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Prevalence and antibiotic resistance of bacteria isolated from ready-to-eat (RTE) food samples of highly patronized eateries in Ogbomoso-Oyo state, Nigeria

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ABSTRACT

The study was designed to determine the prevalence and antibiotic resistance of bacterial isolates of ready- to-eat-rice from four (4) of the major eateries in Ogbomoso, Oyo State, Nigeria. General purpose and non-selective media were used for isolation and a total of ten (10) bacteria were identified. Culture plates with less than 30 colonies and those with more than 300 colonies were removed and not included in the colony counts. The organisms obtained were subsequently sub-cultured and subjected to various biochemical characterization tests for identification. The organisms identified with their percentage of occurrence were Bacillus cereus (30.4%), B. marcescens (4.3%), B. subtilis (4.3%), Streptococcus faecalis (4.3%), S. faecium (13.0%), Staphylococcus aureus, (17.4%) Pseudomonas putida (4.3), Proteus vulgaris (4.3%), Micrococcus luteus (13.0%) and M. acidiophilus (4.3%). Bacteria isolated were tested against six (6) different antibiotics which are Ofloxacin OFL,(5µg); Erythromycin ERY, (10µg); Gentamicin GEN, (10µg); Ceftriaxone CEF, (30µg); Ciprofloxacin CIP, (5µg); and Cotrimoxazole COT, (50µg) in order to determine their susceptibility. Based on the overall effectiveness, Ceftriaxone and Ofloxacin were the least active, while Cotrimoxazole was the most active against the isolates.

Key words: antibiotic, resistance, ready-to-eat-rice, bacterial isolates.

INTRODUCTION

The interactions between microorganisms, plants and animals are natural and constant, and since the human food supply consists basically of plants and animals or products derived from them, it is understandable that our food supply can contain microorganisms in interaction with the food (William and Dennis, 1995). In most cases, these microorganisms use our food supply as a source of nutrient for their own growth. This of course can result in deterioration of the food. When the microorganisms involved are pathogenic, their association with human food is critical from a public health point of view (Bautista *et al.*, 1988). Many of human foods support the growth of pathogenic microorganisms or at least serve as a vector of them. The World Health Organization, thus concluded, 'Food borne disease is perhaps the most widespread health problem in the contemporary world and an important cause of reduced economic productivity'. It is therefore advisable and reasonable to prevent the entrance and growth of microorganisms in foods or eliminate them by processing. Ideally, cooking or heating is expected to reduce the microbial load of cooked foods, but such foods can also become contaminated due to cross contamination, particularly from raw meat or poultry (Ayres, 1960; Singleton, 1997;) and environmental sources such as air and dust, food utensils and food handlers. The effects of microorganisms in food can be to cause spoilage or food-borne illness (Adesiyun, 1984; Johnson, 1984). The properties of food material itself such as moisture content, hydrogen-ion concentration, temperature, and nutritive values are among the important factors that determine the types of microorganisms and the extent to which they are present in a food material (Garbutt, 1997; William and Dennis, 1995; Johnson *et al*., 1983). This piece of work looked into the safety and sterility, the types and prevalence of microorganisms in boiled rice from the selected sites within Ogbomoso on the one hand and the susceptibility of the isolates to the selected antibiotics on the other hand. Boiled rice was chosen because it is one of the most consumed foods around and in order to reduce microbial load as a result of cross contamination, food materials such as meat or chicken, egg, beans, plantain and stew that may be added to boiled rice before eating were not included. The work also looked into likely sources of contamination of cooked or ready-to-eat (RTE) foods, because it is expected that cooking (which involves heating) will reduce, to a reasonable level, the microbial load of foods. The aim of this study is to create awareness to the public about the menace of microbial food contamination among eateries in Ogbomoso and to investigate the degree of resistance and susceptibility of the bacterial isolates to tested antibiotics.

MATERIALS AND METHODS

Sample source and collection

The food sample collected and used for this research was boiled rice only. The samples were collected once from four (4) of the very popular eateries at different locations in Ogbomoso, Oyo State, Nigeria, West Africa. Samples were labeled as; FEG, FEL, FEA and FEU based on location and carefully transferred aseptically to the laboratory immediately for microbial analysis.

