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Antimicrobial Resistance in Bacteria Isolated from Foods in Cuba

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ABSTRACT

INTRODUCTION Antimicrobial drug resistance constitutes a health risk of increasing concern worldwide. One of the most common avenues for the acquisition of clinically-relevant antimicrobial resistance can be traced back to the food supply, where resistance is acquired through the ingestion of antimicrobial resistant microorganisms present in food. Antimicrobial resistance constitutes a health risk, leading to production losses and negative consequences for livelihood and food safety.

OBJECTIVE Determine whether resistant bacteria are present in foods in Cuba.

METHODS A descriptive observational study was conducted in the Microbiology Laboratory of Cuba's National Institute of Hygiene, Epidemiology and Microbiology from September 2004 through December 2018. Researchers analyzed 1178 bacterial isolates from food samples. The isolates were identified as *Escherichia coli, Salmonella, Vibrio cholerae* and coagulase-positive *Staphylococcus*. The antimicrobial susceptibility study was performed using the Bauer-Kirby disk diffusion method, following procedures outlined by the Clinical and Laboratory Standards Institute. The data were analyzed using WHONET version 5.6.

RESULTS Of the total isolates, 62.1% were resistant to at least one antibiotic. Within each group, >50% of isolates showed some type of resistance. *E. coli* and *V. cholerae* exceeded 50% resistance to tetracycline and ampicillin, respectively. *Staphylococcus* showed the highest resistance to penicillin, and *Salmonella* to tetracycline, nalidixic acid and ampicillin. The highest percentages of non-susceptible microorganisms were identified in meats and meat products.

CONCLUSIONS These results serve as an alert to the dangers of acquiring antibiotic-resistant bacteria from food and demonstrate the need to establish a surveillance system and institute measures bacterial control in food products.

KEYWORDS Microbial drug resistance, bacteria, food, foodborne disease, Cuba

INTRODUCTION

Antimicrobial resistance (AMR) is a health risk worldwide, leading to production losses and negative effects on livelihood, food safety and the economy,[1] including in Cuba. Statistics from the national program for prevention and control of healthcare-associated infections show an increase in resistance to the most commonly used hospital antibiotics in the last few years, as well as longer hospitalizations and higher spending on these infections.[2] The public health sector is acting to promote the rational prescription and use of antimicrobials, and is conducting various susceptibility studies on clinically-obtained isolates.[3] However, there are few reports on antimicrobial-resistant foodborne bacteria.

Quantitatively, foodborne AMR is the most common route for the spread of antibiotic-resistant bacteria. The presence of these microorganisms in the food chain, the environment and water can lead to their appearance in the human intestinal microbiome, turning it into a major reservoir for resistant genes in the body. It also increases the risk of their dissemination among commensal bacteria and pathogens that cause intra- and extraintestinal infections.[4]

Among the most clinically important foodborne pathogenic bacteria in AMR are strains of *Salmonella* and *E. coli,* which carry extended-spectrum beta lactamases, fluoroquinolone-resistant *Campylobacter* and *Salmonella*, and methicillin-resistant *Staphylococcus aureus*.[5] However, commensal bacteria also found in foods play a key role in AMR evolution and spread. They predominate in the environment and show greater genetic diversity and host variety in nature, which makes them a potential indicator for AMR. Thus, studying these agents can provide early warning of emerging AMR.[6]

WHO suggests regular, periodic surveillance to address the problem of AMR, with permanent monitoring of changes in its prevalence in humans, animals, foods and the environment.[7] Clearly, it is important to discover foodborne AMR as quickly as possible. This includes studying risks by identifying dangers: antimicrobial-resistant microorganisms, the antimicrobials to which they are resistant, and the food products in which this resistance is found. Cuba has no program dedicated to ongoing surveillance of this problem. For these reasons, this study was performed with the aim of assessing antimicrobial resistance in clinically relevant bacteria isolated from foods in Cuba.

IMPORTANCE This paper highlights the importance of antimicrobial resistance surveillance in foods commonly consumed in Cuba.

