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Prevalence and Antibiotic Resistance Profiles of Bacterial Flora from Maggot-infested, Deteriorated *Iru*

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Abstract

A total of 95 (n = 62 Gram-positive; n = 33 Gram-negative) bacterial strains isolated from 75 deteriorated, maggot-infested iru samples were identified using standard phenotypic taxonomic tools as Bacillus (44.2%), Streptococcus (9.5%), Salmonella parayphii (8.4%), Klebsiella pneumoniae (6.3%), Proteus mirabilis (6.3%), Staphylococcus aureus (6.3%), Micrococcus (5.3%), Pseudomonas aeruginosa (5.3%), E. coli (4.2%), Enterobacter aerogenes (3.2%) and Shigella dysentariae (1.0%) species. Resistance rates among the Gram-positive bacteria towards antibiotic (discs) varied between 69.4% (amoxicillin, cloxacillin, erythromycin, penicillin), 62.9% (chloramphenicol), 54.8% (gentamicin and streptomycin), with overall multiple antibiotic resistance (MAR) rates of 25.0–100%. Very high resistance rates were also exhibited by the Gram-negative bacterial species towards nitrofurantoin (60.0%), ciprofloxacin (63.6%), augmentin, claforan, fortum (66.7%) and cloxacillin (72.7%), with MAR of 25.0-87.5%; while the least resisted was ofloxacin (18.2%). Relatively lower resistance rates (12.9-53.2%) towards clinical antibiotic drugs were recorded among Gram-positive bacteria, except towards septrin (66.1%) and axacef (77.4%), and among the Gram-negative bacteria (15.2-45.5%), except towards erythromycin (60.6%), mediphenicol (63.6%), primpex (66.7%), septrin (75.8%) and axacef (78.7%). MAR of 11.1-100% were recorded among both the Gram-positive and Gram-negative bacteria towards clinical antibiotic drugs, while 59 and 30 different antibiotic resistance profiles were recorded among the Gram-positive and Gram-negative bacteria respectively. Isolated fungal flora were identified as Aspergillus niger, Aspergillus flavus, Penicillium, Rhizopus, Botrydiplodia and Candida spp. Recovery of fungal species and multi-drug resistant bacterial flora from biodeteriorated, maggot-infested iru samples indicated the need for food safety caution in contamination of traditionally-produced fermented food condiments like iru, due to house flies (Musca domestica).

Key words: food deterioration, maggot-infestation, food condiment, food borne pathogens, food safety

Introduction

Food condiments in Nigeria and many other countries of West and Central Africa are popular strong-smelling, fermented food culinary products that give pleasant aroma to soups, sauces and other prepared dishes. They also have great potentials as key protein, fatty acid and good sources of gross energy (Umoh and Oke, 1974, Sarker et al., 1993). Some of the Nigerian indigenous food condiments are *iru* / dawadawa / dadawa, soy *iru* / dawadawa, ogiri, ogiri-igbo, ogiri ugu, ugba, owoh); ukpaka and afiyo / okpehe (Campbell-Platt, 1980; Ogundana, 1980; Anosike and Egwuatu, 1981; Odunfa, 1981; Oyeyiola, 1981; Obeta, 1983; Ogbadu and Okagbue, 1988; Barber et al., 1988; Odibo and Umeh, 1989; Barber et al., 1992; Sanni and Ogbonna 1991; Ogunshe et al., 2007), while newly produced condiments include aisa from Albizia saman seeds, iregi from Delonix regia seeds, etc. (Ogunshe et al., 2006, 2008).

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Iru is a culinary product that can be used to enhance or intensify meatiness in soups, sauces and other prepared dishes. According to Campbell-Platt (1980), *iru* or dadawa / dawadawa (the most popular food condiment in the entire of savannah region of the West and Central Africa) are respective Yoruba and Hausa names for fermented seed cotyledons of African locust beans (Parkia biglobosa). It is an indigenous tree species that is economically and socially important for local people in sub-Saharan Africa (Teklehaimanot, 2004). The fermentation of African locust bean Parkia biglobosa by Bacillus species to produce *iru* is an example of an alkaline fermentation process (Steinkraus, 1995), and after fermentation for 72-96 hours, the cotyledons become soft and dark with a strong characteristic ammoniacal odour (Odunfa, 1986; Achi, 1992). Just like other fermented condiments, *iru* does not keep well if the fermentation is allowed to continue uninhibited for too long or if improperly stored.

If fermentation of *iru* is prolonged, the environment can become suitable for microbial contaminations. Furthermore, over-fermentation has been known to be capable of generating unacceptable levels of volatile fatty acids (Achi, 2005), while improper storage has also been known to cause maggot infestation. Larvae in infested foods produce adult fly, which mate to lay more eggs, and the eggs hatch into other young larvae that can burrow into the food stuff, and any food thus contaminated is rendered unfit for human consumption (http://www.gardening-naturally.com/acatalog/Maggot_In_Food.html). The aims of this study therefore, are to identify the bacterial and fungal flora of maggot-infested, deteriorated *iru* samples and determine the in vitro antibiotic susceptibility / resistance patterns of the isolated bacterial species using antibiotic discs and drugs assays.

Material and Methods

Collection of samples

Seventy five samples of market *iru* used in this study were all obtained from local markets, Agbowo, Bodija, Oja-Oba and Sango within Ibadan metropolis, Oyo state, Nigeria. Microbiological analyses were carried out at the Department of Microbiology, Faculty of Science, University of Ibadan.

The market *iru* samples already wrapped with teak leaves were kept at various open locations at homes for five days after purchase but almost all the samples were already deteriorated and infested with tiny maggots and eggs by the third day. One gram of each maggot-infested *iru* sample was transferred into McCartney bottles containing 9 ml of sterile distilled water and properly homogenised before transferring 1ml aliquots of each serially diluted sample separately into sterile Petri dishes, using the pour-plate method. Each aliquot was plated on molten (450C) plate count agar (Lab M: England), MacConkey agar (MCC; Lab M) at pH 7.2, cystein lactose electrolyte deficient (CLED; Lab M) agar and incubation was done aerobically at 300C for 24-48 hours. Moulds and yeasts were isolated on potato dextrose agar (PDA: Lab M) and Sabouraud dextrose agar (SDA; Lab M) for 3-6 days.

