate 2: Dried seeds of *Irvingia gabonensis*

Some bacteria have the ability of developing resistance to antibiotics. Antibiotics are placed into different categories based on their major mechanisms of action which include interference with protein synthesis (for instance, tetracyclines and macrolides), inhibition of cell wall synthesis (β-lactams and glycopeptide agents), disruption of bacterial membrane structure (daptomycin and polymyxins) and inhibition of nucleic acid synthesis (rifampin and fluoroquinolones), metabolic pathway inhibition (trimethoprim-sulfamethoxazole).[8] Bacteria may exhibit intrinsic resistance to one class of antibiotics, while acquisition of resistance genes from other organisms or *de novo* mutation, is also possible.

Bacterial resistance often results in treatment failure, which can have serious consequences, especially in critically ill patients.[9] Acquired resistance genes may enable a bacterium to produce enzymes that destroy the antibacterial drug. There could be expression of efflux systems to prevent the drug from accessing its intracellular target, which could modify the target site of the drug, or create an alternative metabolic pathway that bypasses the action of the drug. Transformation, conjugation, or transduction could lead to the acquisition of new genetic material by antimicrobial-susceptible bacteria from resistant strains of bacteria, with transposons often mediating the introduction of multiple resistance genes into the host's genome or plasmids. Use of antibacterial agents creates selective pressure for the emergence of resistant strains.[10]

Prolonged therapy with certain antibiotics, such as linezolid or vancomycin, could as well lead to the development of low-level resistance which compromises therapy.[11] The spread of resistant bacteria could become broader infection-control problems within healthcare institutions and communities. Medically important bacteria are increasingly observed in the community.[12,13,14] Obvious additional problems for infection control are as a result of the spread of resistant bacteria and which are rarely traced to some of these food condiments. This work aims at evaluating the antibiogram of bacterial flora of public health significance associated with postharvest *Irvingia gabonensis* seeds.

MATERIALS AND METHODS

Sources of Samples

Irvingia gabonensis seeds were purchased from local sellers from five markets in Lagos State, Nigeria. Samples from twenty-five different locations were separately blended into powdery form using an electric blender (Model 242 NAKAI, JAPAN). These were kept in sterile polythene bags in the refrigerator at 4 ^{o}C and then transported to the laboratory in sterile bags packed in insulated containers with ice packs.

Bacteriological Analyses

Bacteriological analyses were carried out in the Microbial Biotechnology Unit in the Department of Biological Sciences, North-West University, South Africa. Ten grams of ground *I. gabonensis* seeds were added to 90 ml of 0.1% (W/V) sterilized peptone water in a beaker and allowed to stand for 5 mins with occasional stirring. The homogenized samples were serially diluted to up to 10^{-8} and plated in duplicates on respective media using the spread plate technique. All plates were incubated at 32 °C for 24 - 48 hours. The plate count agar (PCA) was employed to enumerate aerobic mesophilic bacteria (AMB) while violet, red bile agar (VRBA) was used for the cultivation of coliforms. Colonies showing purple red colouration surrounded by reddish zone of precipitated bile were considered as coliforms. Enterobacteriaceae were counted on MacConkey agar, and pink to red purple colonies with or without haloes of precipitation were regarded as member of Enterobacteriaceae. *Streptococcus* spp were isolated on blood agar plates and characteristic colonies showing β-haemolysis were confirmed as streptococci. *Bacillus* spp were cultivated on mannitol/eggs yolk/polymyxin agar (MYP). All motile, catalase and Voges Proskauer positive isolates with ellipsoidal spores were confirmed as *B. cereus*. *Staphylococcus aureus* were cultivated on mannitol salt agar (MSA).

Characterization and identification of isolates

Representative colonies were picked and further purified by repeated subculturing on PCA. Pure cultures were preserved on nutrient agar slants at refrigeration temperature of 4 °C. Cell morphology, Gram's reaction, colony characterization and biochemical characterizations of isolates were performed according to standard procedures.[16,17] API 20E and API 20NE were used for additional identification of members of *Enterobacteriaceae* and non-*Enterobacteriaceae*, respectively.