Isolation and Identification of Organisms

The isolation of microorganisms present in the collected food samples was done using the serial dilution procedure (James and Sherman, 2001). 10g of the food sample was transferred

aseptically into 100ml distilled water in 250ml sterile flask. The flask was allowed to stand for about 20minutes and then swirled to ensure even dispersal of food particles. Serial dilution was done by using sterile test tubes labeled T_1, T_2, T_3, T_4 and T_5, each containing 9ml of distilled. 1ml of the suspension in the 100ml flask was transferred aseptically with micro-pipette into tube T_1 and from T_1 to T_2 in the same sequence up to T_5. 100µl of the suspension was then used to inoculate nutrient agar plates for 24 hours at 37^0C. Plates with more than 300 colonies and those with less than 30 colonies tagged 'Too Numerous To Count' (TNTC) and 'Too Few To Count' (TFTC) respectively were not included in the colony-forming unit count Isolated micro-organisms were subjected to various biochemical tests for identification.

Antibiotic susceptibility testing

This was done according to method of the British Society for Antimicrobial chemotherapy, (BSAC) and the National Committee on Clinical Laboratory Standards, (NCCLS). Six different antibiotics were used and these are Erythromycin, ERY, (10µg); Ofloxacin, OFL, (5µg); Ceftriaxone CEF, (30µg); Gentamicin, GEN, (10µg); Cotrimoxazole, COT (50µg); and Ciprofloxacin, CIP, (5µg).

Preparation of inoculum

The growth method was used in the preparation of the inocula. Organism was picked from fresh colonies on plates containing the test organism, inoculated into each of 5ml freshly prepared nutrient broth. The tubes were incubated at 37^0C for 24 hours and the density of the suspension in each tube matched with that of 0.5 McFarland turbidity standards prepared according to the BSAC method.

Test plates inoculation

This was done with the aid of sterile swab sticks within 15minutes after adjusting the turbidity of the inoculum suspensions. The swab in each case was rotated and pressed firmly on the inside wall of the tube containing the broth standardized inoculum suspension above the fluid level. The swab was used to inoculate the surface of a nutrient agar plate by streaking it over the entire agar surface.

Discs application to inoculated agar plates

Antibiotic discs used were mounted aseptically, onto the surface of the inoculated agar plates. Each disc was firmly pressed to ensure complete contact with the agar surface. The discs were evenly distributed so that they are not too close to one another. In an inverted position, the plates were incubated at 37^0C for 18 hours.

Statistics

The results obtained were analyzed with Origin8 Lab software (2007 version). One paired sample t-test and ANOVA were used for assessing the test of significance at 5% level of probability at df = (n-1).

RESULTS AND DISCUSSION

Isolation and characterization of bacterial isolates

Ten (10) different organisms were isolated from the four collected food samples. Isolates were subjected to biochemical characterization and identified using Bergey's Manual of Systemic Bacteriology (Table 6). The colony-forming units (cfu) of the isolates from each site, distribution and the frequencies of occurrence are as shown in Tables 1(a-d), Table 2 and Table 3 respectively.

Plate reading and results interpretation

The six (6) different genera isolated from the food samples from the four selected eateries have been implicated in either food infection or food spoilage at one time or the other (Dainty *et al*., 1983; Gill, 1982; Griffiths, 1990; Jay, 1987). The colony count and the colony forming units per gram of the isolates Tables 1(a-d) showed the microbial load and the extent to which the food sample from each eatery was contaminated. From all the bacterial isolates identified, the genus *Bacillus* was the most prevalent (Tables 2 and 3), and this agrees with the report of Johnson (1984) that the genus is well known for its close association with cereal and flour products. *Bacillus cereus* produces different toxins (the emetic toxins), one of which is heat- stable with heat stability at 126^{o}C for 90 minutes, and a wide pH tolerance of 2 to 11 These factors and the ability to produce aerophilic spores have contributed greatly to the most dominant and prevalent character exhibited by *Bacillus spp.* in this work. The genera, *Staphylococcus, Enterococcus, Micrococcus, Pseudomonas* and *Proteus* were also isolated due to various ways through which they can get into foods. Apart from *Staphylococcus* which is involved in food infection, other genera isolated have been implicated in spoilage of other food materials apart from cereals (Hobbs, 1983). Such foods include vegetables, meats, poultry, and sea foods. These genera are not usually associated with cereal contamination and their isolation from boiled rice is likely due to cross contamination (William and Dennis, 1995).

