ANTIMICROBIAL RESISTANCE IN SEAFOODS, AND NON-THERMAL FOOD PROCESSING

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ABSTRACT

Seafoods may carry strains of bacterial pathogens resistant to clinically-used antibiotics. This review summarizes recent work on antimicrobial resistance from seafoods. Since ready-to-eat seafoods may also be at risk of transmitting antimicrobial resistance through the food chain, it is important to analyse how food processing methods may affect the bacterial populations in foods. Recent studies based on high-hydrostatic pressure applied on seafoods in combination with natural antimicrobials (bacteriocins and essential oils) indicate that the combined treatments achieve a greatest reduction of bacterial loads in the food. Nevertheless, application of high-throughput sequencing technology to study the dynamics of bacterial population surviving treatments reveals complex changes in bacterial populations during food storage. This makes necessary to further study the antimicrobial resistance in those strains surviving treatments and which may be consumed along with the processed food.

Keywords: antimicrobial resistance; seafood; high hydrostatic pressure; biopreservation

ANTIMICROBIAL RESISTANCE IN SEAFOODS

The market for seafood products reached 175 million tons in 2016 (FAO, 2016). Control of antimicrobial resistance in the food chain, including seafoods, has become a matter of general concern (EFSA-ECDC, 2018; FAO, 2017; Watts et al., 2017), and is now being considered as one of the cornerstones in the one-health concept launched by the European Union (Queenan et al., 2016; European Commission, 2017).

Seafoods may contain both saprophytic and human pathogenic bacteria, all of which are suspect of carrying antimicrobial resistance traits (Elbashir et al., 2018). The main causes for antimicrobial resistance in seafoods have been attributed to anthropogenic contamination and the use of antimicrobials in aquaculture (Cabello et al., 2016; FAO, 2017; Han et al., 2017). Furthermore, the microbiota of seafoods may change considerably during processing, becoming enriched with antimicrobial resistant strains from many other sources by cross contamination (FAO, 2017; Noor Uddin et al., 2016). Ready-to-eat seafoods pose a higher risk for transmission of antimicrobial resistance to humans. Fresh marine seaweeds represent an additional category of seafoods which are becoming increasingly popular in Western diets. In spite of the fact that seaweeds may contain bacteria pathogenic to humans (Gupta et al., 2010; Martin et al., 2015; Singh et al., 2015), there are still limited numbers of studies on their antimicrobial resistances.

Several studies have shown antimicrobial resistance in bacteria isolated from seafoods, mainly in strains belonging to the genera *Vibrio* (Lopatek et al., 2015; Dubert et al., 2016; Elmahdi et al., 2016; Kang et al., 2016; Elbashir et al., 2018), *Listeria* (Wieczorek and Osek, 2017), *Pseudomonas* (Tran et al., 2011; Boss et al., 2016; Maravic et al., 2018), *Shewanella* (Kang y So, 2016), *Arcobacter* (Morejón et al., 2017), *Aeromonas* (Piotrowska and Popowska, 2015; Yano et al., 2015), *Staphylococcus* (Sergelidis et al., 2014; Boss et al., 2016; Dong et al., 2017), *Enterococcus* (Hammad et al., 2014; Boss et al., 2016; Valenzuela et al., 2010) and also Enterobacteria (Brillhart and Joens, 2011; Wang et al., 2011; Ryu et al., 2012; Changkaew et al., 2014; Nawaz et al., 2015; Rees et al., 2015; Boss et al., 2016; Vignaroni et al., 2016; Grevskott et al., 2017; Obaidat et al., 2017; Li et al., 2018). Within last group, the detection of multiresistant strains and extended-spectrum beta-lactamase (ESBL) strains deserves special attention because extended-spectrum beta-lactam antibiotics are widely used to treat hospital infections (Ryu et al., 2012; Ahmed et al., 2015; Vignaroli et al., 2016; Grevskott et al., 2017; Roschanski et al., 2017; Said et al., 2017; Singh et al., 2017).

In the food chain, bacteria are exposed to different types of antimicrobial substances, including biocides used for cleaning and sanitation purposes and chemical preservatives. Tolerance to biocides has been linked with specific biocide-tolerance genes, specially efflux pumps. Some efflux pumps have a broad-sustrate specificity, and can also export a range of substances, including antibiotics. Furthermore, biocide-tolerance genes have been found to be linked to gene casettes that contain an array of antimicrobial resistance genes (Ortega Morente et al., 2013; Pal et al., 2015; Li et al., 2017). Therefore, exposure to one of the antimicrobials will coselect for other antimicrobial resistance genes that are physically linked in the casette. In addition, antimicrobial resistance gene clusters are often linked to mobile genetic elements (such as transposons or plasmids), facilitating horizontal gene transfer and spread to other bacteria.