Antibiotic susceptibility of isolates

The bacterial isolates were investigated for their susceptibilities to various antibiotics using the agar disc diffusion as described by Clinical and Laboratory Standard Institute Guidelines[18]. Antibiotics discs used were penicillin (PEN; 25µg), chloramphenicol (CHL; 30µg), gentamicin (GEN; 10µg), cloxacillin (CXC; 30µg), ampicillin (AMP 30µg), erythromycin (ERY; 5µg), streptomycin (STR; 10µg) and tetracycline (TET; 30µg) for Gram-positive bacteria. For Gram-negative bacteria, cloxacillin (CXC; 30µg), ceftazidime (CAZ; 30mg), ciprofloxacin (CRX; 10µg), gentamicin (GEN; 10µg), cefotaxime (CTX; 30µg), augmentin (AUG; 30µg), nitrofurantoin (NIT; 250µg) and ofloxacin (OFL; 30µg) were investigated. The entire surface of each sterile Mueller-Hinton agar plate was inoculated with 500 µl of each bacterial isolate, using sterile swab sticks. The plates were left for about 15 minutes before aseptically placing the antibiotic discs on the agar surfaces with sterile forceps, followed by incubation at 35 ^{o}C for 18-24 hours. The diameters of zones of inhibition were measured and recorded in millimetre, while zones of inhibition less than 10.0 mm in diameter or absence of zones of inhibition were recorded as resistant or negative.

RESULTS

Table I shows the morphological and biochemical characteristics of bacteria isolated from postharvest *I. gabonensis* seeds. A total of 263 bacteria were isolated from samples with 129 Gram-positive and 134 Gram-negative isolates. These were characterized into twelve bacterial species. The Gram-positive isolates were identified as *Bacillus cereus*, *Bacillus subtilis*, *Micrococcus luteus*, *Staphylococcus aureus*, *Staphylococcus epidermidis,* and *Streptococcus pyogenes* while the Gram-negative bacteria were *Enterobacter aerogenes, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Proteus mirabilis* and *Serratia rubidaea. P. aeruginosa* had the highest percentage occurrence of 14.07%, followed by *S. aureus* (12.17%), *S. epidermidis* (9.51%), *B. subtilis* (9.13%), *K. pneumoniae* (8.75%), *P. mirabilis* (7.99%), *S. rubidaea* (7.61%), *E. coli* (6.46%), *M. luteus* (6.46%), *E. aerogenes* (6.08%), *B. cereus* (6.08%) while the lowest was *S. pyogenes* with the percentage occurrence of 5.7%.

Table II shows the antibiotic resistance profile of Gram-positive bacterial isolates from postharvest *I. gabonensis* seeds in Lagos, Nigeria. Out of 129 Gram-positive bacterial isolates, 85 (66%) exhibited resistance to penicillin, gentamicin 65 (50.4%), erythromycin 69 (53.5%), cloxacillin 63 (43.8%), chloramphenicol 73 (56.6%), amoxicillin 75 (58.1%), tetracycline 58 (45%) while 69 (53.5%) showed resistance to streptomycin. *B. cereus* exhibited percentage resistance ranging from 31 to 68.8% as encountered in tetracycline and penicillin, respectively. The percentage resistances of *B. subtilis*, *M. luteus*, *S. epidermidis*, *S. aureus* and *Streptococcus pyogenes* ranged from 37.5 - 70.8%, 29.4 - 100%, 40 – 60%, 37.5 – 62.6% and 46.7 – 86.7%, respectively. The highest percentage of resistance was shown to penicillin by the Gram-positive bacterial isolates while the lowest was shown to tetracycline.

Similarly, from 134 Gram-negative bacterial strains, 87 (65%) were resistant to cloxacillin, ceftazidime 82 (61.2%), ciprofloxacin 67 (50%), gentamicin 77 (57.5%), cefotaxime 74 (55.2%), augmentin 84 (62.7%), nitrofuratoin 61 (45.5%) and 24 (17.9%) to ofloxacin. *E. aerogenes*, *E. coli* and *P. mirabilis* strains showed percentage resistances ranging from 0 – 100% while *K. pneumoniae*, *P. aeruginosa* and *S. rubidaea* strains exhibited percentage resistances ranging from 26.1 - 73.9%, 29.8 – 78.4% and 0 – 75%, respectively. Gram-negative bacterial strains exhibited highest level of resistance to cloxacillin and the lowest to ofloxacin (Table III).

