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Research Note

Enterobacteriaceae Antibiotic Resistance in Ready-to-Eat Foods Collected from Hospital and Community Canteens: Analysis of Prevalence

SARA VINCENTI,[1] MATTEO RAPONI,[2]* ROMINA SEZZATINI,[1] GABRIELE GIUBBINI,[2] AND PATRIZIA LAURENTI[1,2]

[1]*Fondazione Policlinico Universitario Agostino Gemelli, Largo Francesco Vito 1, 00168 Rome, Italy; and* [2]*Istituto di Sanità Pubblica, Sezione Igiene, Università Cattolica del Sacro Cuore, Largo Francesco Vito 1, 00168 Rome, Italy*

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ABSTRACT

Foodborne diseases and antibiotic resistance are serious widespread health problems in the contemporary world. In this study, we compared the microbiological quality of ready-to-eat (RTE) foods found in community canteens versus hospital canteens in Rome, Italy, focusing on detection and quantification of *Enterobacteriaceae* and the antibiotic resistance of these bacteria. Our findings show a remarkable difference in *Enterobacteriaceae* contamination between RTE foods distributed in community canteens (33.5% of samples) and those distributed in hospital canteens (5.3% of samples). This result highlights greater attention to good manufacturing practices and good hygiene practices by the food operators in hospitals compared with food operators in community canteens. As expected, a higher percentage of cold food samples (70.9%) than of hot food samples (10.8%) were positive for these bacteria. Excluding the intrinsic resistance of each bacterial strain, 92.3% of the isolated strains were resistant to at least one antibiotic, and about half of the isolated strains were classified as multidrug resistant. The prevalence of multidrug-resistant strains was 50% in the community samples and 33.3% in hospital canteens. Our results indicate that approximately 38% of RTE foods provided in community canteens is not compliant with microbiological food safety criteria and could be a special risk for consumers through spread of antibiotic-resistant strains. Hygienic processing and handling of foods is necessary for both hospital and community canteens.

Key words: Antibiotic resistance; Drug resistance; *Enterobacteriaceae*; Food safety; Ready-to-eat foods

Foodborne disease is the most widespread health problem in the contemporary world and an important cause of reduced economic productivity. In the United States, approximately 46 million people become ill every year from eating contaminated foods. Thousands of people are hospitalized, and approximately 3,000 people die *(3)*. Foodborne diseases occur after contact with foods contaminated with bacteria, viruses, or parasites. Changes in pathogen populations are relevant for food safety. Food is an excellent vehicle by which many pathogens (bacteria, viruses, prions, and parasites) can reach an appropriate colonization site in a new host.

Despite the changes in food production practices, well-recognized foodborne pathogens (e.g., *Salmonella* and *Escherichia coli*) evolve to exploit novel opportunities and generate new public health challenges, such as antimicrobial resistance. Antimicrobial resistance is a serious threat to public health worldwide, leading to increases in health care costs, treatment failures, and deaths. Antibiotic resistance among foodborne microorganisms is an ongoing public health threat that continues to be a challenge, as indicated by the data published by the Centers for Disease Control and Prevention *(8)*. Even though efforts are necessary to limit the misuse of antibiotics, approximately 440,000 foodborne infections are caused by antibiotic-resistant microorganisms in the United States each year *(7)*. One of the main routes of transmission of antibiotic-resistant pathogens is ready-to-eat (RTE) foods, both of animal and plant origin, that are treated with antibiotics to preserve their original characteristics. RTE foods are defined by European Commission Regulation No 1441/2007 *(12)* as foods intended by the producer or manufacturer for direct human consumption without the need for cooking or other forms of processing that are effective for eliminating or reducing microorganisms of concern to an acceptable level. Among RTE food types, vegetables (e.g., salads) have been identified as posing a risk to human health *(4, 10, 14, 18, 28)*.