Taxonomic studies were carried out on the purified bacterial and fugal isolates from the differently analysed *iru* samples on the basis of their cultural, morphological, microscopic and biochemical characteristics (Bailey and Scott, 1974; Buchanan and Gibbons, 1974; Cruickshank et al., 1975; Harrigan and McCance, 1976; Vera and Power, 1980; Cheesbrough, 1998, 2000).

Antibiotic susceptibility / resistance determination (discs)

Using the agar disc-diffusion method, the antibiotic susceptibility / resistance patterns of the bacterial strains from maggot-infested *iru* samples to various antibiotics (discs)- 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 and cloxacillin (CXC; 30µg), fortum (CAZ; 30mg), ciprofloxacin (CRX; 10µg), gentamicin (GEN; 10µg), claforan (CTX; 30µg), augmentin (AUG; 30µg), nitrofurantoin (NIT; 250µg) and ofloxacin (OFL; 30µg) for Gram-negative were determined.

The entire surface of each sterile Mueller-Hinton agar plate was seeded with 500µl 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 350C for 18-24 hours. Zones of inhibition were measured and recorded in millimetre diameter, while zones of inhibition less than 10.0mm in diameter or absence of zones of inhibition were recorded as resistant or negative (Bauer et al., 1966; NCCLS (2003).

Antibiotic susceptibility determination (clinical drugs)

Antibiotic susceptibility / resistance determination of the Gram-positive and Gram-negative bacterial species from deriorated *iru* samples to various antibiotic drugs was by modifying the agar well-diffusion method of Tagg et al. (1976), in which 1.0% plain agar powder was sterilised and added as sterile soft agar to the aqueous suspensions of the antibiotics to avoid spreading of the antibiotic suspensions from the agar wells to the surface of the seeded agar plates. Antibiotic drugs used in this study were AMX 1 = amoxil (amoxicillin: 250 mg); AMX 2 = amoxil (amoxicillin: 500mg); AMP = ampiclox (ampicillin/ cloxacillin: 500mg); NOB = nobax (azithromycin: 250mg); SEP = septrin (cotrimoxazole : 250mg); LOX = loxaprim (cotrimoxazole: 450mg); AXA = axacef (cefuroxime: 250mg); ERY 1 = erymycin (erythromycin: 500mg); ERY 2 = erythromycin (erythromycin: 250mg); ETO = etocin (erythromycin: 250mg); OFLO = oflomed (ofloxacin 200mg); MED = mediphenicol (chloramphenicol 100mg); MEF = mefacol (chloramphenicol: 100mg); PRIM = primpex (trimethoprim and sulfamethoxazole mg); AUG 1 = augmentin 1

(amoxicillin and clavulanate potassium: 100mg); AUG 2 = augmentin 2 (amoxicillin and clavulanate potassium: 375mg); AUG 3 = augmentin 3 (amoxicillin and clavulanate potassium: 625 mg); TET = tetracycline (mg);.
Using sterile cork borers, wells of 6 mm in diameter were bored into the sterile Mueller-Hinton agar plates followed by surface flaming of the agar surfaces. The test bacterial strains previously inoculated into sterile peptone water and incubated at 370C for 18-24 hours were separately seeded on the agar plates by streaking the entire surface of the sterile plates with 500 µl of each bacterial broth culture. Different antibiotic suspensions, prepared by dissolving each antibiotic capsules and caplets in 30 ml sterile distilled water were separately dispensed into each set of agar wells and then incubated un-inverted at 350C for 24–48h. Diameter of zones of inhibition surrounding the wells were measured and recorded in mm diameter, while wells with no inhibition zones or inhibition zones less than 10.0mm in diameter were recorded as resistant

Results and Discussion

Seventy six market *iru* samples (pete = 39; woro = 37), which were microbiologically analysed in this study had pH range of 6.8 - 8.5. Using standard phenotypic taxonomic tools, a total of 95 bacterial strains (Gram-positive = 62; Gram-negative = 33) isolated from deteriorated, maggot-infested *iru* samples were identified as Bacillus spp. 42 (44.2%), Streptococcus spp. 9 (9.5%), Salmonella parayphii 8 (8.4%), Klebsiella pneumoniae 6 (6.3%), Proteus mirabilis 6 (6.3%), Staphylococcus aureus 6 (6.3%), Micrococcus spp. 5 (5.3%), Pseudomonas aeruginosa 5 (5.3%), E. coli 4 (4.2%), Enterobacter aerogenes 3 (3.2%) and Shigella dysentariae 1 (1.0%). Isolated fungal isolates were identified as Aspergillus niger, Aspergillus flavus, Penicillium, Rhizopus and Botrydiplodia and Candida species.

Table 1: *In vitro* antibiotic resistance rates of Gram-positive bacterial species from deteriorated maggot-infested *iru* samples (discs)

	Antibiotics (µg/l)								
Bacterial species	PEN	GEN	ERY	CXC	CHL	AMX	TET	STR	% MAR
Overall [62]	69.4	54.8	69.4	69.4	62.9	69.4	48.4	54.8	25.0-100
Bacillus spp. [42]	66.7	59.5	64.3	61.9	59.5	69.0	40.5	50.0	25.0-100
Micrococcus spp. [5]	100	40.0	60.0	100	80.0	80.0	60.0	80.0	50.0-100
Streptococcus spp. [9]	66.7	44.4	100	77.8	77.8	55.6	55.6	66.7	50.0-100
Staphylococcus aureus [6]	66.7	50.0	66.7	83.3	50.0	83.3	83.3	66.7	37.5-100

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

Table 2: *In vitro* antibiotic resistance rates of Gram-negative bacterial species from deteriorated maggot-infested *iru* samples (discs)

