International Journal of Food Microbiology 269 (2018) 80–86

Contents lists available at ScienceDirect

International Journal of Food Microbiology

journal homepage: www.elsevier.com/locate/ijfoodmicro

Increased exposure to extended-spectrum β-lactamase-producing multidrug-resistant *Enterobacteriaceae* through the consumption of chicken and sushi products

Ana Isabel Vitas*, Dixita Naik, Lara Pérez-Etayo, David González

Department of Microbiology and Parasitology, University of Navarra, C/Irunlarrea 1, 31008 Pamplona, Spain
IDISNA, Navarra Health Research Institute, Pamplona, Spain

ARTICLE INFO

Keywords:
ESBL
Chicken
Sushi
Multidrug resistances
CTX-M
SHV-12

ABSTRACT

The aim of this study was to determine the occurrence and patterns of resistance of extended-spectrum β-lactamase (ESBL) producing *Enterobacteriaceae* in food products purchased in Navarra, northern Spain. A total of 174 samples of fish and chicken were analyzed from September 2015 to September 2016, including raw and ready-to-eat products: trout (n = 25), salmon (n = 28), panga (n = 13), chicken nuggets and chicken scalopes (n = 32), sushi (n = 31) and sliced cooked poultry (n = 45). Cefpodoxime-resistant strains were isolated on ChromID ESBL agar and further phenotypic (antimicrobial study on MicroScan© NM37 panel) and genotypic characterization (multiplex PCR, sequencing and multi-locus sequence typing, MLST) was performed to confirm and characterize ESBL producers. Raw chicken and sushi have been determined as the most risky products regarding transmission of ESBL-producing *Enterobacteriaceae* (occurrence 53.1% and 19.4%, respectively), while sliced cooked poultry products appear to be a safe product in this aspect. With regard to raw fish, prevalence in salmon was lower (3.6%) than in trout and panga (16.0%). Ninety-eight per cent of ESBL isolates (n = 50) show multidrug-resistant profiles, highlighting the high resistances against quinolones and tetracyclines observed in chicken isolates, as well as against ertapenem and chloramphenicol in sushi strains. Predominant β-lactamase type was SHV-12 (50.1%), followed by TEM-type (24.5%) and CTX-M (20.8%). In addition, CTX-M type was only detected in chicken products. The phylogenetic study showed the prevalence of groups A (35%), F (25%) and B1 (15%), usually related to nonvirulent strains. MLST *E. coli* isolates (n = 20) were grouped into 5 clonal complexes (CC) and 15 sequence types (ST), showing high clonal diversity. ST117 was the prevalent sequence type, while the human pathogen ST131 was not detected in this study. The high prevalence of ESBL-producing multidrug-resistant *Enterobacteriaceae* detected in products of widespread consumption such as chicken and sushi, increases the concern regarding human exposure to superbugs and encourages the need to improve surveillance of this public health issue.

1. Introduction

During the last decade extended-spectrum β-lactamase-producing *Enterobacteriaceae* (ESBL-E) have become a major public health concern because of the possible transmission of these bacteria to humans (Wang et al., 2006). In fact, the number of infections caused by these bacteria has increased throughout the world (Al-mayahie et al., 2016; Ouedraogo et al., 2016; WHO, 2014). Furthermore, ESBL-E usually exhibit co-resistance to multiple classes of antibiotics, increasing the risk of clinical treatment failure.

In addition to clinical environments and healthy carriers (Villar et al., 2013), ESBL-E producers have been isolated from a wide range of sources, such as foods (Denis et al., 2015; Ojer-Usoz et al., 2013; Petternel et al., 2014; Rasmussen et al., 2015), water environments (Hu et al., 2013; Korzeniewska et al., 2013; Ojer-Usoz et al., 2014) and wild and domestic animals (Alcalá et al., 2016; Bogaerts et al., 2015). Among other reasons, the wide dissemination of β-lactam resistances in different environments is related to the association of ESBL- and AmpC encoding genes with mobile genetic elements (Schultsz and Geerlings, 2012), which facilitates the spread of resistances within intense bacterial communities, such as intestinal microbiota of livestock or wastewater treatment plants.