METHODS

A descriptive observational study was conducted from September 2004 through December 2018 on 1178 isolates identified in foods (381 isolates of *E. coli*, 402 of *Salmonella*, 113 of *V. cholerae* and 282 of coagulase-positive *Staphylococcus*). The isolates were performed at the Provincial Hygiene, Epidemiology and Microbiology Centers in 13 Cuban provinces and in the Microbiology Laboratory of the National Hygiene, Epidemiology and Microbiology Institute (INHEM) in Havana, following current standards in Cuba.[8–11]

The microorganisms were identified in a variety of 146 foods subject to microbiological surveillance in the study of foodborne disease outbreaks and health inspections of foods before sale. These were categorized in 14 groups, according to Cuban microbiological criteria standard NC 585, 2017.[12] The food types were:

- Ready-to-eat foods
- Beverages (juices and soft drinks)
- Broths, soups and creams
- Meats and meat products—processed fresh meats sold in pieces and fresh ground meats (poultry, pork, beef); semiprocessed meat products: protein mix, hamburger, sausages, chorizos; processed meat products: mortadella, bologna, smoked products
- Cocao derivatives
- Spices and condiments
- Nutritional supplements of vegetable origin
- Fruits and vegetables
- Eggs and derivatives—prepared eggs: omelets, scrambled eggs and other products; pastry products and egg-based creams
- Milk and dairy products—pasteurized liquid milk, ice cream, cheeses, yogurt
- Fish, seafood and fish products
- Grain-based products

Antimicrobial susceptibility was determined using the Bauer-Kirby disk diffusion method, strictly adhering to procedures established for this purpose by the Clinical and Laboratory Standards Institute (CLSI).[13] The antimicrobial disks (CPM-SCIENTIFICA, Italy) contained the following loads:

Antimicrobial disk	Antibiotic load (µg)
Nalidixic acid	30
Amikacin	30
Ampicillin	10
Azithromycin	15
Carbenicillin	100
Cefotaxime	30
Ceftazidime	30
Ceftriaxone	30
Ciprofloxacin	5
Chloramphenicol	30
Doxycycline	30
Erythromycin	15
Streptomycin	10
Gentamicin	10
Kanamycin	30
Oxacillin	5
Penicillin	10 IU
Sulfamethoxazole/trimethoprim	1.25/23.75
Tetracycline	30

IU: International Units

As part of quality control, *Staphylococcus aureus* ATCC 25923, *E. coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 reference strains were used.

Antimicrobials were selected according to bacterial species. For *Salmonella* and *E. coli:* nalidixic acid, amikacin, ampicillin, carbenicillin, cefotaxime, ceftriaxone, ceftazidime, ciprofloxacin, chloramphenicol, streptomycin, gentamicin, kanamycin, sulfamethoxazole/trimethoprim and tetracycline were chosen. For *Staphylococcus:* amikacin, cefotaxime, ceftriaxone, chloramphenicol, ciprofloxacin, erythromycin, gentamicin, kanamycin, penicillin, oxacillin, sulfamethoxazole/trimethoprim and tetracycline were selected. For *V. cholerae*: ampicillin, ciprofloxacin, sulfamethoxazole/trimethoprim, tetracycline, doxycycline and azithromycin were chosen.

Extended-spectrum beta lactamase (ESβL) detection was performed on 97 *E. coli* isolates from fresh meats. Isolates with inhibition halos equal to or less than the following diameters were classified as presumptive carriers: cefotaxime ≤27 mm, ceftazidime ≤22 mm, and ceftriaxone ≤25 mm. The disk combination method (CLSI, 2015) and ETEST strips (BioMérieux, France) containing the following combinations were used for confirmation: ceftazidime (0.5–32 µg/mL) and ceftazidime/clavulanic acid (0.064–4 µg/mL) (Liofichem, Italy). Results were interpreted following the manufacturer's criteria. *E. coli* ATCC 25922 strains were tested as a negative control, with ESβL *Klebsiella pneumoniae* ATCC 700603 strains tested as a positive control.