	Antibiotics (µg/l)								
	CXC	CXZ	CRX	GEN	CTX	AUG	NIT	OFL	% MAR
Overall [33]	72.7	66.7	63.6	45.5	66.7	66.7	60.6	18.2	25.0-87.5
Enterobacter aerogenes [3]	33.3	33.3	0.0	66.7	100	100	33.3	0.0	37.0-50.0
Escherichia coli [4]	75.0	100	75.0	100	75.0	50.0	75.0	0.0	50.0-75.0
Klebsiella pneumoniae [6]	83.3	83.3	83.3	16.7	66.7	83.3	66.7	50.0	50.0-87.5
Proteus mirabilis [6]	100	66.7	83.3	16.7	50.0	50.0	50.0	0.0	25.0-75.0
Pseudomonas aeruginosa [5]	80.0	60.0	40.0	60.0	80.0	100	60.0	20.0	37.5-87.5
Salmonella paratyphii [8]	62.5	50.0	62.5	37.5	62.5	50.0	62.5	25.0	25.0-75.0
Shigella dysentariae [1]	0.0	100	100	100	0.0	0.0	100	0.0	50.0

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

The isolated Gram-positive (40.0-100%) and Gram-negative (16.7-100%) bacterial species were highly resistant to the test antibiotic discs, and additionally exhibited multiple antibiotic resistance rates (MAR) of 25.0-100% and 25.0-87.5% respectively; however, very low resistance rates (0.0-50.0%) were recorded towards ofloxacin (Tables 1 & 2). A total of 48 and 32 different in vitro antibiotic resistance profiles were respectively exhibited by the Gram-positive and Gram-negative bacterial species isolated from deteriorated, maggot-infested *iru* samples in this study (Tables 3 & 4).

Table 3: *In vitro* antibiotic resistance profiles of Gram-positive bacterial species from deteriorated, maggot-infested *iru* samples (discs)

	Antibiotics (µg/l)								
S/N	PEN	GEN	ERY	CXC	CHL	AMX	TET	STR	%MAR
1 *Bacillus* sp.					CHL	AMX		STR	37.5
2 *Bacillus* sp, *Staphylococcus*					CHL	AMX	TET		37.5
4 sp					CHL	AMX	TET		37.5
3 *Bacillus* sp				CXC	CHL	AMX	TET	STR	62.5
4 *Bacillus sp*				CXC	CHL				25.0
5 *Bacillus sp*				CXC			TET		25.0
6 *Bacillus sp*			ERY		CHL	AMX			37.5
7 *Bacillus sp*			ERY		CHL		TET	STR	50.0
8 *Streptococcus sp*			ERY		CHL	AMX	TET	STR	62.5
9 *Bacillus sp*			ERY	CXC		AMX			37.5
10 *Bacillus sp*			ERY	CXC	CHL		TET		50.0
11 *Staphylococcus* sp			ERY	CXC	CHL		TET	STR	62.5
12 *Bacillus sp*		GEN	CXC		CHL	AMX	TET		62.5
13 *Bacillus sp*		GEN	ERY					STR	37.5
14 *Bacillus sp*		GEN	ERY		CHL	AMX		STR	62.5
15 *Bacillus sp*		GEN	ERY		CHL				37.5
16 *Bacillus sp*		GEN	ERY	CXC		AMX	TET	STR	75.0
17 *Streptococcus sp* [2]		GEN	ERY	CXC	CHL				50.0
7 *Streptococcus sp*		GEN	ERY	CXC	CHL				50.0
18 *Bacillus* sp.	PEN		ERY			AMX			37.5
19 *Bacillus*	PEN		ERY	CXC					37.5
20 *Bacillus* sp	PEN		ERY	CXC				STR	50.0
21 *Bacillus* sp	PEN		ERY	CXC	CHL	AMX		STR	75.0
22 *Bacillus* sp	PEN		ERY	CXC		AMX			50.0
23 *Micrococcus* sp	PEN		ERY	CXC		AMX		STR	62.5
24 *Streptococcus* sp	PEN		ERY	CXC		AMX	TET		62.5
25 *Streptococcus* sp	PEN		ERY		CHL		TET	STR	62.5
26 *Streptococcus* sp	PEN		ERY	CXC		AMX		STR	62.5
27 *Streptococcus* sp	PEN		ERY	CXC	CHL			STR	62.5
28 *Staphylococcus* sp	PEN			CXC		AMX			37.5
29 *Micrococcus* sp	PEN			CXC	CHL		TET	STR	62.5
30 *Bacillus* sp *Micrococcus* sp.	PEN			CXC	CHL	AMX			50.0
31 *Bacillus* sp	PEN				CHL	AMX	TET	STR	62.5
32 *Bacillus* sp	PEN							STR	25.0
33 *Bacillus* sp	PEN	GEN				AMX			37.5
34 *Bacillus* sp [2]	PEN	GEN		CXC		AMX	TET	STR	75.0
35 *Bacillus* sp	PEN	GEN			CHL		TET	STR	62.5
36 *Bacillus* sp p[2]	PEN	GEN			CHL	AMX		STR	62.5
37 *Bacillus* sp	PEN	GEN					TET		37.5
38 *Bacillus* sp	PEN	GEN	ERY	CXC		AMX	TET		75.0
39 *Bacillus* sp [3]	PEN	GEN	ERY	CXC		AMX		STR	75.0
40 *Bacillus* sp	PEN	GEN	ERY	CXC	CHL			STR	75.0
41 *Bacillus* sp	PEN	GEN	ERY	CXC	CHL	AMX			75.0
42 *Bacillus* sp p[3]	PEN	GEN	ERY	CXC	CHL	AMX	TET		87.5
43 *Bacillus* sp	PEN	GEN	ERY		CHL	AMX		STR	75.0
44 *Bacillus* sp	PEN	GEN	ERY	CXC	CHL		TET	STR	87.5
45 *Bacillus* sp	PEN	GEN	ERY	CXC		AMX			62.5
46 *Staphylococcus aureus* [2]	PEN	GEN	ERY	CXC		AMX	TET	STR	87.5
47 *Bacillus* sp	PEN	GEN	ERY	CXC	CHL	AMX			75.0
48 *Bacillus* sp [1] *Micrococcus* sp [2]	PEN	GEN	ERY	CXC	CHL	AMX	TET	STR	100

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

Table 4: Overall *in vitro* antibiotic resistance profiles of Gram-negative bacterial isolates from deteriorated maggot-infested *iru* samples (discs)

