# Antimicrobial and antibiofilm effects of selected food preservatives against *Salmonella* spp. isolated from chicken samples

Buket Er,*[1] Burak Demirhan,* Fatma Kaynak Onurdağ,† Selda Özgen Özgacar,‡ and Aysel Bayhan Öktem*

*Department of Food Analysis, Faculty of Pharmacy, Gazi University, 06330 Ankara, Turkey; †Department of Pharmaceutical Microbiology, Faculty of Pharmacy, Trakya University, 22030 Edirne, Turkey; and ‡Ministry of Health of Turkey, General Directorate of Pharmaceuticals and Pharmacy, 06520 Ankara, Turkey

**ABSTRACT** *Salmonella* spp. are widespread foodborne pathogens that contaminate egg and poultry meats. Attachment, colonization, as well as biofilm formation capacity of *Salmonella* spp. on food and contact surfaces of food may cause continuous contamination. Biofilm may play a crucial role in the survival of salmonellae under unfavorable environmental conditions, such as in animal slaughterhouses and processing plants. This could serve as a reservoir compromising food safety and human health. Addition of antimicrobial preservatives extends shelf lives of food products, but even when products are supplemented with adequate amounts of preservatives, it is not always possible to inhibit the microorganisms in a biofilm community. In this study, our aims were i) to determine the minimum inhibitory concentrations (MIC) and minimum biofilm inhibitory concentrations (MBIC) of selected preservatives against planktonic and biofilm forms of *Salmonella* spp. isolated from chicken samples and *Salmonella* Typhimurium SL1344 standard strain, ii) to show the differences in the susceptibility patterns of same strains versus the planktonic and biofilm forms to the same preservative agent, and iii) to determine and compare antimicrobial and antibiofilm effects of selected food preservatives against *Salmonella* spp. For this purpose, *Salmonella* Typhimurium SL1344 standard strain and 4 *Salmonella* spp. strains isolated from chicken samples were used. Investigation of antimicrobial and antibiofilm effects of selected food preservatives against *Salmonella* spp. was done according to Clinical and Laboratory Standards Institute M100-S18 guidelines and BioTimer assay, respectively. As preservative agents, pure ciprofloxacin, sodium nitrite, potassium sorbate, sodium benzoate, methyl paraben, and propyl paraben were selected. As a result, it was determined that MBIC values are greater than the MIC values of the preservatives. This result verified the resistance seen in a biofilm community to food preservatives and highlighted this subject, not to be ignored in food applications.

**Key words:** biofilm, *Salmonella* spp., food preservative, antimicrobial, antibiofilm

2014 Poultry Science 93:695–701
http://dx.doi.org/10.3382/ps.2013-03404



## INTRODUCTION

Biofilm, which was defined as "a structured community of bacterial cells enclosed in a self-produced polymeric matrix and adherent to an inert or living surface" (Balaban, 2008), has some advantages conferring resistance to environmental conditions and antimicrobial agents (Ganesh Kumar and Anand, 1998). One of the most common mechanisms resulting in resistance is the general inability of antimicrobial agents to pass through the biofilm barrier and affect microorganisms in the biofilm environment as easily as they affect the planktonic cells. The resistance genes may also be transferred from one microorganism to another more easily in a biofilm community (Amorena et al., 1999).

*Salmonella* spp. are capable of attachment, colonization, and biofilm formation on food and contact surfaces of food (Lianou and Koutsoumanis, 2012; Wang et al., 2013b). Occurrence of *Salmonella* spp. biofilm on a contact surface of food is a continuous contamination source. It was reported that bacterial biofilm protects the bacteria from antibacterial agents, disinfectants, and sanitizers (Joseph et al., 2001; Lianou and Koutsoumanis, 2012; Steenackers et al., 2012). Thus, contamination of the food with biofilm-forming bacteria is a serious problem for food industry, resulting in insufficient cleaning and sanitation needed to eliminate biofilm (Lianou and Koutsoumanis, 2012; Wang et al., 2013b). As a result, biofilm formation seems to be a serious problem on food processing, with the potential of being a reservoir of contamination that compromises


Received June 11, 2013.
Accepted November 3, 2013.
[1]Corresponding author: erbuket@gmail.com

food safety and human health (Lianou and Koutsoumanis, 2012).