Results were analyzed using a database created in WHONET version 5.6, a WHO digital platform for surveillance of antimicrobial resistance and infection control.[14] The antibiogram interpretation criteria cutoff points were updated according to CLSI standards. Susceptibility was analyzed by isolate source, for which contingency tables were established, and the chi-square test was applied with a significance level of 0.05%. The data were processed using the EPIDAT program (EpiData Association, Denmark) for epidemiological analysis of tabular data, version 3.0 of 2004.[15]

Results of the *in vitro* susceptibility tests were expressed as absolute frequencies and percentages. Isolates with full growth around the antibiotic disk or those in which growth inhibition did not reach the diameter established for the CLSI susceptibility criterion (reduced susceptibility) were considered resistant. Otherwise, they were considered sensitive to the antibiotic.

Ethical considerations No clinical assays were performed on persons or animals in this study, and the study was authorized by INHEM's scientific council. This document contains no company, institution or brand names of foods from which the isolates were obtained.

RESULTS

AMR was analyzed according to the microorganisms retrieved from different food types (Table 1). Of all isolates, 62.1% (731/1178) were antibiotic-resistant; of all bacteria studied, AMR was observed in 32.3% (236/731) of *Salmonella* isolates, 30.1% (220/731) of *E. coli*, 29.9% (212/731) of *Staphylococcus* and 8.6% (63/731) of *V. cholerae*. Resistant microorganisms were most often identified in meats and meat products, with *Salmonella* and *E. coli* isolates predominating.

Resistance was detected less frequently in bacteria isolated from milk and dairy products, with *Staphylococcus* and *E. coli* the most common. In egg-based products, *Salmonella* and *Staphylococcus* isolates predominated. A low frequency of isolates was found in all other foods.

V. cholerae was isolated in fruits and vegetables, and in fish, seafood and fishery products, which had the highest percentage of resistant isolates at 69.3%.

Table 1: Antimicrobial resistance of microorganisms according to food type from which they were recovered. INHEM 2004–2018

Food Type	*Escherichia coli*			*Salmonella*			*Staphylococcus*			*Vibrio cholerae*			*Total*		
	No.	AMR	%[a]	No.	AMR	%[a]	No.	AMR	%[a]	No.	AMR	%[a]	No.	AMR	%[b]
Meats and meat products	215	141	36.7	284	173	45.1	132	70	18.2	0	0	0.0	631	384	52.5
Milk and dairy products	85	35	36.5	4	4	4.2	62	57	59.4	0	0	0.0	151	96	13.1
Eggs and derivatives	27	14	17.7	67	37	46.8	28	28	35.4	0	0	0.0	122	79	10.8
Fish, seafood and fish products	8	5	6.7	16	2	2.7	28	16	21.3	98	52	69.3	150	75	10.3
Ready-to-eat foods	22	20	30.3	29	19	28.8	27	18	27.3	0	0	0.0	69	66	9.0
Fruits and vegetables	5	0	0.0	0	0	0.0	4	4	26.7	15	11	73.3	24	15	2.1
Nutritional supplements	0	0	0.0	1	0	0.0	8	8	100.0	0	0	0.0	9	8	1.1
Beverages (juices and soft drinks)	13	4	100.0	0	0	0.0	0	0	0.0	0	0	0.0	13	4	0.5
Cocao derivatives	1	1	33.3	0	0	0.0	2	2	66.7	0	0	0.0	3	3	0.4
Spices and condiments	0	0	0.0	1	1	100.0	0	0	0.0	0	0	0.0	1	1	0.1
Grain-based products	2	0	0.0	0	0	0.0	0	0	0.0	0	0	0.0	2	0	0.0
Broths, soups and cream-based soups	3	0	0.0	0	0	0.0	0	0	0.0	0	0	0.0	3	0	0.0
Total %	381	220	30.1	402	236	32.3	282	212	29.0	113	63	8.6	1178	731	100.0

[a] Percentage refers to total number of isolates in category [b] Percentage refers to total number of foods analyzed per microorganism
AMR: Antimicrobial resistance INHEM: National Institute of Hygiene, Epidemiology and Microbiology

Table 2 shows the relation between AMR in *Salmonella, E. coli* and *Staphylococcus* and their isolate sources. *Salmonella* was not associated with any specific food type. The highest percentage of resistant isolates was found in meats and meat products. *E. coli* had a higher proportion of resistant isolates compared to subgroup size in meats and meat products. Additionally, *Staphylococcus* had a higher proportion of resistant isolates found in meat and dairy products.