The purpose of this study was to verify the microbiological quality of a range of RTE foods collected from hospital and community canteens. We compared the microbiological quality of RTE foods from community canteens and hospital canteens in Rome, Italy, focusing on the detection and quantification of *Enterobacteriaceae*. We also evaluated the prevalence of antibiotic resistance in strains isolated from RTE foods from community and

* Author for correspondence. Tel: +39 06-3015.4396; Fax: +39 06-3500.1522; E-mail: matteo.raponi01@icatt.it.

TABLE 1. *Microbiological quality of RTE foods according to Health Protection Agency guidelines (15)*

Bacterial level (CFU g^{-1})	No. (%) of positive samples	Interpretation
$>10^4$	61 (38.6)	Unsatisfactory
10^2–$\leq 10^4$	62 (39.2)	Borderline
$<10^2$	35 (22.2)	Satisfactory

hospital canteens, with special attention to the potential public health implications of the use of antibiotics.

MATERIALS AND METHODS

Sample collection. RTE foods were collected from two hospital canteens and four community canteens in Rome from 2011 to 2016. Room temperature samples were collected from RTE foods that had not been cooked or reheated (i.e., cold dishes such as sandwiches, salads, and fresh vegetables) and from foods that had been cooked or reheated (i.e., hot dishes such as pasta, burgers, pizza, and meat).

Microbiological analysis. Ten grams of each sample was diluted in 90 mL of buffered peptone water (BPW; Oxoid, Unipath, Basingstoke, UK), and 1 mL of the homogenate was introduced into 9 mL of BPW in a test tube labelled as a 1:10 (10^{-1}) dilution. This dilution was serially diluted 10-fold into three other test tubes labeled 10^{-2}, 10^{-3}, and 10^{-4} in agreement with standard methods for initial suspension and decimal dilutions of test samples for microbiological examination (ISO 6887-1:2000) *(16)*. An aliquot (1 mL) of each of these four 10-fold dilutions was inoculated in duplicate onto a violet red bile glucose agar (VRBGA) plate (Oxoid, Unipath), overlain with 10 to 15 mL of VRBGA, and then incubated at 37°C for 24 h. At least five presumptive colonies (pink, red, or purple with or without precipitation halos) were subcultured for biochemical confirmation following standard methods (ISO 21528-2:2004) *(17)*. Colonies that were oxidase negative and glucose positive were confirmed as *Enterobacteriaceae* and subsequently identified via the ID 32E (bioMérieux, Marcy l'Étoile, France) bacterial identification system according to the manufacturer's instructions. The data from the identified isolates were recorded into a specific database. Based on the bacterial levels, a judgment was made regarding the microbiological quality (satisfactory, borderline, or unsatisfactory) of the RTE foods in accordance with Health Protection Agency guidelines *(15)*.

Antimicrobial susceptibility testing. The antibiotic sensitivity of the isolates was determined using the ATB G-EU method (bioMérieux) *(8)*. This method evaluates the sensitivity of *Enterobacteriaceae* to antibiotics in a semisolid medium under conditions similar to those specified for the agar dilution or microdilution methods. The ATB G-EU strip was designed following the recommendations of the European Committee on Antimicrobial Susceptibility Testing *(13)* with the exception of cephalothin and cefoxitin, which complied with the Comité de l'Antibiogramme de la Société Française de Microbiologie *(6)* and Clinical and Laboratory Standards Institute *(9)* recommendations. A 0.5 McFarland suspension was prepared with the bacterial colony to be tested (10^8 CFU mL^{-1}), transferred into the culture medium, and then manually inoculated into the strip. After 18 to 24 h of incubation at 36 ± 2°C under aerobic conditions, growth was determined automatically with a mini API system (bioMérieux).

The tested bacterial strains were classified as sensitive, intermediate, or resistant. Intermediate susceptibility to an antibiotic was considered resistance. Isolates were classified as multidrug resistant (MDR) and non-MDR based on the method of Magiorakos et al. *(20)*. Multidrug resistance was defined as acquired nonsusceptibility to at least one agent in at least three antimicrobial categories. Isolates were defined as non-MDR when they were not susceptible to at least one agent in one or two antimicrobial categories. Data on antibiotic resistance patterns and relative percentages were recorded in the database.

