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Ready-to-eat salads: a vehicle of bacteria and clinically relevant antibiotic resistance genes

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Background. The increase demand for fresh vegetables is causing an expansion of the market for minimally processed vegetables along with new recognized food safety problems. The aim of this study was to analyze the microbiological quality of Portuguese ready-to-eat salads (RTS) and their role in the spread of bacteria carrying antibiotic resistance (ABR) genes.

Methods. RTS (n=50; 7 brands; split or mixed leaves, carrot, cornmeal) were collected in 5 supermarkets in Porto (2010). We screened for Salmonella, Listeria monocytogenes and aerobic mesophilic counts, coliforms and Escherichia coli (standard methods). Samples were also plated in different selective media with/without antibiotics before and after enrichment. ABR was studied by agar diffusion/E-test (CLSI/EUCAST) and detection of extended-spectrum β-lactamase (ESBL) by double disk synergy test and sequencing. Species were identified by PCR (Gram positive), API ID32GN/16S PCR (Gram negative). ABR genes, integron types and E. coli phylogenetic groups (PHG) by PCR, clonality by MLST/PPGE in E. coli and conjugation assays in specific isolates were performed.

Results. A high number of RTS presented poor microbiological quality (86% for aerobic mesophilic, 74% coliforms, 4% E. coli), despite the absence of pathogens. E. coli was detected in 13 samples (n=78; all types and 4 brands; PHG A, B1, B2, D) with resistance to tetracycline (72%; tetA and/or tetB), streptomycin (56%; streptomycin), sulfamethoxazole (50%; sul1 and/or sul2), trimethoprim (50%; dfrA1 or dfrA2), ampicillin (47%; ampicillin), nitrofurazone (36%; ciprofloxacin) (5% or chloramphenicol (3% ciprofloxacin). Two integron types (dfrA1-ermC1, dfrA2-ermC1) were detected in 12 multidrug resistant isolates (MDR), which includes E. coli (n=3; D) belonged to the widespread clonal lineage ST89. Among coliforms, were detected 2 Raoultella sp (2 samples) carrying an ESBL, Shigella 2 and 1 Enterobacter freundii with a qnrB gene. Among Enterococcus (n=108; 35 samples): E. casseliflavus-40, E. faecalis-20, E. faecium-18, E. hirae-9, E. gallinarum-5, and Enterococcus spp-16. ABR was detected for tetracyclines (6%; tetA and/or tetB), erythromycin (3%; ermB), nitrofurazone (1% or ciprofloxacin (1%).

Conclusions. The present study positions RTS within the spectrum of ecological niches that may be reservoirs/vehicles for antibiotic resistance bacteria/genes with clinical interest (e.g. E. coli-DST69; E. faecalis-76,599-2; qnrB) being these findings worthy of attention as their spread to humans by ingestion cannot be dismissed.

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A review of the availability and consumption of fish and seafood in Portugal

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Fish consumption has been associated to a range of health benefits. Fish is low in fat, high in protein and an excellent source of omega 3 fatty acids. With a huge seaside border, Portugal has a privileged position for fish and seafood access. In Portugal, eating fish has not only an important dietary value but also an important role in cultural heritage. The aim of this work was to review the available data on fish and seafood supply/consumption in Portugal, evaluating both time trends and self-sufficiency.

The searched data sources included national or regional representative studies: FAO food balance sheets (FBS) (1961 to 2009), household budget surveys (HBS) (1990 to 2005) and individual consumption obtained through the Epiporto study circa 2000, the National Health Survey (INS) (1965 to 2000) and the SPNCA/Nestlé survey in 2009. The first two sources used provide data about the availability of fish and seafood: the FBS at national level, focusing on the major fish groups and the HBS at household level, listing the fish and seafood species; the individual data translates the real consumption of fish and seafood either in frequency or quantity.

In 1961 the fish and seafood self-sufficiency ratio (production/(production + imports -exports)) for Portugal was 1.01, which indicated the independence of the country for the supply of products. Along time this ratio has decreased and in 2009 its value was 0.37, which is explained for the decrease in production and the large increase in the imports. However, Portugal still has a high supply of fish and seafood. In 2009 FBS, a value of 167g/capita/day was registered for Portugal, ranking the country in the 4th position among the world countries with largest supply and the 1st position among the Mediterranean countries. By contrast, the EU-27 registered only 63g/capita/day in 2009. From the 2005 HBS it was observed that around 70% of the household acquisitions of fish and seafood rely on cod fish (18.6%), hake (13.7%), jack-fish/mackerel (13.8%), sardines (9.8%), tuna (4.3%), squid/saltfish (4.2%), octopus (3.9%) and swordfish (2.4%). Data from the INS revealed that the proportion of people consuming fish has decreased from 55% in 1995 to 49% in 2006. The 2009 SPNCA/Nestlé survey found out similar results, a value of 49% for fish and of 5.5% for seafood. Additionally, data from the Epiporto study reveals a daily consumption of fish and seafood of 75.6g±34.3g/day for women and 78.6±37.9g/day for men.

The work to be presented will allow concluding that fish plays an important role in the Portuguese diet. However, as the characterization of food patterns is an essential tool for building shaped nutritional policies and to assess the associated food consumption environmental impacts, efforts to keep the data updated and comparable along time are necessary.