Table I: Morphological and biochemical characteristics of bacteria isolated from postharvest *I. gabonensis* seeds in Lagos, Nigeria

Gram Reaction	Cellular morphology	Catalase	Oxidase	Coagulase	Indole	Motility	Methyl-Red	Voges Proskauer	Urease Activity	Citrate Utilization	Starch Hydrolysis	Gelatin Hydrolysis	Casein Hydrolysis	Spore Test	NO_3 Reduction	Glucose	Sucrose	Arabinose	Maltose	Mannitol	Xylose	Galactose	Sorbitol	Invositol	Raffinose	Frauction	No. of Isolates	% Occurrence	Most Probable Identity
+ve	R	+	+	-	-	+	-	+	-	+	-	+	-	+	-	+	+	-	-	+	-	-	-	-	+	+	16	6.08	*B. cereus*
+ve	R	+	+	-	-	+	-	+	+	+	-	+	-	+	-	+	+	-	-	+	-	-	-	-	-	+	24	9.13	*B. subtilis*
+ve	C	+	+	+	-	-	-	-	±	-	-	+	-	-	-	+	+	-	+	+	-	-	-	-	ND	-	17	6.46	*M. luteus*
+ve	C	+	-	+	-	-	-	+	+	-	-	+	+	-	+	+	+	-	+	+	-	+	ND	ND	ND	+	32	12.17	*S. aureus*
+ve	C	+	-	-	-	-	-	+	+	-	-	+	-	-	-	+	+	-	-	-	-	-	ND	ND	ND	+	25	9.51	*S. epidermidis*
+ve	C	-	-	-	-	-	-	-	-	-	-			-		+	+	-	+	-	-	+	-	ND	-	ND	15	5.7	*S. pyogenes*
-ve	R	+	-	-	-	+	-	+	-	+	+	-	-	-	+	+	+	+	+	+	+	+	+	+	ND	+	16	6.08	*E. aerogenes*
-ve	R	-	+	-	-	+	+	+		+	-	-	+	+	+	+	+	+	-	+	+	-	-	-	-	+	17	6.46	*E. coli*
-ve	R	-	-	-	-	-	+	+	+	+	+	-	-	-	-	+	+	+	+	+	-	+	-	+	-	-	23	8.75	*K. pneumoniae*
-ve	R	+	+	-	-	+	-	+	-	+	-	+	-	-	+	+	+	+	+	+	+	+	-	-	+	+	37	14.07	*P. aeruginosa*
-ve	R	+	-	-	-	+	+	-	+	+	-	+	-	-	+	+	-	-	-	-	+	+	-	-	-	ND	21	7.99	*P. mirabilis*
-ve	R	+	+	-	-	+	-	+	-	+	+	+	-	-	-	+	+	+	+	+	-	-	-	-	+	+	20	7.61	*S. rubidaea*

Keys: R = Rods; + = Positive reaction; - = Negative reaction; ND = Not determined; A = Zone A; B = Zone B; C =Zone C; D = Zone D and CT = Control.

Table II: Percentage antibiotic resistance of Gram-positive bacteria from postharvest *I. gabonensis* seeds in Lagos, Nigeria