Table 2: Relation between antibiotic resistance of *Escherichia coli, Salmonella* and *Staphylococcus* and food type from which isolates were recovered (n = 1065). INHEM 2004–2018

Susceptibility	By Isolate Source			p Value
	Escherichia coli (n = 381)			
	Meats and meat products	Milk and dairy products	Other	
Sensitive %[a]	74 (34.4)	50 (58.8)	37 (45.7)	0.0000
Resistant %[a]	141 (65.6)	35 (41.2)	44 (54.3)	
Total %[b]	215 (56.4)	85 (22.3)	81 (21.3)	
X^2 22.7709				
Susceptibility	*Salmonella* (n = 402)			
	Meats and meat products	Eggs and derivatives	Other	
Sensitive %[a]	111 (39.1)	30 (44.8)	25 (49.0)	0.3397
Resistant %[a]	173 (60.9)	37 (55.2)	26 (51.0)	
Total %[b]	284 (70.6)	67 (16.7)	51 (12.7)	
X^2 2.1666				
Susceptibility	*Staphylococcus* (n = 282)			
	Meats and meat products	Milk and dairy products	Other	
Sensitive %[a]	52 (42.6)	5 (8.1)	13 (13.3)	0.0003
Resistant %[a]	70 (57.4)	57 (91.9)	85 (86.7)	
Total %[b]	122 (43.3)	62 (22.0)	98 (34.8)	
X^2 16.7991				

[a] Percentage refers to total number of isolates in category
[b] Percentage refers to total number of foods analyzed per microorganism
INHEM: National Institute of Hygiene, Epidemiology and Microbiology

Resistance by antibiotic type was low overall, except for tetracycline in *E. coli* and ampicillin in *V. cholerae*, for which resistance was over 50% (Table 3). Of the 19 antibiotic agents analyzed (14 for *Salmonella* and *E. coli*, 12 for *Staphylococcus* and 6 for *V. cholerae*) *Salmonella* expressed in vitro resistance to 12, and *E. coli*, to 14. Tetracycline, nalidixic acid and ampicillin showed the highest resistance levels. More than 75% of *Staphylococcus* isolates were resistant, mainly against penicillin, erythromicin and tetracycline, in decreasing order. *V. cholerae* was resistant to three antibiotics, namely tetracycline,

Table 3: Percentage of resistance by antibiotic and microorganism. INHEM 2004–2018

Antibiotic	*Salmonella* n = 236		*E. coli* n = 220		*Staphylococcus* n = 212		*V. cholerae* n = 63	
	No.	%	No.	%	No.	%	No.	%
Tetracycline	140	59.3	91	41.4	44	20.8	3	4.8
Nalidixic acid	70	29.7	102	46.4	-	-	-	-
Ampicillin	55	23.3	117	53.2	-	-	54	85.7
Carbenicillin	31	13.1	27	12.3	-	-	-	-
Ceftriaxone	14	5.9	23	10.5	59	27.8	-	-
Ceftazidime	16	6.8	13	5.9	-	-	-	-
Streptomycin	8	3.4	12	5.5	-	-	-	-
Cefotaxime	7	3.0	13	5.9	0	0	-	-
Sulfamethoxazole/ trimethoprim	4	1.7	40	18.2	0	0	6	9.5
Chloramphenicol	2	0.8	38	17.3	0	0	-	-
Kanamycin	2	0.8	15	6.8	6	2.8	-	-
Ciprofloxacin	2	0.8	19	8.6	8	3.8	0	0
Amikacin	0	0	11	5.0	2	0.9	-	-
Gentamicin	0	0	12	5.5	1	0.5	-	-
Penicillin	-	-	-	-	88	41.5	-	-
Oxacillin	-	-	-	-	43	20.3	-	-
Erythromycin	-	-	-	-	52	24.5	-	-
Azithromycin	-	-	-	-	-	-	0	0
Doxycycline	-	-	-	-	-	-	0	0