Statistical analysis. The descriptive statistical analysis was conducted using absolute and relative frequencies for qualitative variables. Associations between positive results for *Enterobacteriaceae* and the qualitative variables were tested with a chi-square test or Fisher exact test, as applicable. These two tests also were used to assess differences in qualitative variables between the *Enterobacteriaceae* strains resistant to antibiotics. For this analysis, the strains with intermediate susceptibility were combined with those classified as resistant. The level of statistical significance was set at $P = 0.05$, and the analysis was performed with SPSS version 22 (IBM, Armonk, NY).

RESULTS

A total of 680 RTE food samples were collected in Rome and analyzed between 2011 and 2016: 433 samples from community canteens and 247 samples from hospital canteens. Of these, 141 samples were from cold dishes and 539 samples were from hot dishes.

Of all the analyzed samples, 158 (23.2%) were positive for *Enterobacteriaceae*. Pathogen prevalence was significantly higher in samples from community canteens (33.5%, $n = 145$) than in samples from hospital canteens (5.3%, $n = 13$) ($P < 0.001$) and significantly higher in the cold dishes (70.9%, $n = 100$) than in the hot dishes (10.8%, $n = 58$) ($P < 0.001$). A total of 182 *Enterobacteriaceae* strains were isolated from the RTE food samples: 164 from community canteen samples and 18 from hospital canteen samples. Several samples were contaminated by more than one *Enterobacteriaceae* strain.

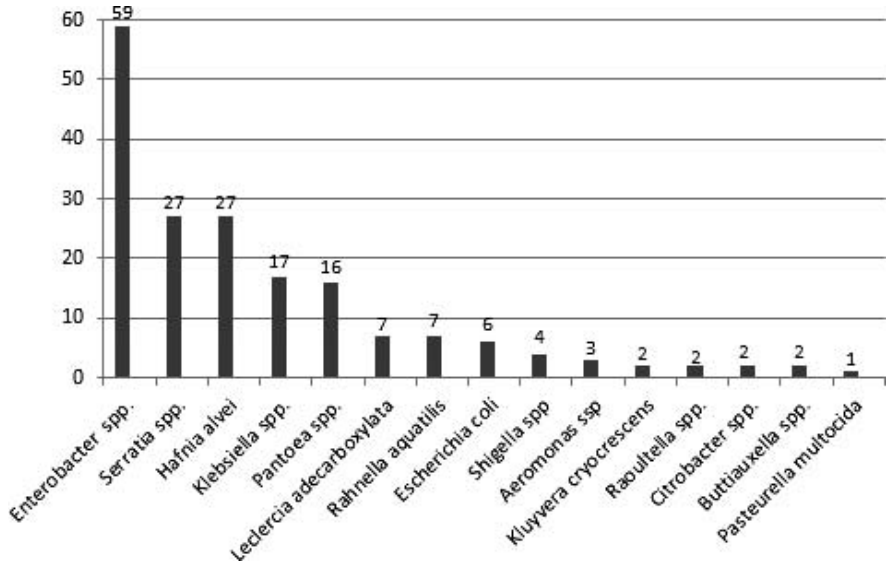
The *Enterobacteriaceae* level was $>10^4$ CFU g^{-1} in 61 (38.6%) samples (17 from hot dishes and 44 from cold dishes) (Table 1). Of these samples, only one (1.64%) was from a hospital canteen. Sandwiches and pasta salads were the foods with the highest number of unsatisfactory samples (13 and 17, respectively).

The most commonly isolated *Enterobacteriaceae* strains were *Enterobacter* spp. (59 strains), *Serratia* spp. (27 strains), *Hafnia alvei* (27 strains), *Klebsiella* spp. (17 strains), and *Pantoea* spp. (16 strains). *Salmonella* and *L. monocytogenes* were not detected in any of examined samples (Fig. 1).