Table 1a: Colony count and colony forming unit (CFU) per gram of food sample from "FEG"

Tube	Colony Number	CFU/g
T_1	TNTC	nd
T_2	104	1.0 X 10^5
T_3	TFTC	nd
T_4	TFTC	nd
T_5	TFTC	nd

*nd; not determined,

Table 1b: Colony count and colony forming unit (CFU) per gram of food sample from "FEL"

Tube	Colony Number	CFU/g
T_1	TNTC	nd
T_2	184	1.0 X 10^5
T_3	83	8.3 x 10^5
T_4	TFTC	nd
T_5	TFTC	nd

**nd; not determined,*

Table 1c: Colony count and colony forming unit (CFU) per gram of food sample from "FEA"

Tube	Colony Number	CFU/g
T_1	TNTC	nd
T_2	239	2.4×10^5
T_3	193	1.9×10^6
T_4	72	7.2×10^6
T_5	TFTC	nd

**nd; not determined,*

Table 1d: Colony count and colony forming unit (CFU) per gram of food sample from "FEU"

Tube	Colony Number	CFU/g
T_1	TNTC	nd
T_2	115	1.2×10^5
T_3	TFTC	nd
T_4	TFTC	nd
T_5	TFTC	nd

**nd; not determined,*

Table 2: Distribution of bacterial isolates from each of the sample source

Sample source	Isolates
FEG	*Streptococcus faecium*
	Micrococcus luteus
	Staphylococcus aureus
	Pseudomonas putida
FEL	*Bacillus cereus*
	Micrococcus luteus
	S. aureus
FEA	*Bacillus cereus*
	Streptococcus faecium
	Streptococcus faecalis
	S. aureus
FEU	*Bacillus marcescens*
	Streptococcus faecium
	Proteus vulgaris
	Micrococcus acidiophilus
	B. cereus
	B. subtilis

Table 3: Frequency of occurrence of each bacterial isolate

Isolate	Frequency	Cumulative frequency	% occurrence
Bacillus cereus	7	7	30.4
B. subtilis	1	8	4.3
B. marcescens	1	9	4.3
Micrococcus luteus	3	12	13.0
M. acidiophilus	1	13	4.3
Pseudomonas putida	1	14	4.3
Proteus vulgaris	1	15	4.3
Streptococcus faecalis	1	16	4.3
S. faecium	3	19	13.0
Staphylococcus aureus	4	23	17.4

Bacterial response to antibiotics

Plates were examined for susceptibility and resistance to the tested antibiotics after 24 hours of incubation at 37^{o}C. The diameters of zones of complete inhibition were recorded as shown in Table 4 and interpreted, by comparing the results with the zone diameter Interpretative standards of BSAC and NCCLS. The isolates were reported as susceptible (S), intermediate (I), resistant, (R) to the tested antibiotics (Table 5). Table 6 shows the percentage antibiotic resistance of each of the bacterial isolates to the tested antibiotics. The results of the antibiotic susceptibility test showed that all the bacterial isolates were totally resistant to Ofloxacin and Ceftriaxone (100%), followed by Gentamicin (70%). Only 20% of the isolates were resistant to Erythromycin, 10% resistant to Ciprofloxacin and none to Cotrimoxazole (Table 4). Moreover, the zones of inhibition of each of the bacterial isolates to all the tested antibiotics are significantly different from each other (Pr < 0.05) with the highest critical difference (C.D) in *B. cereus* (12.01) and lowest in *Micrococcus acidiophilus* (1.53). In summary, the susceptibility pattern shown by all the isolates to the tested antibiotics and their diameters of zones of inhibition (Table 4) reveals the activity of the tested antimicrobials in this order of increasing activity. Ceftriaxone/Ofloxacin–→Gentamicin–→Erythromycin—→Ciprofloxacin–→ Cotrimoxazole.

