# Food processing as a risk factor for antimicrobial resistance spread along the food chain

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**Abstract**

Farms and food industries rely to a large extent on the use of biocides as disinfectants and other antimicrobial agents and preservatives with antimicrobial properties in order to provide food of high microbiological quality and safe for consumers. However, in the last decades it has become apparent that long-term sub-lethal exposure to these antimicrobial agents can exert a selective pressure leading to the emergence and spread of microbial strains with a reduced susceptibility to the used antimicrobials, which can persistently colonize food-processing environments and recurrently contaminate food. In addition, it may induce resistance to unrelated and clinically relevant antibiotics, in a phenomenon known as cross-resistance. This review aims to provide insights on how antimicrobial resistance emergence and spread can be affected by certain food processing activities and to discuss recent research focused on different pathways through which biocides and other antimicrobials could co-select for bacteria resistant to clinically relevant antibiotics.

## Introduction

Antimicrobial resistance (AMR) is currently a global threat that affects human and animal health. Considerable importance is being paid to pathogenic bacteria which have become resistant to different classes of antibiotics in clinical [1,2], veterinary and food related settings [3,4]. Infections caused by antibiotic resistant microorganisms have a significant and increasing economic impact [1,5,6]. In addition, other antimicrobials, such as disinfectants, used to control or reduce the bacterial burden in hospitals and agro-food industries are becoming less effective due to the decreased susceptibility to them of the targeted microorganisms [7]. Many researchers are trying to understand the mechanisms through which bacteria, under certain conditions prevailing at farm, industrial and household settings, become resistant or tolerant to a range of antimicrobials. Concerning the food sector, keeping food safe for consumers relies to a large extent on the use of biocides as disinfectants and other preservatives with antimicrobial properties [7,8]. However, use of biocides and other antimicrobials can induce resistance through long-term sub-lethal exposure and exert a selective pressure favoring isolates which harbor AMR genes (which may also be acquired or transferred, in some occasions, by horizontal gene transfer) [9]. In addition, the exposure of bacteria to low concentrations of biocides and other antimicrobials may induce resistance to unrelated and clinically relevant antibiotics, in a phenomenon known as cross-resistance [7,9].

Scarce data is available on the contribution of the food chain to the global burden of infections caused by acquired antimicrobial-resistant bacteria. Potential sources of AMR in the food chain (graphically summarized in Figure 1) are the use and/or overuse of antimicrobials administered to food producing animals [4] that could contribute to the global burden of human AMR infections [1,4]. Also the role of some environmental niches within the food chain acting as potential hotspots for AMR development and persistence of resistant microorganisms, which may then reach the human host [10,11]. This review aims to provide insights into the spread of AMR through the food chain and how it can be affected by certain food processing activities and discuss recent research focused on different pathways through which biocides and other antimicrobials could co-select for bacteria resistant to clinically relevant antibiotics.

## Determinants of resistance to antimicrobials used in food processing

Bacteria can respond to different types of selective pressures, including antimicrobials, by undergoing various physiological changes resulting in an acquired tolerance towards the inducing agent and/or other antimicrobial agents in an adaptive response mediated through chromosomal mutations, acquisition of new phenotypes *via* horizontal gene transfer or through co-/cross-resistance or cross-protection processes [4,7,12]. In the food chain, AMR can be acquired when bacteria are challenged with antibiotics, preservatives, heavy metals, antiseptics or disinfectants, among others [4,13]. These antimicrobials and food processing aids induce complex bacterial stress responses, commonly triggering the overexpression of efflux pumps, responsible for expelling the antimicrobials from the cell. The relevant efflux systems in Gram-negative bacteria are divided into two groups: primary and secondary transporters. The first group are members of the ATP-Binding Cassette (ABC) family while the second group belong to the Major Facilitator (MFS) and Resistance-Nodulation-Division (RND) Superfamilies, Small Multidrug Resistance (SMR) and Multidrug and Toxic compound Extrusion (MATE) families [14,15]. Intrinsically, greater tolerance to biocides has been noticed in Gram-negative bacteria due to their inherent outer membrane permeability barrier [13] and to the occurrence of

certain resistance genes associated with resistance to quaternary ammonium compounds (QAC) such as *qacE*, *qacEΔ1*, *qacF*, *qacG*, and *qacH* [13].