Excluding the intrinsic resistance of each bacterial strain, 92.3% of the isolates ($n = 168$) were resistant to at least one antibiotic. About half of the isolated strains (48.4%, $n = 88$) were classified as MDR based on the methods of Magiorakos et al. *(20)*. The prevalence of MDR strains was 50% ($n = 82$) in the community samples and 33.3% ($n = 6$) in the hospital samples ($P = 0.179$).

The highest prevalence of antibiotic resistance was observed to cephalothin, with resistance in 79.7% of strains (145 of 182), followed by cefuroxime (48%, 84 of 175 strains) and ticarcillin (42.4%, 70 of 165 strains) (Table 2).

FIGURE 1. Enterobacteriaceae *isolates from RTE food samples.*



The prevalence of *Enterobacteriaceae* was significantly higher in the community samples than in the hospital samples for strains resistant to piperacillin ($P = 0.026$), cefepime ($P = 0.027$), and cephalothin ($P = 0.039$) (Table 3).

Figure 2 shows the heat map relative to antibiotic resistance of the major isolated strains (*Enterobacter* spp., *Serratia* spp., *H. alvei, Klebsiella* spp., and *Pantoea* spp.). Thirty-four (57.63%) of 59 of the *Enterobacter* strains that were isolated were classified as MDR *(20)*. Similar results were observed for the other strains isolated: 14 (51.85%) of 27 *Serratia* strains, 13 (48.15%) of 27 *H. alvei* strains, 3 (17.65%) of 17 *Klebsiella* strains, and 9 (56.25%) of 16 *Pantoea* strains. *Enterobacter* spp. were susceptible to broad-spectrum first- and second-generation cephalosporins (49.15% susceptible to cefuroxin and 89.83% susceptible to cephalothin) (Fig. 2). Moderate susceptibility was found for third- and fourth-generation cephalosporins (30.51% to cephotaxime, 33.90% to ceftazidime, and 25.42% cefepime). Similar results were observed for *Serratia* spp., *H. alvei,* and *Pantoea* spp., which indicates high resistance to first and second generation cephalosporins.

TABLE 2. *Proportion of isolated* Enterobacteriaceae *strains resistant to each antibiotic*

Antibiotic	% (no. resistant/total no. of strains)
Ticarcillin	42.4 (70/165)
Piperacillin	20.3 (37/182)
Piperacillin-tazobactam	13.7 (25/182)
Amoxicillin-clavulanate	33.3 (44/132)
Cefoxitin	36.1 (60/166)
Cefotaxime	27.5 (50/182)
Ceftazidime	28.0 (51/182)
Cefepime	18.7 (34/182)
Cefuroxime	48.0 (84/175)
Cephalothin	79.7 (145/182)
Meropenem	4.4 (8/182)
Imipenem	4.9 (9/182)
Cotrimoxazole	7.7 (14/182)
Tobramycin	4.5 (8/178)
Amikacin	7.3 (13/178)
Gentamicin	5.5 (10/182)
Ciprofloxacin	6.0 (11/182)

The susceptibility to third- and fourth-generation cephalosporins was variable among the isolated strains: *Serratia* spp. (11.1% of strains susceptible to cephotaxime, 11.11% to ceftazidime, and 14.81% to cefepime), *H. alvei* (48.15% to cephotaxime, 62.96% to ceftazidime, and 7.41% to cefepime), and *Pantoea* spp. (37.50% to cephotaxime, 6.25% to ceftazidime, and 18.75% to cefepime). *Klebsiella* strains had a different susceptibility profile: slight resistance to third- and fourth-generation cephalosporins (5.88% to cephotaxime, 11.76% to ceftazidime, and 11.76% to cefepime) and a more variable profile for the first- and second-generation cephalosporins tested (5.88% to cefuroxin and 35.29% to cephalothin).