Biofilm formation may occur on many surfaces used in the food industry, and microorganisms form biofilm according to the characteristics of the surface in their environment. So, biofilm may play a crucial role in the survival of salmonellae under unfavorable environmental conditions, such as in animal slaughterhouses and processing plants. It is also reported that "To date, relatively little research has examined the ability of biofilm formation of *Salmonella* isolated from slaughter plants under the conditions commonly encountered. Moreover, the relationship between antimicrobial resistance and the ability of *Salmonella* isolates to form biofilm is unknown" (Wang et al., 2013a).

Infections caused by *Salmonella* spp. are among the most common zoonotic diseases worldwide, and the most common type of salmonellae infection is enterocolitis. *Salmonella* Enteritidis and *Salmonella* Typhimurium are the most common serotypes isolated from foodborne salmonellae infections, which are capable of forming biofilm on many different surfaces including plastic, glass, stainless steel, and living tissues (Joseph et al., 2001; Bayhan Öktem et al., 2009; Van Houdt and Michiels, 2010; Wong et al., 2010; Rodrigues et al., 2011).

According to the Turkish Food Codex and European Legislation, salmonellae should not be found in 25 g of food (European Parliament and the Council of the European Union, 1998; TFC, 2011b) and microbiological analyses should have been applied before consumption (Bayhan Öktem et al., 2009).

Because food itself is an appropriate medium for growth of microorganisms, antimicrobial preservatives are added to food products to extend shelf life (Fazlara and Ekhtelat, 2012; Çakır and Mehmetoğlu, 2013). Sodium benzoate, potassium sorbate, sodium nitrite, and methyl and propyl parabens, which are included in this study, are common preservatives used in the food industry (Soni et al., 2005; Küçükçetin et al., 2008; Çakır and Mehmetoğlu, 2013).

These antimicrobial additives may have activity to destroy cell membrane, cell wall, or protein structure and enzyme systems of microorganisms and have microbicidal or microbiostatic effects (Stanojevic et al., 2009). It should be emphasized that commonly used food packaging materials may lead to biofilm formation and even the adequate amounts of the preservatives added to the product may not be able to inhibit the microorganisms in biofilm via the mentioned mechanisms of action. These preservatives are also mentioned to be harmful for human health when consumed in high amounts (Stanojevic et al., 2009; Çakır and Mehmetoğlu, 2013). Therefore, preservatives used in food are limited and the limits are notified by the Turkish Food Codex (TFC, 2011a). Thus, increasing the amount of the preservatives does not seem to be possible.

Determination of the resistance to antibacterial agents and disinfectants of *Salmonella* spp. in biofilm is important to prevent cross-contamination and reduce the occurrence of foodborne infections (Wang et al., 2013b). However, the differences between the susceptibility of the biofilm and planktonic forms of the same microorganism have been argued in recent studies and it has been accepted that cells within biofilm are more resistant to biocides than their planktonic counterparts (Russell, 1991). Although biofilm formation changes the susceptibility of the cells to antimicrobial agents, antimicrobial susceptibility tests are still performed only for planktonic forms. The MIC of the agents have known to be lower in planktonic forms than their sessile counterparts. This leads to difficulties in the treatment of infections (Pace et al., 2006). Also, there are no standard recommendations for susceptibility testing to determine the minimum concentration of disinfectants and preservatives that inhibits the activity of microorganisms in a biofilm community. Nonetheless, there are no reported data showing that higher inhibitory concentrations of antimicrobial food preservatives against biofilm forms need to be used than their planktonic counterparts. However, according to the data reported with antimicrobial agents and disinfectants, it should not be wrong to expect the familiar resistance also for preservatives, in a biofilm community.

The packages of food may lead microorganisms to grow in biofilm forms, and thus, the preservatives may not show their antimicrobial effect at their adequate concentrations. For this reason, the importance of determining the susceptibilities of the microorganisms in the biofilm environment to all of the antimicrobial agents is clearly evident.

In this study, our aim was i) to determine the MIC and minimum biofilm inhibitory concentrations (**MBIC**) of selected preservatives against planktonic and biofilm forms of *Salmonella* spp. isolated from chicken samples and *Salmonella* Typhimurium SL1344 standard strain, ii) to show the differences in the susceptibility patterns of same strains versus the planktonic and biofilm forms to the same preservative agent, and iii) to determine and compare antimicrobial and antibiofilm effects of selected food preservatives against *Salmonella* spp.

