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Full Length Research Paper

Antibiotic resistance profile of bacterial isolates from food sold on a University campus in south western Nigeria

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The antibiotic resistance profile of bacterial isolates from cooked food samples sold in different eateries on the campus of the University of Ado-Ekiti was investigated. A total of seventy-eight bacterial isolates belonging to six genera were encountered in the following proportion: *Escherichia coli* (29.5%), *Klebsiella* spp. (25.6%), *Proteus* spp. (18.0%), *Salmonella* spp. (12.8%), *Pseudomonas* spp. (11.5%) and *Enterobacter* spp. (2.5%). The antibiotic resistance pattern of the isolates revealed that resistance to six out of the eight antibiotic tested was above 50%. Nalidixic acid was the only antibiotic with a resistance rate below this range. Resistance to amoxicillin was the highest (89.1%), followed by augmentin (76.9%) and gentamycin (71.8%). The results suggest the need for intensive surveillance of isolates throughout food production continuum to prevent food-borne infections and also to detect emerging antimicrobial resistance phenotypes.

Key words: Antibiotic resistance, vended food quality, surveillance.

INTRODUCTION

Food consumption is an important pathway for bacteria to infect humans, hence the presence of antimicrobial resistant bacteria in foods warrants particular attention. Antimicrobial resistant bacteria have been recovered from both healthy humans (Okeke et al., 2000) and a wide variety of foods, which include vegetables (Osterblad et al., 1999), confectionary (Pinegar and Cooke, 1985) meat and meat products and poultry (Schoeder et al., 2004). Hence food contaminated by faecal material from healthy humans may be an important source of antibiotic resistant organisms that later cause human infections (Teuber, 1999; Schoeder et al., 2004).

Contamination of food may occur during and after processing of such food. Contamination of ready-to-eat food is of primary concern because such organisms may be pathogenic thereby leading to outbreak of food-borne illness. Moreover, non-pathogenic organisms that may contaminate man's food chain from time to time may serve as reservoir of genes for antimicrobial resistance in organisms. These genes are encoded by integrons that occur on plasmids or that are integrated into the bacterial chromosome (Goldstein et al., 2001). Antimicrobial resistant strains of animal or human commensals that do not produce disease may transmit their resistance genes to pathogenic organisms whenever they occur in humans.

Several investigations have been carried out on the microbial quality of ready-to-eat food retailed in low-income restaurants. However, the antimicrobial resistance and the potential risk constituted by such organisms particularly when they are not pathogenic have not been studied extensively. This study was therefore carried out to examine antimicrobial resistance in organisms present in ready-to-eat indigenous food on the campus of the University of Ado-Ekiti.

METHODS

Collection of samples

A total of 85 samples of 8 different food items sold in 22 different eateries on the campus of the University of Ado-Ekiti, Nigeria were

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Table 1. Distribution and proportion of bacterial contaminants in food.

Isolates Identified	Prevalence
E. coli	23 (29.4%)
Klebsiella spp	20 (25.64%)
Proteus spp	14 (17.95%)
Pseudomonas spp.	9 (11.54%)
Salmonella spp.	19 (12.82%)

analysed from February to July 2005. The food items sampled were fried rice, white rice, rice and beans (mixture), 'Eba', 'Amala', Pounded yam and soup. All the food samples were collected in sterile containers and brought immediately to the laboratory and processed within 30 min after collection. The food samples were purchased at the point of sale in the various eateries.

Microbiological analysis of food samples

The total aerobic and coliform counts of all the food samples were carried out using standard procedures. The food samples were also examined for specific food-borne pathogens by direct plating on selective agars such as Salmonella-Shigella Agar (SSA), Sorbitol MacConkey Agar (SMAC) and Thiosulfate Citrate Bile Salts Sucrose Agar (TCBS Agar) for Salmonella-Shigella, presumptive *E. coli* 0157 and *Vibrio* species respectively. All isolates were characterized using standard techniques as described by Olutiola et al. (1993).

Antibiotic susceptibility test

The antibiotic susceptibility test was determined by the disc diffusion method as described by Bauer et al. (1966). Eight different commercially prepared antibiotics discs (Abtek Biologicals Ltd, UK) were used and the concentration of each is as follows: amoxicillin (25 µg), cotrimoxazole (25 µg), nitrofurantoin (30 µg), gentamycin (10 µg), malidixic acid (30 µg), ofloxacin (30 µg), augmentin (30 µg), and tetracycline (30 µg). After 18 h incubation at 37°C, the size of the zone of inhibition was measured and interpreted by comparing with the standard antibiotic sensitivity chart to determine their resistance patterns.

RESULTS

A total number of 78 bacterial isolates belonging to six genera were identified in this study. The distribution of the bacterial isolates in the various food samples is presented in Table 1. *E. coli* was the most common bacterial isolate with an occurrence of 29.49%, followed by *Klebsiella* spp. (25.64%) and *Proteus* spp. (17.59%), *Salmonella* and *Pseudomonas* spp. occurred in 12.82 and 11.54% of the samples, respectively. *Enterobacter* was the organism with the lowest occurrence of 2.5%.