All the major isolated strains were highly susceptibility to the aminoglycoside antimicrobials: *Enterobacter* spp. (11.86% of strains susceptible to amikacin, 3.39% to gentamicin, and 5.08% to tobramycin), *Serratia* spp. (3.70% to amikacin, 3.70% to gentamicin, and 7.4% to tobramycin), *H. alvei* (0% to amikacin, 7.41% to gentamicin, and 0% to tobramycin), *Klebsiella* spp. (5.88% to amikacin, 0% to gentamicin, and 0% to tobramycin), and *Pantoea* spp. (0% to amikacin, 6.25% to gentamicin, and 12.50% to tobramycin). These strains also were susceptible to the carbapenems tested (imipenem and meropenem). For *Enterobacter* strains, 5.08 and 6.78% were resistant to meropenem and imipenem, respectively, 7.41% of *Serratia* strains were resistant to both antibiotics, and *H. alvei* and *Klebsiella* strains were completely susceptible to both. Although 12.50% of *Pantoea* strains were resistant to meropenem, all of these strains were susceptible to imipenem.

DISCUSSION

The *Enterobacteriaceae* is a group of bacteria that can act as indicators of the general hygiene status of a food product. Our findings revealed a remarkable difference in *Enterobacteriaceae* contamination of RTE foods distributed in community canteens (33.5% of samples) compared with

TABLE 3. *Proportion of isolated* Enterobacteriaceae *strains resistant to each antibiotic by source of sample*

Antibiotic	% of resistant strains (no. resistant/total no. of strains)		*P* value
	Hospital samples	Community samples	
Ticarcillin	23.5 (4/17)	44.6 (66/148)	0.122
Piperacillin	0 (0/18)	22.6 (37/164)	0.026[a]
Piperacillin-tazobactam	5.6 (1/17)	14.6 (24/164)	0.475
Amoxicillin-clavulanate	20 (3/15)	35 (41/117)	0.383
Cefoxitin	31.3 (5/16)	36.7 (55/150)	0.788
Cefotaxime	22.2 (4/18)	28 (46/164)	0.783
Ceftazidime	11.1 (2/18)	29.9 (49/164)	0.105
Cefepime	0 (0/18)	20.7 (34/164)	0.027[a]
Cefuroxime	38.9 (7/18)	49 (77/157)	0.414
Cephalothin	61.1 (11/18)	81.7 (134/164)	0.039[a]
Meropenem	0 (0/18)	4.9 (8/164)	1
Imipenem	0 (0/18)	5.5 (9/164)	0.602
Cotrimoxazole	0 (0/18)	8.5 (14/164)	0.367
Tobramycin	5.6 (1/18)	4.4 (7/160)	0.582
Amikacin	5.6 (1/18)	7.5 (12/160)	1
Gentamicin	5.6 (1/18)	5.5 (9/164)	1
Ciprofloxacin	11.1 (2/18)	5.5 (9/164)	0.298

[a] Prevalence of *Enterobacteriaceae* strains resistant to piperacillin, cefepime, and cephalothin was significantly higher in the community samples than in the hospital samples.

those distributed in hospital canteens (5.3% of samples). This discrepancy may indicate greater attention to good manufacturing practices and good hygiene practices by hospital food operators compared with the personnel operating community canteens. As expected, a higher percentage of positive samples was found for cold dishes (70.9%) than for hot dishes (10.8%), which had been cooked or reheated.

Microbes can be introduced into food products during slicing, packaging, portioning, or other handling processes. However, this contamination should be minimized by good hygiene practices for both personnel and equipment. High levels of *Enterobacteriaceae* were expected in some cold RTE foods such as salads, fresh fruits, and vegetables because the bacterial load is not reduced by thermal processes before consumption. Other products such as rice or pasta salads containing raw vegetables, which are not processed before consumption, also can pose a health risk. In contrast, the presence of *Enterobacteriaceae* in heat-treated foods indicates inadequate cooking or storage or postprocessing contamination. The level of bacteria in these RTE foods will depend on the way the foods are handled and stored. In the present study, 38.6% of the RTE foods analyzed were not compliant with microbiological food safety criteria *(15)* (Table 1).