Among different Gram-negative bacterial species, various efflux pumps have been described in *Escherichia coli* and *Salmonella enterica* (AcrAB-TolC), *Pseudomonas aeruginosa* (MexAB-OprM, MexCD-OprJ and MexXY-OprM) and *Campylobacter jejuni* (CmeABC) [14]. Moreover, a TolC multidrug resistance efflux pump associated with biocide resistance has been also detected in members of the *Enterobacteriaceae* family [9,12]. In Gram-positive bacteria, same family groups predominate as efflux proteins which can transport various biocides and disinfectants as well as unrelated compounds, which may result in a multidrug resistance phenotype of great significance [16]. For example, a *norA* gene coding for a multidrug efflux pump has been detected in a *Lactobacillus pentosus* strain [17]. Moreover, Gram-positive bacteria, including *Listeria monocytogenes* and *Staphylococcus aureus,* have demonstrated ability to acquire mutations in efflux systems, which are responsible for multidrug resistance phenotypes and reduced susceptibility to biocides [18,19].

**Persistence in food processing environments as affected by the usage of antimicrobials**

In the last years, large research efforts have been focused on identifying episodes of microbial persistence (long-term colonization) in food processing environments and equipment and on understanding the mechanisms behind this phenomenon and developing strategies for avoiding it, which would contribute to mitigate the transfer of foodborne pathogens during activities such as slicing, washing, or cleaning. For example, *Salmonella* spp. strains were shown to persistently contaminate different points across an Irish pork production facility, and changes in their metabolic activity, leading to a phenotypic switch, have been identified allowing their adaptation and survival during food processing [20]. Other observed adaptive responses linked to microbial persistence in food industries include biocide adaption by *C. jejuni* strains embedded within biofilms [21], and efflux-mediated cross-resistance to selected antimicrobials, induced in strains of *L. monocytogenes* serotypes 1/2a and 4b by exposure to sub-lethal concentrations of benzalkonium chloride compounds (BAC) [22]. Biocide-resistant *L. monocytogenes* strains were also isolated from different processing control points in two tilapia processing factories, and were attributed to incorrect handling of the fillets and to the incorrect application of sanitizing procedures [23]. As to potential control strategies mitigating persistence, researchers have addressed the potential effectiveness of natural antimicrobials, such as oregano essential oil (0.5%) incorporated in detergent solutions used for hand washing and food contact surface cleaning, as useful antimicrobial alternatives for reducing pathogen survival [24]. Similarly, others have looked on the reduction of *E. coli* O157:H7 and *Salmonella* when washing contaminated gloves (used for harvesting ready-to-eat produce) in levulinic acid and sodium dodecyl sulphate (SDS) solutions [25].

**Biocide-induced cross-resistance to antibiotics: implications for food safety**

Biocide-induced cross-resistance to antibiotics has been demonstrated in several occasions (some relevant reports from the last decade are highlighted in Table 1). For example, for *P. aeruginosa*, BAC-resistant strains obtained after long-term exposure to BAC compounds contained mutations in the *pmrB* (polymyxin resistance) gene and suffered a series of physiological adaptations contributing to higher tolerance to such antibiotic and others [26]. Other researchers analysed *P. aeruginosa* strains isolated from different industrial surfaces, such as entrance, slaughter-room, cold-room, cutting-room, freezing tunnel and white-room, of a lamb slaughterhouse or from the lamb products. They

identified cross-resistance events, specifically detected between triclosan or industrial biocide formulations, such as polyhexamethylene guanidine hydrochloride (PHMG), and different antibiotics [27]. Interestingly, a predictive protocol to measure AMR under biocidal usage has been developed in which cross-resistance occurred in *S. aureus* and *E. coli* isolates when exposed to triclosan (0.0004%) and to low concentrations of hydrogen peroxide (0.001%), while no changes in AMR were observed with exposure to chlorhexidine (0.00005%) [28].

The issue of biocides leading to an increase in antibiotic resistance has been investigated in bacteria isolated from organic, dairy and seafood products, or from different manufacturing equipment [13,29,30]. Various isolates from *Lactococcus* spp., *Enterobacter* spp. and *Escherichia* spp. have been found to be resistant to both antibiotics and biocides in a survey testing 120 food-related bacterial isolates [30]. Efflux pump genes *acrB* and *mdfA* were found in the majority of Gram-negative isolates while some *Escherichia* spp. isolates carried also the biocide tolerance gene *qacEΔ1* and the sulphonamide resistance gene *sul1* [30].

