ESBL-PRODUCING ENTEROBACTERIACEAE IN VEGETABLES INTENDED FOR FRESH CONSUMPTION.

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INTRODUCTION

Antimicrobial resistance is one of the main threats to public health nowadays. The contamination of fresh produce with antimicrobial-resistant isolates is particularly important because most food of plant origin is consumed raw, allowing these bacteria to interact with the gut microbiota or to colonise the gut during the intestinal passage, which increases their possibilities of dissemination. There is rising concern about the increased prevalence of ESBL-producing Enterobacteriaceae; whose presence in fresh produce has been previously reported in several studies. However, these studies are diverse in their results and conclusions.

Given the scarcity of data on this subject, there is a need to study the importance of the transmission routes that lead tocontamination with antibiotic-resistant bacteria at primary production and post-harvest stages, and the implications of the transmission of these strains through the food chain. Consequently, the aim of this study was to examine fresh vegetables for direct human consumption as potential vehicles of extended-spectrum β-lactamase producing Enterobacteriaceae and to characterise the isolated resistant-bacteria.

METHODOLOGY

Samples (n=117) of fresh vegetables were collected from three farms and a street market. Additional samples from soil (n=18), water (n=14), air (n=11), and the hands of farm workers (n=12) were also studied. Enterobacteria and E. coli counts were determined by the spread plate technique in ChromAgar Enterobacteria. The detection and isolation of ESBL-producing strains was carried out by streaking a loop of an enriched solution (37ºC/24 h) of each sample onto ChromAgar ESBL plates. The isolates were later identified through MALDI-TOF.

The disc-diffusion method (EUCAST) was selected for the ESBL confirmation of the suspected isolates, using the MAST D72C AmpC and ESBL detection kit, and a selection of antibiotics of different categories were applied for the detection of multidrug-resistant isolates (MDR). In addition, representative isolates were selected to determine the minimum inhibitory concentration (MIC) by microdilution using Sensititre EUVSEC2 plates.

The presence of blaTEM, blaSHV and blaCTX-M genes in phenotypically confirmed ESBL-producing isolates was checked by PCR. Amplified products were purified, sequenced and then compared with sequences contained in a curated database.

RESULTS

The average count of Enterobacteriaceae was 4.2 ± 1.06 logCFU/g or ml, with a maximum value of 6.2 log CFU/g. The presence of E. coliwas detected in 29 samples (16.7 %) with an average value of 2.1 ± 0.75 log CFU/g. The detection of ESBL-producing bacteria was statistically related ($p<0.05$) to the concentration of Enterobacteriaceae.

Phenotypical confirmation resulted in 15 ESBL-producing isolates, 14 from vegetable samples and 1 from a water sample. Thesewere identified as Serratia fonticola (11) and Rahnella aquatilis (4). Five isolates of S. fonticola were determined to be resistant to cefepime, aztreonam and gentamicin, thus being considered MDR.

All isolates showed an intermediate MIC for temocillin. In addition, two S. fonticola isolates showed an MIC above the breakpoint for cefoxitin, one of these also exhibiting an MIC above the breakpoint for cefotaxime and cefepime. Three R. aquatilis isolates had MICs above the breakpoint for cefotaxime, one of which was alsofound to be resistant to cefoxitin.

Four isolates identified as S. fonticola and two isolates identified as R. aquatillis were found to carry a CTX-M gene. The genes from S. fonticola were found to be similar to blaFONA5 and those from R. aquitilis similar to blaRAHN2.

DISCUSSION

The results obtained in this study show that raw vegetables intended to be consumed fresh present high counts of Enterobacteriaceae and, to a lesser extent, E. coli. The detection of ESBL-producing bacteria was related to the Enterobacteriaceae counts, indicating that routine monitoring of this bacterial group in fresh produce can be a good indicator of the presence of antibiotic-resistant bacteria.

The presence of ESBL-producing Enterobacteriaceae in the studied samples confirmed previous reports that identified fresh produce as a vehicle of antibiotic-resistant bacteria and their genes. ESBL-producing S. fonticola and R. aquatilis are environmental Enterobacteriaceae commonly found in raw vegetables. Even though they rarely result in infectionin humans, they can become a reservoir of antibiotic-resistance genes that can be disseminated along the food chain andpotentially transferred to pathogens during the intestinal passage. Moreover, their presence can indicate a high dissemination in the farm environment, whereby water is identified as an important source of contamination.