Sandwiches and pasta salads were the most contaminated RTE foods (data not shown). Although low levels of bacteria may be due to natural contamination of the raw materials used in those foods, the high levels detected in our study suggest faults in the production or subsequent handling of the food, leading to an unacceptable increase in hygienic and public health risk. The most common

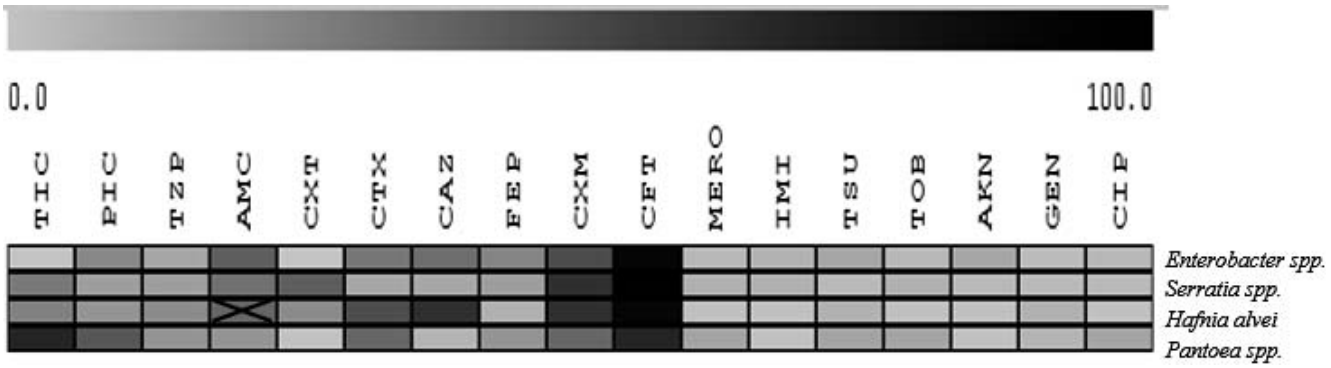


FIGURE 2. *Graphical representation of antimicrobial resistance (% of strains) among five groups of bacteria. A crossed box indicates the antibiotic resistance phenotypes to amoxicillin-clavulanate (AMC) not analyzed because the strains are intrinsically resistant. For the definitions of non-MDR and MDR strains, these antimicrobial categories were not counted. TIC, ticarcillin; PIC, piperacillin; TZP, piperacillin-tazobactam; CXT, cefoxitin; CTX, cefotaxime; CAZ, ceftazidime; FEP, cefepime; CXM, cefuroxime; CFT, cephalothin; MERO, meropenem; IMI, imipenem; TSU, cotrimoxazol (trimethoprim-sulfamethoxazole); TOB, tobramycin; AKN, amikacin; GEN, gentamicin; CIP, ciprofloxacin.*

bacterial strains isolated were *Enterobacter* (59 strains), *Serratia* spp. (27 strains), *H. alvei* (27 strains), *Klebsiella* spp. (17 strains), and *Pantoea* spp. (16 strains) (Fig. 1). These bacteria typically originate from the intestinal tract of animals and humans and can be found on plant products or in the environment. Thus, their presence in RTE foods indicates inadequate food handling practices.

All *Enterobacteriaceae* are killed by heat processes used in food production and are readily removed from equipment and surfaces by appropriate cleaning procedures. Although the presence of these bacteria in RTE products probably represents a very low risk for immunocompetent people, the risk is more significant for immunocompromised and vulnerable groups such as hospital patients.

Samples were tested for *Salmonella* and *L. monocytogenes* because of the implication of these pathogens in disease outbreaks associated with consumption of RTE foods. Unlike previous findings *(5, 22),* these pathogens were not found in the RTE foods we analyzed.