Poultry meat is considered an important reservoir of antibiotic resistances and high prevalence of ESBL-E (especially *E. coli*) has been

* Corresponding author.
E-mail address: avitas@unav.es (A.I. Vitas).

https://doi.org/10.1016/j.ijfoodmicro.2018.01.026
Received 9 October 2017; Received in revised form 19 December 2017; Accepted 30 January 2018
Available online 01 February 2018

reported in different countries (Abdallah et al., 2015; Kola et al., 2012; Overdevest et al., 2011; Tansawai et al., 2018; Trongjit et al., 2016). In fact, previous studies carried out in Navarra (Spain) have shown that 84% of poultry samples collected from 50 retail outlets were positive for ESBL-E (Ojer-Usoz et al., 2013). Due to the extended consumption of this product throughout the world in different types of presentations, the risk of transfer of these resistant bacteria to the population should not be neglected. On the other hand, while published studies on the occurrence of ESBL-producing bacteria in fish and fishery products are limited (Brahmi et al., 2017; Le et al., 2015; Ojer-Usoz et al., 2017), available data show prevalence ranging between 3% and 63%, depending on the country and type of product. This poses special concern regarding the transmission to humans when contaminated fish products are consumed raw, as in the case of sushi. In fact, deficient hygienic practices at domestic level could promote ESBL-E transmission to foods during handling and storage of products as consequence of cross-contamination (Tschudin-Sutter et al., 2014).

Within this framework, the purpose of this study was to evaluate the presence of ESBL-E in raw (fresh fish and chicken) and processed ready-to-eat products (RTE) (sushi and sliced cooked poultry) purchased in Navarra (northern Spain), as well as to characterize their patterns of antimicrobial resistance and β-lactamase (*bla*) genes.

2. Material and methods

2.1. Collection of food samples

A total of 174 food samples were collected from different shops and supermarkets in Navarra (northern Spain) from September 2015 to September 2016. Sampling was randomly carried out in big and small retail outlets of Pamplona (the main city of Navarra).The study includes raw food (trout $n = 25$; salmon $n = 28$; panga $n = 13$; chicken nuggets and scallops $n = 32$) and processed (RTE) (sushi $n = 31$; sliced cooked poultry $n = 45$). All sushi presentations were filled with fishery products (as salmon, tuna, shrimp, swordfish) and vegetables (avocado, carrots, cucumber). These products have been selected to check if prevalence of ESBL-producing *Enterobacteriaceae* still high in chicken, as detected in previous studies (Ojer-Usoz et al., 2013) and to obtain information regarding prevalence of these bacteria in raw ingredients and final presentations of widespread consumed RTE products as sushi.

Samples were immediately transported to the laboratory and maintained at refrigeration temperatures from collection to microbiological analysis, which was carried out within 24 h of reception.

2.2. Bacterial isolation and identification

Samples (25 g) were homogenized in 225 ml of buffered peptone water (BPW, Merck, Darmstadt, Germany) by using a Stomacher (Lab-Blender 400, Seward Medical, England). Following incubation at 37 °C ± 1 °C during 24 h, a second enrichment was performed by adding 1 ml of pre-enriched sample to 10 ml of EE Mossel Broth (Difco, Le Pont de Claix, France) and a new incubation period was applied (37 °C ± 1 °C, 24 h). Resistant strains to Cefpodoxime were isolated on ChromID ESBL agar (Biomerieux, Marcy l'Etoile, France) (37 °C ± 1 °C, 24 h). Isolates were identified by using biochemical tests (oxidase, API 20E, Biomerieux) and were stored at −80 °C for further characterization.