Table 4: Zones of Inhibition of the bacterial isolates after 24 hours of incubation at 37^{o}C

Antibiotics	Zones of Inhibition (mm)									
	BC	**BM**	**BS**	**SF**	**SF^{2}**	**SA**	**PP**	**MA**	**ML**	**PV**
Erythromycin	27	18	26	20	21	21	17	24	31	32
Ofloxacin	21	10	05	00	00	19	18	12	26	24
Ceftriaxone	24	12	14	14	12	16	00	00	19	09
Gentamicin	16	13	18	18	13	11	19	11	21	26
Ciprofloxacin	25	18	17	25	19	0	23	20	27	26
Cotrimoxazole	24	25	20	20	21	25	26	24	34	32
*C.D at 5% (df =5)	12.01	4.71	3.40	2.11	1.92	1.79	2.19	1.53	8.84	4.75

NB: Each value is mean of triplicate values

BC = Bacillus cereus, BM = B. marcescens, SF^{1} = Streptococcus faecium, SF^{2} = S. faecalis, SA = Staphylococcus aureus, PP = Pseudomonas putida, MA = Micrococcus acidiophilus, ML = M. luteus, PV = Proteus vulgaris.

Table 5: Susceptibility Testing Result as Compared with Standards of the BSAC and NCCLS

Antibiotics	Bacteria response to antibiotics										
	BC	**BM**	**BS**	**SF^{1}**	**SF^{2}**	**SA**	**PP**	**MA**	**ML**	**PV**	**% Resistance**
Erythromycin	S	R	S	S	S	S	R	S	S	S	20
Ofloxacin	R	R	R	R	R	R	R	R	R	R	100
Ceftriaxone	R	R	R	R	R	R	R	R	I	R	100
Gentamicin	R	R	R	R	R	R	S	R	S	S	70
Ciprofloxacin	S	S	S	S	I	R	I	S	S	S	10
Cotrimoxazole	S	S	S	S	S	S	S	S	S	S	0

BC = Bacillus cereus, BM = B. marcescens, B = Bacillus subtilis

SF^{1} = Streptococcus faecium, SF^{2} = S. faecalis, SA = Staphylococcus aureus,

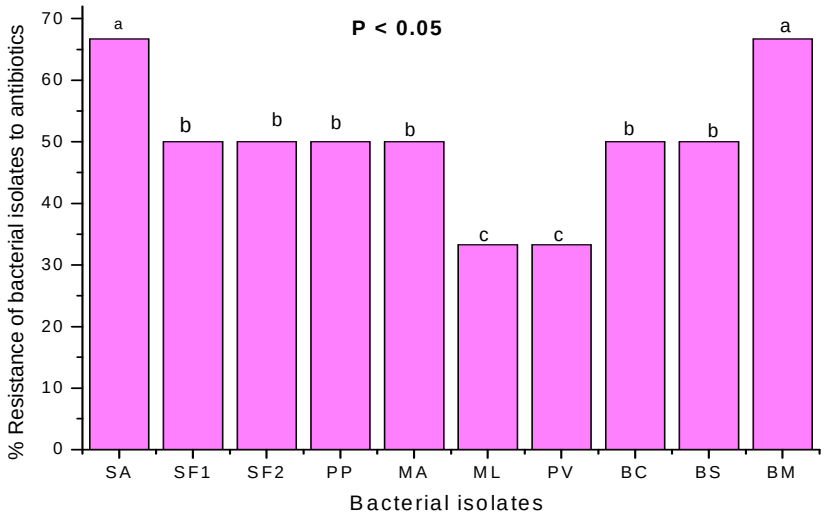
PP = Pseudomonas putida, MA = Micrococcus acidiophilus, ML = M. luteus,PV = Proteus vulgaris.