	Antibiotics (µg/l)									
	CXC	CXZ	CRX	GEN	CTX	AUG	NIT	OFL	MAR	%MAR
1. *Enterobacter* sp.				GEN	CTX	AUG			3	37.5
2. *Enterobacter* sp.		CXZ		GEN	CTX	AUG			4	50.0
3. *Salm. paratyphii*			CRX		CTX	AUG	NIT		4	50.0
4. *Ps. aeruginosa*			CRX	GEN	CTX	AUG	NIT		5	62.5
5. *Kleb. pneumoniae*			CRX		CTX	AUG	NIT	OFL	5	62.5
6 *Salm paratyphii*			CRX	GEN	CTX	AUG	NIT	OFL	6	75.0
7. *Ps. aeruginosa*	CXC				CTX	AUG			3	37.5
8. *Pr. mirabilis, Salm. paratyphii*	CXC						NIT		2	25.0
9. *Enterobacter* sp.	CXC				CTX	AUG	NIT		4	50.0
10. *Pr. mirabilis*	CXC		CRX		CTX	AUG			4	50.0
11. *Salm. paratyphii*	CXC		CRX		CTX	AUG		OFL	5	62.5
12. *Ps. aeruginosa*	CXC	CXZ				AUG			3	37.5
13. *Kleb. pneumoniae*	CXC	CXZ	CRX			AUG			4	50.0
14. *Salm. paratyphii*	CXC	CXZ	CRX				NIT		4	50.0
15. *Pr. mirabilis*	CXC	CXZ	CRX						3	37.5
16. *Pr. mirabilis*	CXC	CXZ	CRX			AUG	NIT		5	62.5
15. *E. coli*	CXC	CXZ	CRX	GEN	CTX		NIT		6	75.0
16. *Pr. mirabilis*	CXC	CXZ	CRX	GEN	CTX	AUG			6	75.0
17. *E. coli*	CXC	CXZ	CRX	GEN		AUG	NIT		6	75.0
18. *Salm. paratyphii*	CXC	CXZ				AUG	NIT		4	50.0
19. *Kleb. pneumoniae*	CXC	CXZ	CRX			AUG			4	50.0
20. *Pr. mirabilis*	CXC	CXZ	CRX		CTX		NIT		5	62.5
21. *Salm paratyphii*	CXC	CXZ	CRX	GEN	CTX				5	62.5
22. *Salm paratyphii*		CXZ		GEN	CTX				3	37.5
23. *Sh. dysentariae*		CXZ	CRX	GEN			NIT		4	50.0
24. *E. coli*	CXZ		CRX	GEN	CTX				4	50.0
25. *Kleb. pneumoniae*	CXC	CXZ		GEN	CTX		NIT	OFL	6	75.0
26. *E. coli*	CXC	CXZ		GEN	CTX	AUG	NIT		6	75.0
27. *Kleb pneumoniae*	CXC	CXZ	CRX		CTX	AUG	NIT		6	75.0
28. *Ps. aeruginosa*	CXC	CXZ		GEN	CTX	AUG	NIT	OFL	7	87.5
29. *Kleb. pneumoniae*	CXC	CXZ	CRX		CTX	AUG	NIT	OFL	7	87.5
30. *Ps. aeruginosa*	CXC	CXZ	CRX	GEN	CTX	AUG	NIT		7	87.5

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

Antibiotic resistance rates of the bacterial species from deteriorated, maggot-infested *iru* samples towards antibiotic drugs were as shown in Tables 5 and 6 respectively. The most resisted antibiotic drugs by the Gram-positive bacterial species were mediphenicol [chloramphenicol 100mg] (51.6%); erymycin [erythromycin: 500mg] / augmentin 2 [amoxicillin and clavulanate potassium: 375 mg] (53.2%); septrin [cotrimoxazole: 250 mg] (66.1%) and axacef [cefuroxime: 250 mg] (77.4%); while the least resisted were augmentin 3 [amoxicillin and clavulanate potassium: 875 mg] (12.9%); ampiclox [ampicilin/cloxacillin: 500 mg] (16.1%); amoxil 2 [amoxicillin: 500mg] / oflomed [ofloxacin: 200 mg] (17.7%) and mefacol [chloramphenicol: 100mg] (25.8%).

Table 6 shows that the Gram-negative bacterial species were mostly resistant towards erymycin [erythromycin: 500mg] (60.6%); mediphenicol [chloramphenicol 100mg] (63.6%); primpex [trimethoprim and sulfamethoxazole mg] (66.7%); septrin [cotrimoxazole: 250mg] (75.8%) and axacef [cefuroxime: 250 mg] (78.8%).

Table 5: *In vitro* antibiotic resistance rates of Gram-positive strains from deteriorated, maggot-infested *iru* samples (clinical drugs)

Bacterial species	Antibiotics (mg/ml)																
	NOB	ERY1	SEP	AXA	ERY2	AUG1	OFLO	MED	TET	LOX	ETO	AMO1	AUG2	AMO2	PRIM	AUG3	MEF	AMP	%MAR
Bacillus	38.1	54.8	71.4	90.5	50.0	42.8	16.7	52.4	50.0	33.3	47.6	50.0	52.4	19.0	50.0	9.5	21.4	16.7	22.2-100
Micrococcus	20.0	20.0	40.0	60.0	40.0	60.0	20.0	40.0	60.0	40.0	40.0	0.0	60.0	0.0	0.0	0.0	20.0	0.0	16.7-66.7*
Staphylococcus	50.0	66.7	83.3	83.3	50.0	66.7	33.3	66.7	66.7	66.7	33.3	50.0	83.3	16.7	66.7	50.0	33.3	16.7	16.7-100
Streptococcus	11.1	44.4	44.4	44.4	11.1	33.3	11.1	33.3	33.3	33.3	33.3	44.4	33.3	22.2	22.2	11.1	44.4	22.2	11.1-100
Overall % Resistance	**33.9**	**53.2**	**66.1**	**77.4**	**41.9**	**45.2**	**17.7**	**51.6**	**38.7**	**38.7**	**43.5**	**45.2**	**53.2**	**17.7**	**43.5**	**12.9**	**25.8**	**16.1**	**11.1-100**

Keys: NOB = nobax (azithromycin: 250 mg); ERY 1 = erymycin (erythromycin: 500mg); SEP = septrin (cotrimoxazole : mg); AXA = axacef (cefuroxime: 250 mg); ERY 2 = erythromycin (erythromycin: 250 mg); AUG 1 = augmentin 1 (amoxicillin and clavulanate potassium: 100 mg); OFLO = oflomed (ofloxacin 200 mg); MED = mediphenicol (chloramphenicol mg); TET = tetracycline (mg); LOX = loxaprim (cotrimoxazole: 450 mg); ETO = etocin (erythromycin: mg); AMO 1 = amoxil (amoxicillin: 250 mg); AUG 2 = augmentin 2 (amoxicillin and clavulanate potassium: 375 mg); AMO 2 = amoxil (amoxicillin: 500 mg); PRIM = primpex (trimethoprim and sulfamethoxazole mg); AUG 3 = augmentin 3 (amoxicillin and clavulanate potassium: 875 mg); MEF = mefacol (chloramphenicol: mg); AMP = ampiclox (ampicilin/cloxacillin :500 mg); * = mono resistance.