## MATERIALS AND METHODS

### Strains

*Salmonella* Typhimurium SL1344 standard strain and 4 *Salmonella* spp. strains isolated from chicken breast meat samples collected from Ankara region, Turkey (Kaynak Onurdağ et al., 2011), were used in the study. The detection of *Salmonella* spp. in chicken meats was investigated by the horizontal method recommended by the International Organization for Standardization (ISO, 2002). Among the isolation process, buffered peptone water was used as preenrichment medium, selenite

cystine broth (107709, Merck, Darmstadt, Germany) and Rappaport Vassiliadis broth (107700, Merck) were used as enrichment mediums, Salmonella-Shigella Agar (107667, Merck), and xylose lysine deoxycholate agar (105287, Merck) were used as selective mediums. The typical and atypical colonies grown on Salmonella-Shigella and xylose lysine deoxycholate agar plates, which were isolated from the samples, were passaged to MacConkey agar (105465, Merck), and then the lactose negative pure colonies, which were isolated from MacConkey agar plates, were used in biochemical tests (ISO, 2002; Kaynak Onurdağ et al., 2011). After biochemical tests, 4 strains that are identified as *Salmonella* spp. were confirmed by API 20 E (bioMérieux, Durham, NC) identification system.

The competence of biofilm formation of the 4 isolates and *Salmonella* Typhimurium SL1344 standard strain was observed by a modified microtiter plate test before the experiments (Abdi-Ali et al., 2006). Bacteria were incubated at 37°C for 24 h on tryptic soy agar (**TSA**; 105458, Merck) plates and subcultured on TSA supplemented with 5% glucose (108342, Merck). Bacteria were then inoculated from 5% glucose-supplemented TSA to 5% glucose-supplemented tryptic soy broth (**TSB**; 105459, Merck). Then, 100 μL of this culture was added in 6 parallel wells of a 96-well polystyrene plate and incubated at 37°C for 24 h for biofilm formation on the polystyrene plate surface. After incubation, the wells were rinsed with buffer (0.01 *M* potassium phosphate buffer made isotonic with saline, pH 7.5) to remove detached cells and then fixed with 150 μL of absolute methanol (106007, Merck) for 10 min. Attached material was then stained by adding 150 μL of crystal violet (HT90132, Sigma, Steinheim, Germany; 1% wt/vol) for 20 min. The plates were rinsed with tap water, and the amount of attached material was observed by solubilization of the crystal violet dye in 150 μL of 33% glacial acetic acid (695092, Sigma).

## Plotting the Correlation Lines

BioTimer assay, described by Pantanella et al. (2008), was used in our study. The medium contained Mueller-Hinton broth (**MHB**; 110293, Merck), 30 g; glucose, 10 g; phenol red (Sigma P3532), 25 mg; and distilled water to 1,000 mL. The pH was adjusted to 7.0 ± 0.1 and 6.0 ± 0.1. After autoclaving at 121°C for 15 min, the media were red and clear. For each *Salmonella* spp., 0.2 mL of the fresh cultures was mixed with 1.8 mL of the phenol-red medium and diluted 2-fold with 1 mL of the medium in 24-well sterile microplates. After dilution, from each of the wells, 10 μL was inoculated to Mueller-Hinton agar (**MHA**; 105437, Merck) plates. The MHA plates and the microplates were incubated at 37°C for 24 h. During incubation, the time required for a complete color change (red to yellow) in every well was noted. The colonies on the MHA plates were counted and the number of cells per 1 milliliter (cfu/mL) was determined. A correlation line linking the time for color switch and the log cfu of the *Salmonella* spp. was plotted. All the experiments were done in 3 parallel series.

## Antibacterial Susceptibility Testing

Pure ciprofloxacin (17850, Fluka, Steinheim, Germany), sodium nitrite (106544, Merck), potassium sorbate (Tekkim Chem. TK, 200970, Bursa, Turkey), sodium benzoate (106290, Merck), methyl paraben (Nipagin M, 47889, Supelco, Steinheim, Germany), and propyl paraben (Nipasol, PHR1010, Fluka) were used as antimicrobial agents. Ciprofloxacin was chosen as a standard for quality control and the preservatives were selected from common preservatives used in the food industry. Methyl paraben and propyl paraben were dissolved in ethanol (100983, Merck), and other preservatives and ciprofloxacin were dissolved in distilled water for preparing the stock solutions. Stock solutions of the preservatives and ciprofloxacin were prepared as 32,768 and 32 μg/mL, respectively. The stock solutions were used in the microdilution method and BioTimer assay.