Bacterial species	N	Antibiotics (µg/l)							
		PEN	GEN	ERY	CXC	CHL	AMX	TET	STR
B. cereus	16	11 68.8%	9 56.3%	9 56.3%	7 43.8%	10 62.5%	9 56.3%	5 31.3%	7 43.8%
B. subtilis	24	13 54.2%	14 53.3%	11 45.8%	9 37.5%	13 54.2%	15 62.5%	12 50%	17 70.8%
M. luteus	17	17 100%	8 47.1%	8 47.1%	9 52.9%	12 70.6%	15 88.2%	5 29.4%	10 58.8%
S. epidermidis	25	12 48%	10 40%	15 60%	12 48%	15 60%	12 48%	14 56%	13 52%
S. aureus	32	21 62.6%	15 46.9%	13 40.6%	19 59.4%	12 37.5%	17 53.15	15 46.9%	13 40.6%
S.pyogenes	15	11 73.3%	9 60%	13 86.7%	7 46.7%	11 73.3%	7 46.7%	7 46.7%	9 60%
TOTAL	129	85 66%	65 50.4%	69 53.5%	63 43.8%	73 56.6%	75 58.1%	58 45%	69 53.5%

Keys: N = Number of isolated strains; PEN = penicillin; GEN = gentamicin; ERY = erythromycin; CXC = cloxacillin; CHL = chloramphenicol; AMX = amoxycillin; TET = tetracycline; STR = streptomycin

Table III: Antibiotic resistance profiles of Gram-negative bacterial isolates from postharvest *I. gabonensis* seeds in Lagos, Nigeria.

Bacterial species	N	Antibiotics (µg/l)							
		CXC	CXZ	CRX	GEN	CTX	AUG	NIT	OFL
E. aerogenes	16	4 25.0%	0 0.0%	0 0.0%	8 50.0%	16 100%	16 100%	0 0.0%	0 0.0%
E. coli	17	9 52.9%	12 70.6%	9 52.9%%	17 100%	10 58.8%	7 41.2%	12 70.6%	0 0.0%
K. pneumonia	23	15 65.2%	17 73.9%	15 65.2%	6 26.1%	14 60.9%	13 56.5%	11 47.8%	11 47.8%
P. aeruginosa	37	29 78.4%	17 46%	11 29.8%	21 56.8%	19 51.4%	30 81.1%	20 54.1%	13 35.2%
P. mirabilis	21	21 100%	9 42.9%	15 71.4%	13 61.9%	8 38.1%	12 57.1%	7 33.3%	0 0.0%
S.rubidaea	20	5 25.0%	15 75.0%	10 50.0%	5 25.0%	5 25.0%	5 25.0%	5 25.0%	0 0.0%
TOTAL	134	87 65%	82 61.2%	67 50%	77 57.5%	74 55.2%	84 62.7%	61 45.5%	24 17.9%

Keys: CXC = cloxacillin; CAZ = ceftazidime; CRX = ciprofloxacin; GEN = gentamicin; CTX = cefotaxime; AUG = augmentin; NIT = nitrofurantoin; OFL = ofloxacin

The multiple antibiotic resistance (MAR) profiles of Gram-positive bacterial species from postharvest *I. gabonensis* seeds is shown in Table IV. The %MAR ranged from 25.0% to 87.5%.

The percentage multiple antibiotic resistances of the Gram-negative isolates from *I. gabonensis* ranged from 25% to 75.0% (Table V). A strain of *Serratia* species showed lowest %MAR of 25% while a strain of *P. aeruginosa* and three strains of *K. pneumoniae* exhibited the highest %MAR of 75.0%.

Table IV: Antibiotic resistance profiles of Gram-positive bacterial species from postharvest *I. gabonensis* seeds in Lagos, Nigeria

