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Antimicrobial Resistance in Bacteria Isolated from Foods in Cuba


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Original Research

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 WHO-NET 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 antimiotics, 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.

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:
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:

<table>
<thead>
<tr>
<th>Antimicrobial disk</th>
<th>Antibiotic load (µg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nalidixic acid</td>
<td>30</td>
</tr>
<tr>
<td>Amikacin</td>
<td>30</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>10</td>
</tr>
<tr>
<td>Azithromycin</td>
<td>15</td>
</tr>
<tr>
<td>Carbenicillin</td>
<td>100</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>30</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>30</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>30</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>5</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>30</td>
</tr>
<tr>
<td>Doxycycline</td>
<td>30</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>15</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>10</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>10</td>
</tr>
<tr>
<td>Kanamicin</td>
<td>30</td>
</tr>
<tr>
<td>Oxacillin</td>
<td>5</td>
</tr>
<tr>
<td>Penicillin</td>
<td>10 IU</td>
</tr>
<tr>
<td>Sulfamethoxazole/trimethoprim</td>
<td>1.25/23.75</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>30</td>
</tr>
</tbody>
</table>

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%.
were recovered (n = 1065). INHEM 2004–2018

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.

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, erythromycin and tetracycline, in decreasing order. *V. cholerae* was resistant to three antibiotics, namely tetracycline,
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]

**Table 4: Isolates studied, by microorganism and province where identified.**

<table>
<thead>
<tr>
<th>Province</th>
<th>E. coli</th>
<th>Salmonella</th>
<th>Staphylococcus</th>
<th>V. cholerae</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. %</td>
<td>No. %</td>
<td>No. %</td>
<td>No. %</td>
<td>No. %</td>
</tr>
<tr>
<td>Havana (INHEM)</td>
<td>263</td>
<td>42.4</td>
<td>98</td>
<td>15.8</td>
<td>250</td>
</tr>
<tr>
<td>Pinar del Río</td>
<td>64</td>
<td>82.1</td>
<td>10</td>
<td>12.8</td>
<td>4</td>
</tr>
<tr>
<td>Santiago de Cuba</td>
<td>39</td>
<td>30.0</td>
<td>67</td>
<td>51.5</td>
<td>9</td>
</tr>
<tr>
<td>Las Tunas</td>
<td>10</td>
<td>15.4</td>
<td>49</td>
<td>75.4</td>
<td>6</td>
</tr>
<tr>
<td>Sancti Spíritus</td>
<td>2</td>
<td>16.7</td>
<td>10</td>
<td>83.3</td>
<td>0</td>
</tr>
<tr>
<td>Villa Clara</td>
<td>2</td>
<td>3.0</td>
<td>65</td>
<td>97.0</td>
<td>0</td>
</tr>
<tr>
<td>Granma</td>
<td>1</td>
<td>1.1</td>
<td>6</td>
<td>6.9</td>
<td>0</td>
</tr>
<tr>
<td>Ciego de Ávila</td>
<td>1</td>
<td>0.0</td>
<td>14</td>
<td>51.9</td>
<td>7</td>
</tr>
<tr>
<td>Camagüey</td>
<td>0</td>
<td>0.0</td>
<td>27</td>
<td>96.4</td>
<td>0</td>
</tr>
<tr>
<td>Cienfuegos</td>
<td>0</td>
<td>0.0</td>
<td>4</td>
<td>66.7</td>
<td>2</td>
</tr>
<tr>
<td>Guantánamo</td>
<td>0</td>
<td>0.0</td>
<td>12</td>
<td>92.3</td>
<td>0</td>
</tr>
<tr>
<td>Holguín</td>
<td>0</td>
<td>0.0</td>
<td>21</td>
<td>100.0</td>
<td>0</td>
</tr>
<tr>
<td>Isla de la Juventud</td>
<td>0</td>
<td>0.0</td>
<td>1</td>
<td>100.0</td>
<td>0</td>
</tr>
<tr>
<td>Matanzas</td>
<td>0</td>
<td>0.0</td>
<td>18</td>
<td>81.8</td>
<td>4</td>
</tr>
<tr>
<td>Total</td>
<td>381</td>
<td>32.3</td>
<td>402</td>
<td>34.1</td>
<td>282</td>
</tr>
</tbody>
</table>

* Percentage refers to total number of isolates for province, † Percentage refers to total number of isolates INHEM: National Institute of Hygiene, Epidemiology and Microbiology * Special Municipality
Original Research

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 bacterial 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 was 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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44

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Original Research


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