Results of several studies suggest that antibiotic resistance in bacteria originates in the environment and then is transmitted to human pathogens *(11, 25)*. Human activities such as use of antibiotics for treatment of human and animal diseases or as part of agricultural systems could contribute to the increase of antibiotic resistance *(2, 27)*. For example, Overdevest et al. *(23)* found a high prevalence of extended-spectrum β-lactamase (ESBL)–coding genes in retail chicken meat (79.8%), and genetic analysis revealed that the predominant ESBL-coding genes in the chicken meat were identical to those in human rectal swab specimens. Thus, foods that are not appropriately processed could be a vehicle for the spread of antibiotic-resistant bacteria.

In our study, the prevalence of MDR strains was higher in the community canteen samples (50%, $n = 82$) than in the hospital canteen samples (33.3%, $n = 6$) ($P = 0.179$). Isolates from the community samples also were more likely to be resistant to the antibiotics tested than were the hospital isolates (Table 3). Lower resistance rates in bacteria from the hospital canteens might be a function of reduced fitness due to appropriate conditions for storage and handling such as cooling and washing. McMahon et al. *(21)* found lower MICs of antimicrobial agents in bacterial suspensions that had been exposed to low-temperature stress than in unstressed control suspensions. Further investigations would be needed to obtain statistically valid results, and more samples of different kinds of foods that undergo different processing steps should be included.

In recent decades, ESBL-producing *Enterobacteriaceae* have become a severe health problem around the world *(19, 30–32)*. With the exception of *Klebsiella* spp. (see Fig. 2), the major isolated strains were resistant to first- and second-generation cephalosporins (37.50% of *Pantoea* strains to 100% of *Serratia* strains). However, resistance to third- and fourth-generation cephalosporins was more variable (5.88% of *Klebsiella* strains to 62.96% of *Serratia* strains). The *H. alvei* and *Serratia* strains isolated from food in our study had a threefold higher prevalence of resistance to cefepime and twofold higher prevalence of resistance to ceftazidime compared with clinical strains previously analyzed *(1, 24, 26)*. In contrast, *Klebsiella* strains isolated from food were less resistant than clinical strains.

To standardize the use of drugs and avoid the widespread occurrence of antibiotic resistance, the 2017 Expert Committee of the World Health Organization *(29)* identified three categories of antibiotics: access (those to use as first or second choices), watch (those with higher resistance potential), and reserve (those to use as last resort treatments). Some cephalosporins that we tested (i.e., cefepime and ceftazidime) are classified in the reserve group for use when alternatives have failed. Regarding other critically important antimicrobial agents *(29)* such as carbapenems and fluoroquinolones, resistance in some isolates is increasing as can be seen with *Pantoea* spp. This phenomenon is worrisome because the loss of efficacy of these drugs due to the spread of resistant bacteria in nonhuman sources could have an important impact on human health. Therefore, it is crucial to maintain a high level of microbiological quality in RTE foods to reduce the spread of antibiotic resistance from the environment to humans.

In our study, almost 38% of the RTE foods administered in the community canteens were not compliant with microbiological food safety criteria and therefore could pose a health risk for the consumer. Preliminary data revealed that isolated strains of *H. alvei* and *Serratia* spp. were more resistant to the third- and fourth-generation cephalosporins tested than were the clinical strains *(1, 24, 26)*. These findings suggest that food might be a reservoir of antibiotic-resistant strains, which could contribute to the spread of antibiotic resistance genes from the environment to humans. To reduce the potential risk for the consumer, hygienic processing and handling of foods is necessary in both hospital and community canteens. Proper food handling is especially important because of the large number of people worldwide who consume RTE foods every day as part of their regular diets or because of hospitalization. In conclusion, it is essential to more strictly monitor the emergence of antibiotic resistance in bacteria from RTE foods to protect consumers from a public health hazard and maintain the effectiveness of antibiotic therapies.

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