Table 6: *In vitro* antibiotic resistance rates of Gram-negative species from deteriorated, maggot-infested *iru* samples (clinical drugs)

Bacterial species	Antibiotics (mg/ml)																		
	NOB	ERY1	SEP	AXA	ERY2	AUG1	OFLO	MED	TET	LOX	ETO	AMX1	AUG2	AMX2	PRIM	AUG3	MEF	AMP	%MAR
E. coli	25.0	75.0	100	75.0	50.0	50.0	25.0	75.0	25.0	25.0	25.0	25.0	50.0	25.0	50.0	25.0	50.0	50.0	16.7-100
Enterobacter	33.3	66.7	66.7	100	33.3	0.0	0.0	66.7	33.3	66.7	33.3	0.0	0.0	0.0	100	0.0	0.0	33.3	22.2-444*
Klebsiella	50.0	83.3	83.3	83.3	33.3	50.0	33.3	33.3	16.7	16.7	66.7	33.3	50.0	16.7	66.7	16.7	500	50.0	22.2-100
Proteus	33.3	16.7	16.7	83.3	33.3	0	16.7	66.7	16.7	0.0	16.7	33.3	0.0	0.0	33.3	0.0	33.3	0.0	16.7-33.3
Pseudomonas	20.0	40.0	80.0	40.0	60.0	60.0	20.0	40.0	20.0	0.0	40.0	20.0	60.0	20.0	80.0	0.0	0.0	20.0	11/1-55.6
Salmonella	62.5	75.0	100	87.5	50.0	62.5	25.0	87.5	37.3	37.5	50.0	25.0	75.0	25.0	75.0	25.0	37.5	25.0	27.8-100
Shigella	100	100	100	100	100	100	0.0	100	100	0.0	0.0	0.0	100	0.0	100	100	100	100	61.1
Overall % Resistance	**42.4**	**60.6**	**75.8**	**78.8**	**42.4**	**42.4**	**21.2**	**63.6**	**27.3**	**21.2**	**39.4**	**24.2**	**45.5**	**15.2**	**66.7**	**15.2**	**27.3**	**27.3**	**11.1-100**

Keys: NOB = nobax (azithromycin: 250 mg); ERY 1 = erymycin (erythromycin: 500mg); SEP = septrin (cotrimoxazole : mg); AXA = axacef (cefuroxime: 250 mg); ERY 2 = erythromycin (erythromycin: 250 mg); AUG 1 = augmentin 1 (amoxicillin and clavulanate potassium: 100 mg); OFLO = oflomed (ofloxacin 200 mg); MED = mediphenicol (chloramphenicol mg); TET = tetracycline (mg); LOX = loxaprim (cotrimoxazole: 450 mg); ETO = etocin (erythromycin: mg); AMX 1 = amoxil (amoxicillin: 250 mg); AUG 2 = augmentin 2 (amoxicillin and clavulanate potassium: 375 mg); AMX 2 = amoxil (amoxicillin: 500 mg); PRIM = primpex (trimethoprim and sulfamethoxazole mg); AUG 3 = augmentin 3 (amoxicillin and clavulanate potassium: 875 mg); MEF = mefacol (chloramphenicol: mg); AMP = ampiclox (ampicilin/cloxacillin :500 mg); * = mono resistance.

Table 7: Overall in vitro antibiotic resistance profiles of Gram-positive bacterial species from deteriorated maggot-infested *iru* samples (clinical drugs)