Table 2 presents the number of antibiotics to which resistance was demonstrated. Seventy isolates (89.1%) were resistant to amoxicillin, 60 (76.9%) to augmentin, 56 (71.8%) to gentamycin, 53 (67.9%), 43 (55.1%) and 42 (53.8%) were resistant to tetracycline, nitrofurantoin and cotrimoxazole, respectively. A smaller percentage of isolates (23.1%) were resistant to nalidixic acid while none of the isolates was resistant to ofloxacin (Table 3). A total of 26 different resistance patterns were observed in the isolates. The commonest resistance pattern was amoxicillin/ gentamycin/augmentin (AMX, GEN, AUG), which occurred in ten *E. coli* isolates (Table 4).

Table 2. Number of antibiotics to which resistance was shown by food isolates.

No. of Antibiotics to which resistance was demonstrated	No. of organisms that demonstrated resistance
0	5 (6.4%)
1	3 (3.8%)
2	4 (5.1%)
3	16 (20.5%)
4	8 (10.3%)
5	17 (21.8%)
6	9 (11.5%)
7	16 (20.5%)
8	- (0%)
Total	78

DISCUSSION

There is need for continued surveillance of emerging antimicrobial resistant organisms isolated from both food and healthy humans. This is because there is steadily accruing evidence from around the world, which indicate food as a source of antimicrobial-resistant organisms (Schoeder et al., 2004). The results from this study revealed that various food samples and in particular, ready-to-eat food samples get contaminated with bacteria, which may either be pathogenic or non-pathogenic.

In the study, *E. coli* was the most common bacterial isolate with an occurrence of 29.49%. *E. coli* when found in water and food supplies, is indicative of a recent faecal contamination and is a threat to public (Monica et al., 2000; Mora et al., 2005). Its presence is a major health concern especially in cases of verotoxin producing *E. coli* (VTEC) Serogroup 0157, a major cause of haemorrhagic colitis. Faecal contamination of food cannot be prevented entirely, particularly in this setting where hygienic standard of food production is low and not monitored. Such organisms will continue to be an inhabitant of food for the foreseeable future, at least in this part of the world. The frequency of isolation of *Salmonella* was also reasonably high (12.82%). According to Animashaun (1991), there is no surveillance system for *Salmonella* in Nigeria; individual studies and laboratory records have however revealed that typhoid fever is endemic in Nigeria (Famurewa and Moro, 1989; Moro et al., 2000, 2001). Adding to the problem is the emergence of *Salmonella*

Table 3. Distribution and proportion of antibiotic resistance among bacterial isolates.

Isolates Identified	Total Number	Total number percentage resistance exhibited to antibiotics							
		AMX	COT	NIT	GEN	NAL	OFL	AUG	TET
E. coli	23	23 (100%)	7 (30.4%)	6 (26.1%)	21 (91.3%)	2 (8.70%)	- (0%)	22 (95.7%)	8 (34.8%)
Klebsiella spp.	20	12 (60%)	11 (55%)	4 (20%)	5 (25%)	3 (21.4%)	- (0%)	4 (20%)	11 (55%)
Proteus spp.	14	14 (100%)	10 (100%)	13 (92.9%)	10 (71.4%)	3 (21.4%)	- (0%)	14 (100%)	13 (92.9%)
Pseudomonas spp.	9	9 (100%)	9 (100%)	9 (100%)	8 (88.9%)	8 (88.9%)	- (0%)	8 (88.9%)	9 (100%)
Salmonella spp.	20	10 (100%)	5 (50%)	10 (100%)	10 (100%)	4 (40%)	- (0%)	10 (100%)	10 (100%)
Enterobacter spp.	2	2 (100%)	- (0%)	1 (50%)	2 (100%)	- (0%)	- (0%)	2 (100%)	2 (100%)
Total	78	70 (89.1%)	42 (53.8%)	43 (55.1%)	56 (71.8%)	18 (23.1%)	- (0%)	60 (76.9%)	53 (67.9%)

AMX: Amoxycillin (25 µg), COT: Cotrimoxazole (25 µg), NIT: Nitrofurantoin (30 µg), GEN: Gentamicin (10 µg), NAL: Nalidixic acid (30 µg), OFL: Ofoxacin (30 µg), AUG: Augmentin (30 µg), TET: Tetracycline (30 µg).

Table 4. Antibiotic resistance patterns in isolates identified.