INHEM: National Institute of Hygiene, Epidemiology and Microbiology

Table 4: Isolates studied, by microorganism and province where identified. INHEM 2004–2018

Province	*E. coli*		*Salmonella*		*Staphylococcus*		*V. cholerae*		Total	
	No.	%[a]	No.	%[a]	No.	%[a]	No.	%[a]	No.	%[b]
Havana (INHEM)	263	42.4	98	15.8	250	40.3	10	1.6	621	52.7
Pinar del Río	64	82.1	10	12.8	4	5.1	0	0.0	78	6.6
Santiago de Cuba	39	30.0	67	51.5	9	6.9	15	11.5	130	11.0
Las Tunas	10	15.4	49	75.4	6	9.2	0	0.0	65	5.5
Sancti Spíritus	2	16.7	10	83.3	0	0.0	0	0.0	12	1.0
Villa Clara	2	3.0	65	97.0	0	0.0	0	0.0	67	5.7
Granma	1	1.1	6	6.9	0	0.0	80	92.0	87	7.4
Ciego de Ávila	0	0.0	14	51.9	7	25.9	6	22.2	27	2.3
Camagüey	0	0.0	27	96.4	0	0.0	1	3.6	28	2.4
Cienfuegos	0	0.0	4	66.7	2	33.3	0	0.0	6	0.5
Guantánamo	0	0.0	12	92.3	0	0.0	1	7.7	13	1.1
Holguín	0	0.0	21	100.0	0	0.0	0	0.0	21	1.8
Isla de la Juventud*	0	0.0	1	100.0	0	0.0	0	0.0	1	0.1
Matanzas	0	0.0	18	81.8	4	18.2	0	0.0	22	1.9
Total	381	32.3	402	34.1	282	23.9	113	9.6	1178	100.0

[a] Percentage refers to total number of isolates for province, [b] Percentage refers to total number of isolates
INHEM: National Institute of Hygiene, Epidemiology and Microbiology   * Special Municipality

ampicillin and sulfamethoxazole/trimethoprim (Table 3). A low percentage (2.8%) of ESβL enzyme was detected in 97 *E. coli* isolates obtained from fresh meats.

Geographical distribution of isolates (Table 4) showed that the highest percentage, 52.7% of the total, was identified in Havana Province at INHEM's laboratory. The percentage of isolates sent from provinces outside Havana was low. The highest percentage came from Santiago de Cuba (11.0%); the rest were less than 10.0%.

DISCUSSION

More than half of the bacterial isolates recovered from foods were resistant to at least one of the drugs tested. The most clinically important isolates were *E. coli* and *Salmonella,* since they often cause gastrointestinal disease or extraintestinal infections requiring treatment. The least effective antibiotics administered *in vitro* were tetracycline, ampicillin, nalidixic acid and penicillin, as also found in international studies.[16–20]

For WHO-classified antibiotics,[18] specifically those appropriate for only limited use in humans (including ciprofloxacin, cefotaxime, ceftriaxone and ceftazidime), resistance was low and observed more often in *E. coli* and *Staphylococcus.* The international literature reports resistance percentages higher than those in this study.[19–21] The foods that most often contained resistant isolates were meats and meat products; for *Salmonella,* this result is consistent with those of other researchers, which show that these products are among the main sources of resistant bacteria in this genus.[22,23]

The 173 *Salmonella* isolates from meats and meat products were obtained from 31 different foods. Hamburger showed the highest number of resistant isolates. Among fresh meats, resistance was most often found in poultry, where isolates from ground turkey were predominant, followed by those from ground chicken and mechanically deboned meat. These results agree with international reports, which found that in ground meats, the *Salmonella* detected often presents with high virulence and high levels of AMR.[24,25]

Since most poultry meats in Cuba are imported,[26] this could be considered a route for spreading resistance, in addition to antibiotics found in imported meat that are not used in domestic animal production, such as cefotaxime, ceftriaxone and ceftazidime.