A recent study from our group on antimicrobial resistance in bacteria isolated from seafoods sold at Spanish supermarkets indicated that most bacterial isolates from fish belonged to genus *Pseudomonas* (Romero et al., 2017). Although the incidence of genes encoding for ESBL was very low, one *Pseudomonas synxantha* isolate from anchovies carried *bla*$_{NDM-1}$ gene. Furthermore, boiled, ready-to-eat foods (pranws, Norway lobster) showed high microbial loads (of 4 to 5 log colony forming units per gram). One *Acinetobacter oleivorans* strain isolated from boiled prawns carried *bla*$_{CTX-M}$ and *bla*$_{TEM}$ genes, and one multiresistant *Pseudomonas putida* strain isolated from Norway lobster carried *bla*$_{TEM}$ along with copper resistance *pcoA/copA* genes. These results suggest that ready-to-eat seafoods may act as vehicles for transmission of antimicrobial resistance genes.

FOOD PROCESSING AS A WAY OF REDUCING MICROBIAL LOAD AND ANTIMICROBIAL RESISTANCE

Approaches to reduce the burden of antimicrobial resistance in seafoods should aim both at avoiding selection of antimicrobial resistance during food production and processing, and combating transmission of antimicrobial resistant strains in the food chain. One way to combat antimicrobial resistance could be the use of food processing technologies that do not rely on chemical antimicrobials. As an example, high-hydrostatic pressure (HHP) is a non-thermal process that is widely applied on foods in order to reduce microbial load and prolong the food product shelf life, with minimal changes on the food nutritional and organoleptic properties (Balasubramaniam et al., 2015; Wang et al., 2016). HHP is usually applied on the packaged food, avoiding the risk of cross-contamination. HHP treatments have been applied on seafods with several purposes such as reducing the total microbial load (Ginson et al., 2015; Li et al., 2016) and inactivating human pathogenic bacteria such as *Vibrio*, *Listeria*, *Escherichia*, *Salmonella* o *Staphylococcus* (Fioretto et al., 2005; Kural et al., 2008; Ye et al., 2012; Kaur y Rao, 2017; Lebow et al., 2017) and viruses (Uema et al., 2017).

One of the limitations of HHP treatments is that they may affect the color and texture of proteinaceous seafoods as the pressure of treatment increases. In order to improve the efficacy of treatments, HHP can be used as part of hurdle technology in combination with other natural antimicrobials such as bacteriocins or plant essential oils or phenolic compounds. Previous studies have addressed possible applications of bacteriocins for food preservation, including seafoods (Galvez et al., 2014; Johnson et al., 2017). Bacteriocins are ribosomally synthesized antimicrobial peptides or proteins, which can be posttranslationally modified or not (Jack et al., 1995). Enterocin AS-48 is a broad-spectrum circular bacteriocin with a generally-recognised as safe (GRAS) status (Cebrián et al., 2012; Grande Burgos et al., 2014). Inmersion in an enterocin AS-48 solution for 1 min delayed bacterial growth and reduced biogenic amine production in sardines during refrigerated storage (Ananou et al., 2014). Spray-application of an enterocin AS-48 solution reduced viable counts of a cocktail of *Listeria monocytogenes* strains on raw hake and salmon fillets as well as on smoked salmon, an effect that was potentiated by bacteriophage P100 (Baños et al., 2016). So far, no cross-resistance has been reported for this bacteriocin and antibiotics used to treat human or animal infections. The antibacterial activity of enterocin AS-48 can be potentiated by other antimicrobials such as essential oils and phenolic compounds (Abriouel et al., 2010). Essential oils have proven to be effective for extension of the shelf life of fresh fish (Harpaz et al., 2003; Giatrakou et al., 2008; Quitral et al., 2009; Çoban et al., 2014). Thyme oil has been reported to improve the preservation of bluefish (Erkan et al., 2011), smoked rainbow trout (Erkan, 2012), fresh Mediterranean swordfish fillets (Kykkidou et al., 2009), Asian sea bream (Harpaz et al., 2003), sea bass (Kostaki et al., 2009) and peeled shrimps (Mastromatteo et al., 2010).

A recent study from our group addressed the application of a mild HHP treatment (300 MPa, 5 min) on the preservation of seabream fillets packed under vacuum in films that were previously activated or not with enterocin AS-48 and thymol (Ortega Blázquez et al., 2018). The activated film and the HHP treatment applied singly reduced aerobic mesophiles viable counts by 1.46 and 2.36 log cycles, respectively, while the combined treatment achieved a reduction of 4.13 log cycles. Both HHP and combined treatments resulted in longer delays in bacterial growth during refrigerated storage of the samples. The bacterial diversity of fillets was investigated by high-throughput sequencing with Illumina technology. *Proteobacteria* were the dominant phyla in sea bream fillets, but the relative abundance of *Firmicutes* increased by the end of storage both in controls and in samples treated by HHP singly or in combination with the activated films. There were large differences in the dynamics of bacterial populations in the controls and in the treated samples during storage. The HHP treatments (singly or in combination with activated films) determined a high relative abundance of *Acinetobacter* (followed by *Pseudomonas* and *Shewanella*) during early storage as well as a higher relative abundance of lactic acid bacteria by the end of storage. These results highlight the importance to also determine the presence of antimicrobial resistance traits in bacteria surviving HHP treatments and proliferating in the processed food during storage.

In conclusion, antimicrobial resistance is a complex problem to be dealt with during food processing. Seafoods are complex ecosystems that change during the product shelf life depending on the applied preservation treatments. Ongoing studies in our laboratory are aiming at understanding how these changes in the bacterial communities of the food influence the prevalence of antimicrobial resistance in foods.

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