Isolates	No of isolates that demonstrated resistance	Antibiotics resistance pattern observed	Number of antibiotics to which resistance was shown
E. coli	1	AMX, AUG	2
	10	AMX, GEN, AUG	3
	1	AMX, COT, TET	3
	2	AMX, COT, GEN, AUG	4
	3	AMX, GEN AUG, TET	4
	2	AMX, NIT, GEN, AUG, TET	5
	2	AMX, COT, NIT, GEN, AUG	5
	2	AMX, COT, NIT, GEN, NAL, AUG, TET	7
***Klebsiella* spp.**	3	AMX	1
	1	COT, TET	2
	1	COT, GEN	2
	1	AMX, COT	2
	4	AMX, COT, TET	3
	1	COT, GEN, TET	3
	3	AMX, COT, NIT, GEN, AUG, TET	6
	1	AMX, COT, NIT, NAL, AUG, TET	6
***Proteus* spp.**	1	AMX, NIT, GEN, AUG	4
	1	AMX, NIT, AUG, TET	4
	2	AMX, NIT, GEN, AUG, TET	5
	1	AMX, COT, GEN, AUG, TET	5
	3	AMX, COT, NIT, AUG, TET	5
	3	AMX, COT, NIT, GEN, AUG, TET	6
	3	AMX, COT, NIT, GEN, NAL, AUG, TET	7
	1	AMX, COT, GEN, AUG, TET	5
	3	AMX, COT, NIT, AUG, TET	5
	3	AMX, COT, NIT, GEN, AUG, TET	6
	3	AMX, COT, NIT, GEN, NAL, AUG, TET	7
***Enterobacter* spp.**	1	AMX, GEN, AUG, TET	4
	1	AMX, NIT, GEN, AUG, TET	5

Table 4. Contd.

***Salmonella* spp.**	5	AMX, NIT, GEN, AUG, TET	5
	1	AMX, COT, NIT, GEN, AUG, TET	6
	4	AMX, COT, NIT, GEN, NAL, AUG, TET	7
***Pseudomonas* spp.**	1	AMX, COT, NIT, NAL, TET	5
	1	AMX, COT, NIT, GEN, AUG, TET	6
	7	AMX, COT, NIT, GEN, NAL, AUG, TET	7

strains resistant to chloramphenicol and cotrimoxazole, two antibiotics routinely prescribed for the treatment of typhoid fever.

A high percentage (94%) of the bacterial isolates were resistant to one or more drugs and 85% of the resistant isolates were multiple drug resistant (Table 2). The source of these organisms isolated from the food samples is likely to be via the hands of workers and/or cooking utensils used for the preparation of the food. Since most organisms are likely destroyed by the high temperature used for cooking, the contamination is most likely to be post-cooking. Food handlers and vendors have been associated with contamination of food with various types of aetiologic agents (Moro et al., 2001).

Various reports have also established food animals such as beef from cattle and poultry as important reservoirs for antimicrobial-resistant organisms (Schoeder et al., 2004). Moreover, antimicrobial-resistant *E. coli* have been traced from the gut contents of pigs, calves and chicken, to carcasses at slaughter and ultimately shown to colonize the gut of human volunteers handling and eating meat (Linton, 1986). Epidemiological data from Huang et al. (2001) suggests that humans become colonized with antimicrobial-resistant *E. coli* from food consumption. Upon colonization, these organisms may transfer antimicrobial determinants to other bacteria including potential pathogens in the intestinal flora of man (Mizan et al., 2002).

A closer look at the antibiotic resistance pattern demonstrated by the bacterial isolates in this study revealed that resistance was highest to amoxicillin (89.1%), followed by augmentin (76.9%) and gentamycin (71.8%). Amoxicillin and tetracycline are drugs commonly used in veterinary medicine, while the others such as nitrofurantoin and nalidixic acid among others are employed in humans. Reports from different parts of Nigeria have observed temporal trends in the prevalence of resistance among enteric organisms such as *E. coli* and *Shigella* (Okeke et al., 2005). In multiple studies, resistance to commonly used antimicrobial trimethoprim-sulphamethoxazole (TMP-SMX), ampicillin and chloramphenicol has shown increasing prevalence in the last 15 years. These studies have consistently found low prevalence of resistance to nalidixic acid and the fluoroquinolones. It is interesting to note that clinical management of a number of food-borne infections has been complicated by antimicrobial resistant bacteria (Mangel et al., 2001). Initially, organisms resistant to multiple drugs were found mostly in hospitals, where antimicrobial agents are used most extensively, but resistance is currently found almost as frequently in the community. This study calls for more emphasis on the value of intensive surveillance of isolates throughout the food production continuum to detect emerging antimicrobial resistance phenotypes in developing countries like Nigeria.

Summary and concluding remarks

The study demonstrated the occurrence of multiple antibiotic resistance among bacterial isolates in ready-to-eat foods sold on a university campus in south western Nigeria. This study thus emphasizes the need for intensive surveillance of isolates throughout the food production continuum to prevent food-borne infections and also to detect emerging antimicrobial resistance phenotypes especially in the developing world.

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