Exposure to other different physico-chemical stresses at sub-lethal levels (such as salt, ethanol, or UV light) has been also shown to confer AMR onto lactic acid bacteria isolated from naturally-fermented Aloreña table olives [17,31]. Resistance was induced in strains of *Leuconostoc pseudomesenteroides* and *Lactobacillus pentosus* by different stressors, and different susceptibility patterns induced by such stresses were observed. All physico-chemical stressors applied led to cross-resistance events to antibiotics [31]. In addition, changes in the expression profiles of stress/resistance genes, such as *rpsL*, *recA*, *srtA*, and *uvrB* were observed, and depended on the type of stressor, targeted gene and lactic acid bacteria used [31]. The same authors analysed the response of ~~*Lb.*~~*L. pentosus* MP-10 strain to various biocides (triclosan and BAC compounds) or antibiotics (amoxicillin, tetracycline and chloramphenicol) to better understand which key factors are involved in antibiotic resistance or biocide tolerance [17]. Significant differences in the expression of three proteins were observed in the proteome of ~~*Lb.*~~*L. pentosus* MP-10 strain upon treatment with triclosan: glutamyl-tRNA synthetase was up-regulated, the phosphocarrier protein HPr, which is related to carbohydrate metabolism, was down-regulated, and an oxidoreductase (a member of the aldo/keto reductase family) was not detected [17]. Cross-resistance between biocides and antibiotics led to different stress related responses in ~~*Lb.*~~*L. pentosus* MP-10 strain, and a NADH peroxidase (Npx) and a small heat shock protein were over-expressed in the amoxicillin and tetracycline-adapted strains, respectively [17].

**Minimal processing and AMR**

Fresh-cut fruits, vegetables and different minimally processed ready-to-eat foods have become a growing industry in recent years. However, this sort of products require different mild antimicrobial or preservation treatments to reduce or eliminate bacterial growth without degrading the quality of the end-product [32]. For instance, for fresh-cut fruits and vegetables, the washing of fresh produce is a critical process to remove not only organic load but also to inactivate foodborne pathogens, and many different combinations of treatments have been developed to this end. Some authors have suggested the use of free chlorine solutions for washing cabbage, Romaine or Iceberg lettuces in order to prevent pathogen cross-contamination in produce industries from United States [32]. Although chlorinated washing water samples were microbiologically tested for mesophilic bacterial counts after chlorine neutralization, potential cross-contamination of washed produce could occur due to bacterial survival [32] and little is known on the phenotypic and genotypic backgrounds of these microbial communities under different stress conditions. A water-assisted UV light treatment used in

combination with chlorine and hydrogen peroxide has been validated for inactivating *Salmonella* spp. on fresh produce [33]. A similar approach has been applied to reduce *E. coli* O157:H7 contamination on iceberg lettuce at a commercial processing facility using flume water, peroxyacetic acid and mixed peracid [34]. Results indicated a five-log CFU/mL reduction of *E. coli* O157:H7 in wash water with a small organic load when using peroxyacetic acid and mixed peracid treatment [34]. With numerous studies focused on the use of chlorine-based sanitizers, such studies provide valuable information on the use of alternative sanitizers and their increased effectiveness in the presence of various organic loads to be found in the wash water. However, such sanitizers, and other antimicrobial strategies aimed at controlling microbial hazards in ready-to-eat products, should be used under controlled conditions, and in right doses to reduce microbial load but also to avoid foreseen physiological adaptation that could lead to AMR and cross-resistance phenomena between biocides or other antimicrobials/stresses and antibiotics.

**Conclusions**

Microbial persistence in food processing environments represents a huge challenge for food safety, as can serve as a source of recurrent contamination of food with resistant microorganisms. Resistance to biocides and other antimicrobials has been considered as one of the main determinants of microbial persistence in food industries. Different pathways have been described in which biocides and other antimicrobials used in low-level concentrations for disinfection or processing purposes could co-select for antibiotic resistant bacteria, with efflux pumps playing a central role in this phenomenon. Nevertheless, the use of antimicrobials remains essential to maintain the hygiene of food processing premises and the microbiological quality and safety of produced foods and, therefore, the cost-benefit of antimicrobial usage in food processing must be considered in any risk assessment exercise. Consequently, cleaning and disinfection protocols and processing regimes should be carefully designed to avoid the exposure of microbes to sub-lethal doses of antimicrobials, which can lead to the emergence and spread of resistant colonizing strains. In addition, there is a need to develop novel and more effective antimicrobial strategies to control the microbial burden in food processing facilities, while preventing the problem of AMR to occur. Some of the new alternatives being proposed by researchers include the use of biocontrol approaches involving phage, competitive exclusion strategies and bacteriocins, among others. These biocontrol agents have demonstrated antimicrobial potential, although there are concerns about their overall efficacy in industrial settings. However, their efficacy is constantly improving with the discovery of novel agents with a broader spectrum of activity or a stronger antibacterial activity, and the likelihood of inducing resistance phenomena is much lower for this type of novel biocontrol strategies.