Resistant *E. coli* isolates were most often found in pork, mortadella and smoked pork loin. Three isolates carrying ESβL were found in imported poultry meat and beef, and in domestically produced pork, at a lower percentage than has been reported in other countries.[27,28]

Globally, antimicrobial susceptibility of *E. coli* is studied in different foods depending on geographic region. In the European Union and the United States, emphasis is on meats and antibiotics such as cephalosporins and fluoroquinolones.[29,30] In Asia and Latin America, there are more studies on ready-to-eat foods.[31,32] This could be due to greater availability of industrially processed ready-to-eat foods in developed countries, while in developing nations there are more prepared foods sold by small-scale manufacturers who generally do not monitor product preparation, potentially allowing bacterial contaminants to survive and multiply. In this study, which analyzed meats and ready-to-eat foods, antibiotic resistance was frequent regardless of food type.

Currently, AMR in commensal bacteria such as *E. coli* is cause for growing concern because resistant genes can be replaced with bacteria that are pathogenic to humans. The scientific literature has demonstrated transfer of multidrug resistance through *E. coli* plasmids to other enterobacteria such as *Salmonella*.[33]

Most antibiotic-resistant *Staphylococcus* isolates were identified in meats and meat products such as sausages, ground meats and hamburger. In milk and dairy products, most isolates were found in cheese, mainly artisanal cheeses. This last food group was shown to be associated with resistant isolates. Other countries report varying percentages of AMR to at least one of the antibiotics tested, among which *S. aureus* was the most prevalent in meats and cheeses.[21,34]

It should be noted that foodborne staphylococcal intoxication does not require antibiotic treatment, and there is no evidence that consuming foods contaminated with this bacteria is associated with infection in humans.[35] However, there is now special interest in antimicrobial susceptibility studies because of the possible transfer of resistant genes between microorganisms, and thus from the environment to humans.[7]

V. cholerae is a species endemic to aquatic environments, and thus may be an indicator of antibiotic resistance in bacteria found in these ecosystems. In this study, it was mainly found in fish, seafood and other fish products. Its expressed resistance was low except to ampicillin, to which resistance was seen in >50% of isolates. No resistance was found to ciprofloxacin, azithromycin or doxycycline, which are often used as first-line treatments for infections of toxigenic agents of this species. For *V. cholerae*, the international literature reports AMR usually higher than that found in this study.[36,37]

The highest percentage of isolates analyzed came from foods inspected at INHEM as part of the institution's responsibilities in sanitary registration including imported products and those domestically produced by various Cuban companies. Foods that do not meet the bacterial limits in the standard[11] are not approved for sale. However, there are currently no trade regulations that address antibacterial resistance, which is why studies focusing on risk are needed to accurately determine the scope of the problem.[38]

We observed an unequal distribution in both the number and geographic origin of isolates received from laboratories in other provinces participating in the study, as well as in numbers of isolates of each bacteria type received. There were low percentages of *E. coli*, *Staphylococcus* and *V. cholerae*, which made it impossible to analyze antibiotic resistance for each region of the country. This would be possible if a national antimicrobial resistance surveillance system were established to obtain standardized information that would allow comparisons by region and over time.

One of the study's main limitations was the unequal numbers of bacterial isolates sent from each province. The study was based on the isolates received, which did not allow nationally based analysis of a resistant bacterial load for each food. In addition, the information presented was obtained more than a year ago, which makes it invalid for immediate surveillance purposes, but does not affect its usefulness as a resource for illustrating a problem that demands surveillance and control. Despite these limitations, a broad range of antibiotics were analyzed, including most classes used in human and veterinary treatment, and the number of isolates studied for each bacterial genus was sufficient for making preliminary estimates of AMR prevalence in each case, although without claims as to their representativity.

CONCLUSIONS

Resistant phenotypes were identified in more than half the bacteria isolated from foods, with a higher percentage found in animal products such as meat, dairy, eggs and foods made from these ingredients. Low percentages of AMR were found for antibiotics classified as critical for human use. These results may serve as an alert to the dangers of acquiring foodborne antibiotic-resistant bacteria and demonstrate the need to establish a surveillance system and institute related control in Cuba.

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