Bacterial Species	No. of Strains	Antibiotics									
		PEN	GEN	ERY	CXC	CHL	AMX	TET	STR	MAR	%MAR
B. cereus	4				CXC	CHL	AMX			3	37.5
B. cereus	5					CHL	AMX	TET	STR	4	50.0
B. cereus	3				CXC	CHL			STR	3	37.5
B. cereus	2	PEN	GEN				AMX			3	37.5
B. cereus	2	PEN	GEN		CXC		AMX	TET	STR	6	75.0
B. subtilis	3	PEN		ERY	CXC		AMX	TET		5	62.5
B. subtilis	5	PEN		ERY	CXC		AMX		STR	5	62.5
B. subtilis	8	PEN	GEN	ERY	CXC	CHL			STR	6	75.0
B. subtilis	5	PEN	GEN	ERY	CXC	CHL	AMX			6	75.0
B. subtilis	3	PEN	GEN	ERY	CXC	CHL	AMX	TET		7	87.5
M. luteus	3		GEN	ERY		CHL	AMX		STR	5	62.5
M. luteus	2	PEN	GEN	ERY	CXC	CHL		TET	STR	7	87.5
M. luteus	3	PEN	GEN	ERY	CXC		AMX			5	62.5
M. luteus	9					CHL	AMX	TET		3	37.5
S. aureus	8	PEN	GEN		CXC		AMX	TET	STR	6	75.0
S. aureus	4		GEN	ERY	CXC		AMX	TET		5	62.5
S. aureus	2		GEN	ERY	CXC		AMX		STR	5	62.5
S. aureus	7		GEN	ERY	CXC	CHL	AMX			5	62.5
S. aureus	5					CHL	AMX	TET		3	37.5
S. aureus	9	PEN	GEN		CXC		AMX	TET	STR	6	75.0
S. epidermidis	6		GEN	ERY	CXC		AMX	TET		5	62.5
S. epidermidis	3		GEN	ERY	CXC		AMX		STR	5	62.5
S. epidermidis	8				CXC				STR	2	25.0
S. epidermidis	5		GEN	ERY	CXC	CHL	AMX			5	62.5
S. epidermidis	3					CHL	AMX	TET		3	37.5
S. pyogenes	2			ERY		CHL	AMX	TET	STR	5	62.5
S. pyogenes	1	PEN	GEN	ERY	CXC		AMX	TET	STR	7	87.5
S. pyogenes	5					CHL	AMX		STR	3	37.5
S. pyogenes	4				CXC	CHL	AMX	TET	STR	5	62.5
S. pyogenes	3				CXC	CHL	AMX			3	37.5

Keys: PEN = penicillin; GEN = gentamicin, ERY = erythromycin; CXC = cloxacillin; CHL = chloramphenicol, AMX = amoxicillin; TET = tetracycline; STR = streptomycin; MAR = Multiple antibiotic resistance; %MAR = Percentage multiple antibiotic resistance

Table V: Antibiotic resistance profiles of Gram-negative bacterial isolates from *I. gabonensis* seeds in Lagos, Nigeria

Bacterial Strains	No. of Strains	Antibiotics								MAR	%MAR
		CXC	CXZ	CRX	GEN	CTX	AUG	NIT	OFL		
E. aerogenes	7				GEN	CTX	AUG			3	37.5
E. aerogenes	6		CXZ			CTX	AUG			3	37.5
E. aerogenes	1	CXC				CTX	AUG	NIT		4	50.0
E. aerogenes	2				GEN	CTX	AUG			3	37.5
E. coli	4	CXC	CXZ	CRX		CTX		NIT		5	62.5
E. coli	7	CXC	CXZ	CRX		CTX		NIT		5	62.5
E. coli	3	CXC	CXZ	CRX	GEN	CTX				5	62.5
E. coli	3	CXC	CXZ		GEN	CTX		NIT		5	62.5
E. coli	1	CXC	CXZ	CRX	GEN	CTX		NIT		6	75.0
K. pneumoniae	5	CXC	CXZ				AUG			3	37.5
K. pneumoniae	4	CXC	CXZ	CRX			AUG	NIT		5	62.5
K. pneumoniae	2	CXC	CXZ	CRX	GEN	CTX		NIT		6	75.0
K. pneumoniae	6	CXC	CXZ	CRX			AUG			4	50.0
K. pneumoniae	3	CXC	CXZ	CRX		CTX		NIT		4	50.0
K. pneumoniae	3	CXC	CXZ	CRX	GEN	CTX				4	50.0
P. aeruginosa	6			CRX		CTX	AUG	NIT	OFL	5	62.5
P. aeruginosa	5			CRX	GEN	CTX	AUG	NIT	OFL	6	75.0
P. aeruginosa	2	CXC		CRX		CTX	AUG		OFL	5	62.5
P. aeruginosa	6		CXZ		GEN	CTX				3	37.5
P. aeruginosa	7		CXZ	CRX	GEN			NIT		4	50.0
P. aeruginosa	5		CXZ	CRX	GEN	CTX				4	50.0
P. aeruginosa	6		CXZ	CRX	GEN	CTX				4	50.0
P. mirabilis	4	CXC				CTX	AUG			3	37.5
P. mirabilis	5	CXC				CTX		NIT		3	37.5
P. mirabilis	5	CXC					AUG	NIT		3	37.5
P. mirabilis	3	CXC		CRX		CTX				3	37.5
P. mirabilis	4	CXC		CRX		CTX				3	37.5
S. rubidaea	7	CXC				CTX	AUG			3	37.5
S. rubidaea	5	CXC						NIT		2	25.0
S. rubidaea	4	CXC				CTX	AUG	NIT		4	50.0
S. rubidaea	4	CXC		CRX		CTX	AUG			4	50.0