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*of special interest

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**Table 1.** Summary of relevant research studies in the last decade showing evidence of cross-resistance between biocides and clinically relevant antibiotics.

| Reference | Microbial species | Biocides used | Antibiotics to which resistance was developed | Methodological approach followed |
|---|---|---|---|---|
| [26] | *P. aeruginosa* | BAC | Polymyxin B, tetracycline, ciprofloxacin | BAC-fed bioreactors inoculated with river sediment selected for resistant *P. aeruginosa*. Adaptive selective experiments were carried out for *P. aeruginosa* strains in the presence of BAC for more than 300 generations. |
| [35] | *E. coli* | Triclosan | Levofloxacin, amoxicillin, tetracycline and chloramphenicol | Cells were exposed for 30 days to triclosan at a concentration of 0.2 mg/L. |
| [36] | *Pseudomonas* spp. | Didecyldimonium chloride and sodium hypochlorite | Colistin, ceftazidime, amikacin, meropenem, gentamicin, piperacillin-tazobactam, ciprofloxacin | The minimal inhibitory concentrations (MICs) of each antibiotic were compared before and after exposure to sub-inhibitory concentrations of didecyldimonium chloride and sodium hypochlorite. |
| [37] | Bacteria from organic foods, including *Bacillus cereus* and *E. faecalis* | Cetrimide and chlorhexidine | Ceftazidime and cefotaxime, among others | Serial inoculation (up to 20 passages) on Tryptic Soy Broth (TSB) media supplemented with a range of concentrations of the biocides. |
| [29] | Bacteria from organic foods, mainly from *Bacillus* spp. *Enterococcus* spp. and *Staphylococcus* spp. | BAC and hexadecylpyridinium chloride | Ampicillin, sulfamethoxazole, cefotaxime | Serial inoculation (up to 20 passages) on TSB supplemented with a range of concentrations of the biocides. |
| [38] | *Klebsiella pneumoniae* | Chlorhexidine | Colistin | Serial inoculation (up to 6 passages) in the presence of the biocide. |
| [39] | *E. coli, C. coli, S. enterica, L. monocytogenes* | Didecyl dimethyl ammonium chloride | Ampicillin, cefotaxime, ceftazidime, chloramphenicol and ciprofloxacin | The strains were daily exposed to increasing sub - inhibitory concentrations of the biocide for 7 days. |
| [21] | *C. jejuni* | Trisodium phosphate, sodium hypochlorite, acetic acid and a commercial alkaline biocide | Kanamycin, streptomycin | Serial inoculation (several passages) in the presence of the biocides. |

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|---|---|---|---|---|
| | | which contains sodium lauryl ether sulfate, linear alkyl benzene sulfonic acid, sodium salt, propylene glycol monomethyl ether, and dipropylene glycol *n*-propyl ether | | |
| [9] | *S. enterica* serovar Typhimurium | mixture of aldehydes and QAC; a QAC; an oxidative compound; a halogenated tertiary amine compound | Nalidixic acid, ciprofloxacin, chloramphenicol, tetracycline | Bacterial cultures were repeatedly sub-cultured over 4 days (eight subcultures) in each biocide. |
| [8] | *S. enterica* serovar Typhimurium | a mixture of aldehydes and QAC; a halogenated tertiary amine compound | Nalidixic acid, chloramphenicol, tetracycline, ciprofloxacin | After 5 hours exposure to biocides, live cells were sorted by flow cytometry and their AMR profile was assessed. |
| [40] | *S. enterica* serovar Typhimurium | a blend of oxidizing compounds; a QAC containing formaldehyde and glutaraldehyde; a biocide composed of organic acids and surfactants | Ciprofloxacin, chloramphenicol, tetracycline, and ampicillin | Strains were passaged daily for 7 days in sub-inhibitory concentrations, and separately for 16 days in gradually increasing concentrations of the biocides. |
| [41] | *S. enterica* serovar Typhimurium | an aldehyde based disinfectant | Ciprofloxacin | Agar plates containing the biocide were inoculated with the microbial suspension and incubated for up to 7 days. Then, some isolates were randomly selected and characterized. |

**FIGURE LEGENDS**

**Fig. 1.** Schematic overview of the main sources of antimicrobials and routes of transmission of antimicrobial resistance along the food chain.