

ANTIMICROBIAL RESISTANCE PROFILE IN *ENTEROBACTERIACEAE* ISOLATED FROM READY-TO-EAT RAW VEGETABLE

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Introduction

The expansion of antimicrobial-resistant bacteria is a global problem, including mainly *Enterobacteriaceae*. Foods consumed raw may pose a potential risk to consumer health, due to the possible transmission of pathogenic and/or antimicrobial-resistant *Enterobacteriaceae*. Antimicrobial resistance is encoded by mobile genes, which can be transferred between strains of the same or different species. Resistance gene transfer can occur in food or in the gastrointestinal tract, thus reducing treatment options for *Enterobacteriaceae* infections¹. Beta-lactam-resistant *Enterobacteriaceae* has great clinical importance and they are involved in serious infections². Beta-lactam is an antimicrobial that acts by preventing the formation of the bacterial cell wall. The indiscriminate use of beta-lactams has increasingly led to the emergence of beta-lactamase-producing bacteria, which break the beta-lactam ring, leading to resistance to beta-lactams¹⁻⁴.

The investigation of antimicrobial resistance is an important tool for providing data for epidemiological surveillance. The objective of this study is to evaluate the antimicrobial resistance profile in *Enterobacteriaceae* strains isolated from ready-to-eat raw vegetable.

Material and Methods

A total of 150 suspected *Enterobacteriaceae* isolates were evaluated. They belong to the culture collection of the Food Hygiene and Microbiology Laboratory (LHIMA), of the Faculty of Pharmacy, UFF. They were previously isolated from 20 samples of ready-to-eat raw vegetable salads, purchased at 16 self-service restaurants located in Niterói, RJ, using the isolation methodology described by Kornacki *et al.* 2015,⁵. The bacterial colonies were sent to the Laboratório de Investigação em Microbiologia Médica-LIMM, UFRJ, for identification by matrix-assisted laser desorption and ionization mass spectrometry (MALDI-TOF MS).

The phenotypic profile of antimicrobial resistance of *Enterobacteriaceae* was evaluated using the disk diffusion test, as recommended by the Clinical and Laboratory Standards Institute (CLSI)⁶. The isolates were classified as sensitive (S), intermediate (I) or resistant (R), according to the interpretation of the CLSI manual (2021). The strains that showed resistance to at least three antimicrobials of different classes was classified as multidrug resistant (MDR)⁷. *E. coli* ATCC 25922 was used as a test control. The isolates that showed resistance to cefotaxime, ceftazidime and aztreonam were identified as suspected of producing extended-spectrum beta-lactamase (ESBL) and was tested in the presence of clavulanic acid. Isolates that showed resistance to imipenem or meropenem and/or ertapenem was considered suspicious for carbapenemase producers⁶. Ertapenem resistance is often the most sensitive indicator for carbapenemase production⁶.

Results and Discussion

Some of the 150 isolates were not viable, thus resulting in 145 final isolates. It was possible to identify seven different genera, with the highest prevalence of *Enterobacter* (65/145; 44.82%), followed by *Klebsiella* (59/145; 40.68%). Fourteen different species of *Enterobacteriaceae* were identified. *Klebsiella pneumoniae* was the most prevalent species (52; 35.86%), followed by *Enterobacter cloacae* (32; 22.07%).

The antimicrobial resistance profile was evaluated according to the CLSI (2021) recommendations, using 16 antimicrobials belonging to 12 different classes. Eighteen strains (18/145, 12.41%) were sensitive to all antimicrobials. Seventy-eight strains (78/145, 53.79%) showed an intermediate resistance phenotype for at least one antimicrobial. Ninety-three strains (93/145, 64.13%) showed resistance to at least one antimicrobial. Thirty-nine strains (39/145, 26.90%) showed both a resistance and an intermediate resistance phenotype to at least one antimicrobial. Seven (4.82%) strains showed resistance to three or more classes of antimicrobials, being classified as MDR. Ampicillin resistance (53; 56.99%) was the most observed profile among the 93 resistant *Enterobacteriaceae*, followed by amoxicillin clavulanic acid (44; 47.31%), nitrofurantoin (11; 11.83%), fosfomicin (4; 4.30%), tetracycline (4; 4.30%), aztreonam, cefuroxime, ceftazidime, levofloxacin (3; 3.22% each), imipenem, trimethoprim (2; 2.15%, each), ertapenem and gentamicin (1; 1.07%). All strains were sensitive to piperacycline, tazobactam and chloramphenicol.

The ESBL phenotype was observed in *K. pneumoniae* (2/7, 28.57%), *S. marcescens* (2/7, 28.57%), *E. coli* (1/7, 14.29%), *E. cloacae* (1/7, 14.29%) and *E. bugandensis* (1/7, 14.29%). In addition, three (2.07%) *Enterobacteriaceae* (*E. bugandensis*, *Enterobacter* spp. and *K. pneumoniae*) showed resistance to at least one carbapenem (imipenem and/or ertapenem), being classified as CRE (Carbapenem-resistant *Enterobacteriaceae*) suspected. The use of antimicrobials is essential for the treatment of non-intestinal infections by *Enterobacteriaceae* with carbapenems being the last antimicrobials of choice^{3, 4, 7}.

The *Enterobacter* spp. (F4R16/4) showed two halos around the ertapenem disc. A smaller (16 mm) ertapenem-resistance halo between the antimicrobial disc and the weak bacterial growth, and a larger (23 mm) outer halo of ertapenem sensitivity between the antimicrobial disc and the dense bacterial growth. Bacterial colonies are formed by identical or strictly similar single cells (clone), originating from the multiplication of a single cell, however, individual variations can be observed in the same colony⁸.

Conclusion

Ready-to-eat raw vegetable salads are an important carrier of antimicrobial-resistant *Enterobacteriaceae*, especially *Enterobacter* spp. and *Klebsiella pneumoniae*. MDR, ESBL and CRE strains are present in ready-to-eat raw vegetable salads, being a problem that can affect the public health system.

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