S/N	Bacterial species	Antibiotics (mg/ml)																	%MAR
		NOB	AXA	AMX1	AMX2	AUG1	AUG2	AUG3	AMP	MED	MEF	LOX	SEP	ERY1	ERY2	ETO	OFL	TET	PRIM	
1	*Micrococcus*					AUG1				MED			SEP							5.6 *
2	*Streptococcus*											LOX	SEP	ERY1		ETO		TET		11.1
3	*Staphylococcus*							AUG3			MEF									16.7
4	*Streptococcus*			AMX1	AMX2	AUG1					MEF	LOX	SEP	ERY1		ETO		TET		16.7
5	*Streptococcus*		AXA							MED					ERY2				PRIM	16.7
6	*Micrococcus*		AXA																	16.7
7	*Streptococcus*		AXA	AMX1												ETO				16.7
8	*Bacillus*		AXA					AUG3				LOX				ETO		TET		16.7
9	*Bacillus*	NOB								MED			SEP	ERY1		ETO				22.2
10	*Bacillus*					AUG1		AUG3				LOX	SEP				OFL		PRIM	22.2
11	*Streptococcus*					AUG1					MEF			ERY1						22.2
12	*Bacillus*		AXA	AMX1					AMP	MED					ERY2			TET		22.2
13	*Bacillus*		AXA	AMX1	AMX2									ERY1		ETO				22.2
14	*Bacillus*		AXA	AMX1						MED					ERY2			TET		22.2
15	*Bacillus*		AXA										SEP	ERY1		ETO				22.2
16	*Bacillus*		AXA									LOX	SEP		ERY2			TET		22.2
17	*Streptococcus*		AXA									LOX	SEP	ERY1						22.2
18	*Streptococcus*		AXA	AMX1						MED		LOX			ERY2	ETO		TET		27.8
19	*Bacillus*					AUG1		AUG3		MED		LOX	SEP							27.8
20	*Micrococcus*										MEF					ETO		TET		27.8
21	*Streptococcus*			AMX1					AMP					ERY1					PRIM	27.8
22	*Bacillus*	NOB		AMX1					AMP					ERY1		ETO		TET	PRIM	27.8
23	*Bacillus*	NOB	AXA	AMX1										ERY1					PRIM	27.8
24	*Bacillus*		AXA	AMX1									SEP	ERY1		ETO				27.8
25	*Bacillus*		AXA			AUG1		AUG3		MED						ETO				27.8
26	*Bacillus*		AXA					AUG3		MED		LOX	SEP							27.8
27	*Bacillus*		AXA							MED			SEP			ETO			PRIM	27.8
28	*Bacillus*		AXA					AUG3				LOX				ETO			PRIM	27.8
29	*Micrococcus*		AXA					AUG3			MEF				ERY2	ETO		TET	PRIM	33.3
30	*Bacillus*			AMX1							MEF	LOX				ETO		TET	PRIM	33.8
31	*Bacillus*	NOB	AXA										SEP	ERY1	ERY2				PRIM	33.8
32	*Bacillus*	NOB	AXA								MEF		SEP	ERY1	ERY2					33.8
33	*Staphylococcus*		AXA	AMX1			AUG2	AUG3					SEP						PRIM	33.3
34	*Bacillus*	NOB	AXA	AMX1						MED			SEP		ERY2			TET		38.8
35	*Bacillus*	NOB	AXA					AUG3		MED				ERY1	ERY2			TET		38.8
36	*Bacillus*	NOB	AXA					AUG3		MED			SEP	ERY1	ERY2				PRIM	38.8
37	*Bacillus*		AXA	AMX1	AMX2	AUG1		AUG3		MED			SEP							38.8
38	*Bacillus*		AXA	AMX1		AUG1		AUG3					SEP		ERY2				PRIM	38.8
39	*Bacillus*		AXA	AMX1		AUG1				MED			SEP	ERY1		ETO				38.8
40	*Bacillus*		AXA			AUG1		AUG3		MED			SEP	ERY1				TET		38.8
41	*Bacillus*	NOB	AXA			AUG1		AUG3		MED			SEP	ERY1	ERY2					44.4
42	*Bacillus*		AXA			AUG1	AUG2	AUG3		MED	MEF		SEP	ERY1						44.4
43	*Bacillus*		AXA	AMX1						MED		LOX	SEP		ERY2	ETO		TET		44.4
44	*Staphylococcus*		AXA	AMX1		AUG1		AUG3		MED		LOX				ETO		TET		44.4
45	*Bacillus*		AXA			AUG1		AUG3					SEP		ERY2		OFL	TET	PRIM	44.4
46	*Bacillus*		AXA	AMX1				AUG3				LOX	SEP	ERY1	ERY2				PRIM	44.4
47	*Bacillus*	NOB	AXA	AMX1	AMX2		AUG2						SEP	ERY1	ERY2			TET		50.0
48	*Bacillus*		AXA	AMX1		AUG1		AUG3		MED			SEP	ERY1	ERY2	ETO	OFL		PRIM	50.0
49	*Bacillus*	NOB	AXA			AUG1		AUG3		MED			SEP	ERY1	ERY2		OFL		PRIM	55.6
50	*Staphylococcus*	NOB	AXA			AUG1		AUG3		MED			SEP	ERY1	ERY2		OFL	TET	PRIM	55.6
51	*Bacillus*		AXA			AUG1		AUG3	AMP		MEF	LOX	SEP	ERY1		ETO			PRIM	55.6
52	*Bacillus*		AXA	AMX1		AUG1		AUG3		MED		LOX	SEP		ERY2	ETO		TET	PRIM	55.6
53	*Bacillus*	NOB	AXA	AMX1	AMX2				AMP	MED	MEF	LOX	SEP			ETO			PRIM	61.1
54	*Bacillus*	NOB	AXA	AMX1	AMX2			AUG3					SEP	ERY1	ERY2		OFL	TET		61.1
55	*Micrococcus*	NOB	AXA			AUG1		AUG3		MED			SEP	ERY1	ERY2	ETO	OFL	TET	PRIM	66.7
56	*Staphylococcus*	NOB	AXA			AUG1	AUG2	AUG3		MED	MEF	LOX	SEP	ERY1	ERY2			TET	PRIM	72.2
57	*Bacillus*	NOB	AXA			AUG1		AUG3		MED		LOX	SEP	ERY1	ERY2	ETO	OFL		PRIM	72.2
58	*Bacillus*		AXA	AMX1	AMX2	AUG1		AUG3	AMP	MED	MEF	LOX	SEP	ERY1	ERY2	ETO		TET	PRIM	77.8
59	*Bacillus* [2]	NOB	AXA	AMX1	AMX2	AUG1	AUG2	AUG3	AMP	MED	MEF	LOX	SEP	ERY1	ERY2	ETO	OFL	TET	PRIM	100
	Streptococcus	NOB	AXA	AMX1	AMX2	AUG1	AUG2	AUG3	AMP	MED	MEF	LOX	SEP	ERY1	ERY2	ETO	OFL	TET	PRIM	100
	Staphylococcus	NOB	AXA	AMX1	AMX2	AUG1	AUG2	AUG3	AMP	MED	MEF	LOX	SEP	ERY1	ERY2	ETO	OFL	TET	PRIM	100

Keys: NOB = nobax (azithromycin: 250 mg); ERY 1 = erymycin (erythromycin: 500mg); SEP = septrin (cotrimoxazole : mg); AXA = axacef (cefuroxime: 250 mg); ERY 2 erythromycin (erythromycin: 250 mg); AUG 1 = augmentin 1 (amoxicillin and clavulanate potassium: 100 mg); OFLO = oflomed (ofloxacin 200 mg); MED = mediphenic (chloramphenicol mg); TET = tetradox [tetracycline: mg]; LOX = loxapnim (cotrimoxazole: 450 mg); ETO = etocin (erythromycin: mg); AMX 1 = amoxil (amoxicillin: 250 mg); AUG 2 augmentin 2 (amoxicillin and clavulanate potassium: 375 mg); AMX 2 = amoxil (amoxicillin: 500 mg); PRIM = primpex (trimethoprim and sulfamethoxazole mg); AUG 3 = augmentin (amoxicillin and clavulanate potassium: 625 mg); MEF = mefacol (chloramphenicol: mg); AMP = ampiclox (ampicillin/cloxacillin :500 mg). * = mono resistance