Keys: CXC = cloxacillin; CAZ = ceftazidime; CRX = ciprofloxacin; GEN = gentamicin; CTX = cefotaxime; AUG = augmentin; NIT = nitrofurantoin; OFL = ofloxacin; MAR = Multiple antibiotic resistance; %MAR = Percentage multiple antibiotic resistance

DISCUSSION

Multiple antibiotic resistances were recorded among the bacterial flora associated with *I. gabonensis* seeds. It was noted that strains of same bacterial species exhibited varying susceptibility patterns to antibiotics. There was no single bacterial species whose strains were solely resistant or susceptible to one antibiotic. This is the basis for the importance of the roles of combined therapy. The fact that high multiple antibiotic resistance was recorded against most of the commonly used antibiotics in this study is a reflection of high prevalence of multiple antibiotic resistant bacteria in the community.

Despite the enormous health benefits associated with the consumption of *I. gabonensis* seeds as reported by several authors,[19-24] the presence of multidrug-resistant bacterial strains in the food

condiment is of public health concern. The isolation of potential pathogens in this study could be attributed to poor hygienic practices during harvest, transportation and/or storage of the seeds. The organisms encountered in this study have medical implications. For instance, *S. aureus* in food products is employed generally as a sanitation index. This indicates that the postharvest plant seeds had been exposed to conditions that might introduce or allow proliferation of pathogenic microorganisms.[14] *S. aureus* is an important food-poisoning organism because of its cosmopolitan distribution in nature and could be traced back to the environment as they are important normal flora of humans.[13]

S. epidermidis is the most frequently connected species from human epithelia. It is largely present on the axillae, head, and nares. The bacterium belongs to the group of coagulase-negative staphylococci (CoNS), which is distinguished from coagulase-positive staphylococci such as *S. aureus* by lacking the enzyme coagulase. The species displays a high degree of diversity with 74 identified sequence types. Among CoNS, *S. epidermidis* causes the greatest number of infections.[25] The bacterium represents the most frequent causative agent associated with infections of any type of indwelling medical devices, including peripheral or central intravenous catheters (CVCs).[26]

The presence of *B. cereus* in the food product could be attributed to the fact that the organism is usually found in soil, on decaying organic matter, vegetables and fomites, fresh and marine waters, and the intestinal tract of invertebrates, through which food products may become contaminated, and this leads to the transient colonization of the human intestine.[27] This organism is also frequently present in food production environments due to the adhesive nature of its endospores, which enables it to spread to all kinds of food.[28]

B. subtilis possesses the ability to transform itself into a spore and, thus, enters a dormant state, which allows it to withstand extreme environmental conditions. The bacterium holds the record for surviving in space for the longest duration, 6 years on a NASA satellite. *B. subtilis* is usually considered non-pathogenic. However, it has been implicated in food poisoning caused by poor quality bakery products among others. *B. subtilis* food poisoning has a rapid onset, with acute vomiting, commonly followed by diarrhoea.[29]

The contamination of the food product with *M. luteus* could be through soil, dust, water, or human skin. This bacterium can withstand huge doses of UV radiation and has the ability to degrade pollutants such as petrol. *M. luteus* played a crucial role in Fleming's discovery of lysozyme. It possesses the ability to exhibit dormancy without forming spores. *M. luteus* causes odours in humans during the process of breaking down the components of sweat.