R = Resistance, I = Intermediate S = Sensitive/Susceptible

Table 6: Biochemical characterization of bacterial isolates

GRAM STAIN	SHAPE	MOTILITY	CATALASE	OXIDASE	COAGULASE	UREASE	INDOLE	METHUL RED	VOGES PROSKAEUR	GELATIN HYDROLYSIS	STARCH HYDROLYSIS	CAESI NHYDROLYIS	CITRATEUTILIZATION	PIGMENTATION	H_2S PRODUCTION	O_2 RELATIONSHIP	FRUCTOSE	SUCROSE	LACTOSE	MANNITOL	ARABINOSE	XYLOSE	DULCITOL	RAFFINOSE	GLUCOSE	MALTOSE	ADONITOL	SACCHROSE	IDENTIFICATION
+	SR	+	+	+	-	+	-	-	-	+	+	+	-	+	-	A	A/G	A/G	A/G	A/G	A/G	A/G	A/G	A/G	A/G	A/G	A/G	A/G	*B. cereus*
+	SR	+	+	+	-	-	-	-	-	+	-	+	-	-	-	A	A/G	A/G	A/G	A/G	A/G	A/G	A/G	A/G	A/G	A/G	A/G	A/G	*B. marscecens*
+	C	-	+	-	+	+	+	-	-	+	+	-	+	+	+	A	A	A	A	A	A	A	A	A	A	A	A	A	*S. aureus*
+	S	-	+	-	-	+	+	-	-	+	+	-	+	+	+	A	A	A	A	A	A	A	A	A	A	A	A	A	*S. faecium*
-	R	+	+	+	-	+	+	+	-	-	+	-	-	-	-	A	A/G	A/G	A/G	A/G	A/G	d	D	A/G	A/G	A/G	A/G	A/G	*P. vulgaris*
+	S	-	-	-	-	-	-	+	-	-	-	+	-	-	-	A	A	A	A	A	A	A	A	A	d	A	A	d	*S. faecalis*
-	R	+	+	-	-	+	-	+	-	+	+	-	+	-	-	A	A	A	A	A	A	A	A	A	A	A	A	A	*P. putida*
+	C	+	+	+	-	+	+	-	-	-	+	-	+	-	+	A	A	A	A	A	A	A	A	A	A	A	A	A	*M. luteus*
+	C	+	+	+	-	-	-	-	-	+	-	+	-	-	-	A	A	A	A	A	D	A	A	A	A	A	A	A	*M. acidiophilus*
+	SR	+	+	+	-	-	-	-	-	-	-	+	-	+	-	A	A	A	A	A	A	A	A	A	A	A	A	A	*B. subtilis*

+	=	*Positive*	*C*	=	*Cocci*
-	=	*Negative*	*a*	=	*Aerobic*
R	=	*Rod*	*A*	=	*Acid Production*
SR	=	*Short Rod*	*A/G*	=	*Acid/Gas Production*
S	=	*Sphere*	*d*	=	*doubtful*



$a \neq b \neq c$

Fig.1. Percentage resistance of bacterial isolates to tested antibiotics

At the 0.05% probability level, the percentage resistances of the bacterial isolates to the tested antibiotics were significantly different from each other (Fig. 1).

The total resistance (100%) shown by all the isolates to Ceftriaxone; a Cephalosporin (Table 5) a broad-spectrum antibiotic which acts by inhibiting cell wall synthesis in growing or dividing cells (Kathleen and Arthur, 2000) is likely to be due to the presence of ß-lactamase which acts by cleaving the beta-lactam ring of cell wall, inhibiting antibiotics like ceftriaxone (Warren, 2006). Bacterial resistance to antibiotics may be due to R plasmids (Klech, and Lee, 1978; Silva and Hofer, 1995), which can be transferred between various strains of bacteria through conjugation and transformation processes. According to Hermansson *et al* (1997), some strains of bacterial resistance to antibiotics depend on the mobile genetic elements called transposons (Herwig *et al.*, 1997). Resistance can also be associated with the production of enzymes that modify and inactivate antibiotics (Koch, 1981) as earlier said.

Ofloxacin – a floroquinolone, bactericidal and broad spectrum antibiotic also showed a total resistance (100%) by all the isolates (Table 5) unlike ciprofloxacin in the same class of antibiotics that showed 10% resistance. This might be due to lower concentration and potency of these two antibiotics since both were used at the same concentration (5µg). Antibiotic resistant micro-organisms may be associated with reduced penetration of the antibiotic into the cell, or can result from active processes such as changes in the transport of those compounds onto or from the bacterial cells (Hermansson *et al*., 1997). All the isolates showed similar susceptibility pattern to cotrimoxazole- a sulphonamide antibiotic which is a combination of Sulfamethoxazole and trimethoprim in the ratio 1:5.

Summary: From our results, it was very clear that none of the bacterial isolates was resistant to Cotrimoxazole while all isolates were resistant to Ofloxacin and Ceftriaxone antibiotics. Also,

the concentration at which antibiotics were used is a factor that plays a significant role to bacteria response to antibiotics. It is therefore, very important that proper and healthy food hygiene is ensured in food eateries to minimize the risk of microbial cross contamination in ready-to-eat (RTE) foods.

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