Table 8: In vitro antibiotic resistance profiles of Gram-negative species from deteriorated, maggot-infested *iru* samples (clinical drugs)

S/N Bacterial species	Antibiotics (mg/ml)															%MAR
	NOB	AXA	AMX1	AMX2	AUG1	AUG2	AUG3	AMP	MED	MEF	LOX	SEP	ERY1	ERY2	ETO	OFL	TET	PRIM	
1 *Proteus mirabilis*		AXA																	5.6*
2 *Ps aeruginosa*												SEP						PRIM	11.1
3 *E. coli*		AXA						AMP				SEP							16.7
4 *Proteus mirabilis*		AXA								MEF								PRIM	16.7
5 *E. coli*									MED			SEP	ERY1	ERY2					22.2
6 *Kleb pneumoniae*		AXA						AMP				SEP			ETO				22.2
7 *Enterobacter sp*		AXA						AMP			LOX							PRIM	22.2
8 *Proteus mirabilis*		AXA							MED	MEF				ERY2					22.2
9 *Salm typhi*									MED			SEP	ERY1	ERY2			TET		27.8
10 *Proteus mirabilis*									MED			SEP		ERY2		OFL	TET		27.8
11 *Kleb pneumoniae*	NOB					AUG2		AMP					ERY1		ETO				27.8
12 *Proteus mirabilis*	NOB	AXA	AMX1						MED						ETO				27.8
13 *Ps aeruginosa*			AMX1	AMX2								SEP	ERY1		ETO		TET		33.3
14 *Salm typhi*		AXA			AUG1	AUG2			MED			SEP			ETO				33.3
15 *Salm typhi*		AXA			AUG1	AUG2			MED			SEP						PRIM	33.3
16 *Ps aeruginosa*		AXA			AUG1	AUG2			MED					ERY2				PRIM	33.3
17 *Ps aeruginosa*					AUG1	AUG2						SEP	ERY1	ERY2	ETO			PRIM	38.8
18 *Kleb pneumoniae*		AXA	AMX1		AUG1	AUG2					LOX	SEP	ERY1					PRIM	38.8
19 *Enterobacter sp*	NOB	AXA							MED		LOX	SEP	ERY1					PRIM	38.8
20 *Proteus mirabilis*	NOB	AXA	AMX1						MED				ERY1					PRIM	33.3
21 *Kleb pneumoniae*	NOB	AXA								MEF		SEP	ERY1			OFL		PRIM	38.8
22 *Enterobacter sp*		AXA							MED			SEP	ERY1	ERY2	ETO		TET	PRIM	44.4
23 *E. coli*		AXA			AUG1	AUG2			MED	MEF		SEP	ERY1					PRIM	44.4
24 *Salm typhi*	NOB	AXA				AUG2			MED			SEP	ERY1		ETO			PRIM	44.4
25 *Salm typhi*	NOB	AXA								MEF	LOX	SEP	ERY1	ERY2				PRIM	44.4
26 *Salm typhi*	NOB	AXA			AUG1	AUG2			MED			SEP	ERY1					PRIM	44.8
27 *Kleb pneumoniae*		AXA			AUG1				MED	MEF		SEP	ERY1	ERY2	ETO			PRIM	50.0
28 *Ps aeruginosa*	NOB	AXA			AUG1	AUG2		AMP	MED			SEP		ERY2		OFL		PRIM	55.6
29 *Sh dysentariae*	NOB	AXA			AUG1	AUG2	AUG3		MED			SEP	ERY1	ERY2			TET	PRIM	61.1
30 *E. coli*	NOB	AXA	AMX1	AMX2	AUG1	AUG2	AUG3	AMP	MED	MEF	LOX	SEP	ERY1	ERY2	ETO	OFL	TET	PRIM	100
Kleb pneumoniae	NOB	AXA	AMX1	AMX2	AUG1	AUG2	AUG3	AMP	MED	MEF	LOX	SEP	ERY1	ERY2	ETO	OFL	TET	PRIM	100
Salm typhi [2]	NOB	AXA	AMX1	AMX2	AUG1	AUG2	AUG3	AMP	MED	MEF	LOX	SEP	ERY1	ERY2	ETO	OFL	TET	PRIM	100

Keys: NOB = nobax (azithromycin: 250 mg); ERY 1 = erymycin (erythromycin: 500mg); SEP = septrin (cotrimoxazole : mg); AXA = axacef (cefuroxime: 250 mg); ERY 2 = erythromycin (erythromycin: 250 mg); AUG 1 = augmentin 1 (amoxicillin and clavulanate potassium: 100 mg); OFLO = oflomed (ofloxacin 200 mg); MED = mediphenicol (chloramphenicol mg); TET = tetradox [tetracycline: mg]; LOX = loxaprim (cotrimoxazole: 450 mg); ETO = etocin (erythromycin: mg); AMO 1 = amoxil (amoxicillin: 250 mg); AUG 2 = augmentin 2 (amoxicillin and clavulanate potassium: 375 mg); AMO 2 = amoxil (amoxicillin: 500 mg); PRIM = primpex (trimethoprim and sulfamethoxazole mg); AUG 3 = augmentin 3 (amoxicillin and clavulanate potassium: 625 mg); MEF = mefacol (chloramphenicol: mg); AMP = ampiclox (ampicillin/cloxacillin :500 mg); * = mono resistance.

The least resisted however, were amoxil 2 [amoxicillin: 500 mg] / augmentin 3 [amoxicillin and clavulanate potassium: 875 mg] (15.2%); oflomed [ofloxacin 200 mg] / loxaprim [cotrimoxazole: 450 mg] (21.2%); amoxil 1 [amoxicillin: 250 mg] (24.2%) and mefacol [chloramphenicol: 100mg] / ampiclox [ampicilin/cloxacillin: 500 mg] / tetradox tetracycline mg] (27.3%). It was observed in this study that 59 and 30 different antibiotic resistance profiles were exhibited by the Gram-positive and Gram-negative bacterial species from deteriorated maggot-infested *iru* samples respectively (Tables 7 & 8).