The reservoir for *S. pyogenes* infection is humans and through which the food product must have got contaminated. Asymptomatic colonisations of the pharynx are found in up to 20% of the population during winter. Streptococcal pharyngitis is transmissible mainly through droplets, and occasionally through contaminated food and water. *S. pyogenes* can cause skin and soft tissue infections which usually can affect the muscles, the deeper tissue layers, and fascia. Toxin-mediated diseases are scarlet fever and the streptococcal toxic shock syndrome.[30]

E. aerogenes are generally distributed in water, soil, sewage, dairy products and vegetables. It is part of the commensal enteric flora and usually not pathogenic. However, some strains produce shiga-like toxin. The bacterium has also been associated with nosocomial infections and a variety of opportunistic infections involving the urinary and respiratory tracts, and cutaneous wounds.

Enterobacter spp are resistant to most antibiotics, especially the cephalosporins. Their resistance to β-lactam antibiotics, chloramphenicol, quinolones and tetracyclines have been well documented.[31,32]

The occurrence of *P. aeruginosa* in the food product could be attributed to its ubiquity. It is a common opportunistic pathogen in humans, causing a broad range of infections in community and healthcare settings.[33] The most serious manifestations of infection include bacteraemia (particularly in neutropenic patients), pneumonia (particularly in cystic fibrosis patients and critically ill patients), urinary tract infections and wound infections.[34] This organism is intrinsically resistant to many antibiotics and in recent years resistance has emerged to what were previously antimicrobial agents of choice.[35]

P. mirabilis is most commonly found in the human intestinal tract as part of normal human intestinal flora, alongside *Klebsiella* species and *E. coli*.[36] *P. mirabilis* is present in multiple environmental habitats, including hospitals and long-term care facilities. *P. mirabilis* causes 90% of *Proteus* infections and can be considered a community-acquired infection.[36]

E. coli is well recognized as the main commensal inhabitant of mammals' gastrointestinal tract. Pathogenic *E. coli* strains cause a number of human diarrhoea. These strains are serotyped on the basis of their O (somatic), H (flagellar), and K (capsular) surface antigen profiles into six categories: Enteroaggregative (EAEC), (EHEC)/Shiga toxin-producing *E. coli* (STEC), Enteroinvasive (EIEC), Enteropathogenic (EPEC), Enterotoxigenic (ETEC), and diffuse adherent (DAEC). EHEC is a subgroup of VTEC/STEC associated with 45 human diseases which, in addition to the verocytotoxin/shigatoxin producing capacity, harbors additional genes that are important in virulence. Verocytotoxin-producing *E. coli* (VTEC) is a term used to describe strains of *E. coli* characterized by the ability to produce verocytotoxin(s) (VT), or just verotoxins that are capable of killing Vero cells, a tissue culture line of monkey kidney cells.[37] Infections associated with these pathotypes are of public health concern.

The genus *Serratia* is a member of the broad Enterobacteriaceae family and has been differentiated into 10 species.[38] Infections of humans with *Serratia* are not as common as with more virulent members of the Enterobacteriaceae. *S. rubidaea*, which is a less well-described member of the genus, is majorly found in soil, water and food. The isolation of this species from food samples and clinical specimens is rare, but it may cause opportunistic infections in severely ill patients receiving broad-spectrum antimicrobials, or those that have undergone surgery or other invasive procedures.[39,40] When identified in clinical specimens, *S. rubidaea* is largely isolated from respiratory tract samples, skin wounds, faeces and bile.[41]

The increased level of resistance to antimicrobial drugs is a reflection of the indiscrimate use of antibiotics which is becoming a common practice without legal cautions in our environment.

CONCLUSION

This study showed that *I. gabonensis* seeds could be a source of antibiotic-resistant pathogenic bacterial strains despite its enormous medicinal properties, and which is a threat to public health. This work has provided informative data related to the multidrug resistant profile of bacteria of clinical importance associated with *I. gabonensis* seeds, which could be initiated by the transfer of resistant traits among commensal flora through food chain.

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