2.3. Antimicrobial susceptibility testing and ESBL detection

Susceptibility to 33 antibiotics belonging to 9 groups (penicillins, cephalosporins, monobactams, β-lactamase inhibitors, carbapenems, aminoglycosides, quinolones, tetracyclines and others) was determined by using the automatized autoSCAN-4 system (Siemens AG, Germany) with the MicroScan® NM37 panel (Siemens AG) and Lab Pro® 3.5 software. ESBL production was considered to be positive if there was a decrease in MICs of ≥3 two-fold concentration with any of the β-lactams in the presence of clavulanic acid, *versus* its MIC when tested alone due to the ability of clavulanic acid to inhibit β-lactamases (Komatsu et al., 2003). When necessary, ESBL production was confirmed by the combined disk test (CLSI, 2013) and the double-disk synergy test (Jarlier et al., 1988). All antibiotics were purchased from Rosco Diagnostica (Taastrup, Denmark).

2.4. Characterization of β-lactamase genes

For the detection of bla_{TEM}, bla_{SHV} and bla_{OXA-1} genes, the multiplex-PCR described by Colom et al. (2003) was used. The presence of bla_{CTX-M} genes was studied using a modification of the multiplex-PCR used by Woodford et al. (2006), which consists of initial denaturation at 94 °C for 5 min, followed by 30 cycles of DNA denaturation at 94 °C for 45 s, primer annealing at 55 °C for 30 s, primer extension at 72 °C for 50 s and a final elongation at 72 °C for 6 min. Primers were obtained from Sigma and dNTP mix and Taq-polymerase were supplied by Bioline (Bioline, London, England).

PCR products of *bla* genes were analyzed by DNA sequencing (EZ-Seq purification service of Macrogen). The amino acid sequences obtained were compared with those included in the GenBank database using BLAST, as well as with those deposited at the web site http://www.lahey.org/Studies/, in order to ascribe the specific type of β-lactamase gene.

2.5. E. coli phylogenetic characterization

E. coli strains were assigned to one of the main phylogenetic groups (A, B1, B2, C, D, E and F) using a multiplex PCR and two simple PCR methods previously described (Clermont et al., 2013). DNA was purified with the QIAGEN QIAquick PCR purification kit and Multilocus Sequence Typing (MLST) was performed according to Tartof et al. (2005). The primers of seven housekeeping genes, including *adk*, *fumC*, *gyrB*, *icd*, *mdh*, *purA* and *recA*, were PCR-amplified, purified and sequenced. The sequences were then compared with the PubMLST database http://mlst.warwick.ac.uk/mlst/dbs/Ecoli, and each unique combination of alleles (the allelic profile) was designated as a sequence type (ST).

2.6. Statistical analysis

The results were subjected to statistical processing with SPSS 15 software, applying the chi square test for comparison of ESBL with food, with a level of significance of $p < 0.05$.

3. Results and discussion

3.1. Prevalence of ESBL-E in chicken and fish food products

The occurrence of extended-spectrum β-lactamase-producer *Enterobacteriaceae* in 174 samples from the retail market of Navarra (Spain) is presented in Table 1. As expected, chicken raw products showed the highest prevalence of ESBL-E (53.1%; confidence interval 37.3%–68.5%), in a similar way as that reported by other authors (Kola et al., 2012; Leverstein-van Hall et al., 2011; Overdevest et al., 2011). However, this prevalence was lower than the 84% determined in a previous study in the same region of Spain (Ojer-Usoz et al., 2013). This could be due to the different kind of chicken products analyzed (chicken breast in the previous study and semi-processed chicken in this study) and/or the possible improved hygiene conditions during processing and packaging as consequence of HACCP systems implantation. In any case, chicken meat still represents a substantial source for the transmission of ESBL genes or ESBL-producing strains to humans. By contrast, occurrence in sliced cooked poultry meat (chicken and turkey) was 0%, being lower than the value reported in China in 2014 (6.7%)