Conclusion

Studies involving fermentation of legumes for condiment production usually observed a steady increase in pH with fermentation period, which is due to the ability of the dominating Bacillus spp. to hydrolyse proteins into amino acids and ammonia (Whitaker, 1978; Parkouda, 2009) or due to the protease and deaminase enzymes produced by the fermenting Bacillus spp. (Hesseltine, 1979). The preservation and flavour characteristics of these fermented condiments are derived in part from the liberation of ammonia and increased pH concurrent with protein hydrolysis of free amino acids and peptides. The pH of deteriorated, maggot-infested *iru* samples, studied in the current work had the same alkaline range (pH 6.8 - 8.5) as fresh *iru* samples but still, the deteriorated, maggot-infested *iru* samples were not fit for human consumption. Similarly, Wokoma and Aziagba (2001) reported that fresh dawadawa products prepared from African yam bean seeds (Sphenostylis Stenocarpa Harms) and soybean seeds, which were stored at room temperature were highly perishable, showing the most pronounced signs of spoilage with a rapid onset of loss of fresh appearance, production of offensive odour and maggot infestation.

During fermentation, post-fermentation processing and in home-cooking application, it is likely that several volatile molecules are generated, which reflects the characteristic flavour for which *iru* is known. The flavour properties of *iru* are most likely due to its amino acid content, and in particular glutamate, which contribute to flavour enhancement, as well as peptides and aroma volatile constituents. Volatiles may of course be directly produced during fermentation or may evolve as a result of heat on amino acid and fatty acid constituents of *iru*. Although, the contribution of the accompanying bacterial flora to the properties of the legume products is only partly understood (Iwuoha and Eke, 1996), they most probably play a role in flavour development. Evidence for the participation of indigenous enzymes and flora in the development of the flavour of *iru* was presented by Ikenobomeh, et al. (1986), and often, the finished products are of indigenous character, which exhibit sensory properties that result from the unique fermenting bacterial flora. A study on the characterisation of aroma volatile in Bacillus-fermented soybean also indicated the formations of aroma active aldehydes, ketones and acids (Owens et al,, 1997); therefore, the inability of the microbial flora in the maggot-infested *iru* to exhibit the peculiar characteristic flavour and aroma of *iru* could be due to metabolic secretions of the infesting maggots, when feeding on and degrading the condiment.

Each female housefly (Musca domestica), which is considered a pest that can carry serious diseases (Larrañ and Salas, 2008; Wikipedia, 2011) can lay approximately 500 eggs (white and about 1.2 mm in length) in several batches of about 75 – 150 (Bennett, 2003) and within a day, larvae (maggots) hatch from the eggs. The maggots are pale-whitish, 3.0–9.0 mm long, thinner at the mouth end and without legs, normally living for at least one week and feeding on (usually dead and decaying) organic materials, such as decaying food and depending on the species, they develop and consume food quickly (http://www.gardening-naturally.com/acatalog/Maggot_In_Food.html). It has been reported that the dynamics of fermentation in any food matrix is a complex microbiological process involving interactions between quite a number of different microorganisms (Daeschel, 1987), while the contribution of the accompanying flora of fermenting substrates is largely determined by the substrate composition and hygiene during production. During fermentation, the microorganisms use the nutritional components of the seeds and convert them into products that contribute to the chemical composition and taste of the condiments. Bacillus species were reported to be mostly responsible for the alkaline fermentation of the vegetable condiments but several workers have identified other microorganisms associated with *iru* and diverse groups of bacteria comprising of Bacillus, Micrococcus, Leuconostoc, Staphylococcus species and Enterobacteriaceae have been reported in the fermentation of locust bean seeds to *iru* (Anosike and Egwuatu, 1981; Odunfa, 1981; Obeta and Ugwuanyi, 1996; Omafuvbe et al., 2002; Ogunshe and Olasugba, 2008).

The genera of bacteria isolated from the maggot-infested *iru* samples in this study- Bacillus, Staphylococcus, Streptococcus, Staphylococcus, Enterobacter, E. coli, Klebsiella, Salmonella, Shigella, Proteus and Pseudomonas, were similar to those isolated from non maggot-infested *iru* samples by earlier workers. However, multiple antibiotic resistance were recoded among the bacterial flora. Several antimicrobial agents have been evaluated and have become available for use in bacterial infectious conditions but the fact that extremely very high multiple antibiotic resistance were recorded towards most of the commonly used antibiotics / drugs indicated the prevalence of multiple antibiotic resistant bacteria in deteriorated, maggot-infested *iru*, which is a major public health concern of considerable food and medical significance, and this can potentially lead to widespread dissemination of multi-antimicrobial-resistant bacterial pathogens, most especially in foods (Schwarz and Charius-Dancla, 2001). Therefore, usage of

maggot-infested condiments in food preparations after rinsing must be discouraged, and there is also the need for better and safer modes of storing and packaging fermented food condiments like *iru* at the traditional levels of production.

With the exception of Ogudana (1980) who isolated fungal flora, several workers have reported that only bacterial flora are associated with fermentations of protein seeds into food condiments; therefore, the recovery of certain moulds like Aspergillus niger, Aspergillus flavus, Candida, Penicillium, Rhizopus and Botrydiplodia species from deteriorated, maggot-infested *iru* samples in this study can lead to the conclusion that these groups of fungal species may have been introduced by the maggots. It is commonly observed that the only mode of bio-deterioration of *iru* condiment is due to infestation by maggots when improperly preserved or stored, which at times is often over-looked. In the olden days, ogiri were preserved by the Ijebus simply by keeping the ogiri paste already wrapped in leaves within paper layers under their stew / soup pots. As soon as the hot pots are taken off the fire they are immediately placed in wider basins than the cooking pots already with the paper layers containing the ogiri. This is thus a means of heat preservation of the condiment and maggot-infestations were never reported through this traditional preservation technology.

One of the limitations of this study was the inability to culture the gut contents of the maggots in order to determine if their gut contents contained similar microbial flora, and it would have been conclusive to report that the maggots were likely sources of the bacteria species isolated from the deteriorated, maggot-infested *iru* samples. It is however, concluded in this study that maggot infestation of fermented food condiments related to food spoilage and recovery of fungal flora from such foods may be responsible for food poisoning and should be considered as an indication of food spoilage and a method for evaluating food safety and improving microbial food quality of such category of foods.

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