## Microdilution Method

Salmonellae strains were subcultured in TSA plates at 37°C for 24 h. Bacterial susceptibility testing was performed according to the guidelines of Clinical and Laboratory Standards Institute (CLSI, 2008). Cation-adjusted MHB at pH 7.0 and pH 6.0 were prepared. The MHB was added each well of the microplates. Bacterial suspensions used for inoculation were prepared at $10^6$ cfu/mL by diluting fresh cultures at McFarland 0.5 density ($10^8$ cfu/mL). Bacterial suspensions were inoculated to the 2-fold-diluted solution of the preservatives and ciprofloxacin. A 10-μL bacterial inoculum was added to each well of the microplates. There were $10^5$ cfu/mL bacteria in the wells after inoculations. Microplates were incubated at 37°C for 24 h. After incubation, the lowest concentration of the compounds that completely inhibits macroscopic growth was determined and reported as MIC. All solvents and diluents, pure microorganisms, and pure media were used in control wells.

## BioTimer Assay

Salmonellae strains were grown at 37°C for 24 h on TSA supplemented with 5% glucose and inoculated to TSB supplemented with 5% glucose containing sterile glass beads with 5 mm diameter to form biofilm. After incubation, the glass beads were washed 3 times in PBS to remove planktonic bacteria. To ensure biofilm formation on glass beads, salmonellae were grown with 5-mm-diameter glass beads in TSB supplemented with 5% glucose. The glass beads were rinsed with buffer to remove detached cells and then put in the next well containing 150 μL of absolute methanol (106007, Merck) for 10 min. Then the glass beads were taken and put in the next well containing 150 μL of crystal violet

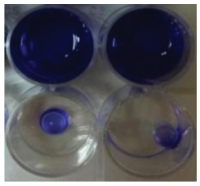

**Figure 1.** Biofilm formation on glass beads. Color version available in the online PDF.

(HT90132, Sigma; 1% wt/vol) for 20 min. The glass beads were rinsed with PBS again, and the amount of attached material was observed by solubilization of the crystal violet dye in 150 μL of 33% glacial acetic acid (695092, Sigma; Figure 1).

One milliliter of the phenol red medium was put in every well of the 24-well microplates. One milliliter of the preservatives and ciprofloxacin was put in the first wells of the microplates and diluted 2-fold. The glass beads on which biofilm formation occurred were placed in each well containing a different concentration of the compounds and incubated at 37°C for 24 h. A well that did not contain any compound was used in each microplate as a positive control, and the time required for color switch of this well was used to determine the number of *Salmonella* spp. in biofilm on the glass beads using the previously prepared correlation line. After incubation, the lowest concentration of the preservatives and ciprofloxacin that inhibited the color change was determined as the MBIC.

## RESULTS AND DISCUSSION

The MIC and MBIC of the preservatives and ciprofloxacin against *Salmonella* spp. are given in Figures 2 and 3, respectively. Correlation lines linking the time for color change and the number of planktonic *Salmonella* spp. were drawn, and for each strain, log cfu/glass beads were calculated using the equations and the linear coefficients of the correlation lines. They were $y = -0.0086x + 7.7118$, $r = 0.9930$ and $y = -0.0102x + 8.5244$, $r = 0.9915$ for *Salmonella* Typhimurium SL1344; $y = -0.0061x + 6.8276$, $r = 0.9904$ and $y = -0.005x + 6.4975$, $r = 0.9946$ for *Salmonella* spp. isolate 1; $y = -0.0064x + 5.1956$, $r = 0.9921$ and $y = -0.0047x + 4.8048$, $r = 0.9902$ for *Salmonella* spp. isolate 2; $y = -0.0055x + 6.6647$, $r = 0.9902$ and $y = -0.0041x + 6.4303$, $r = 0.9903$ for *Salmonella* spp. isolate 3; $y = -0.0063x + 6.7479$, $r = 0.9904$ and $y = -0.0058x + 6.8272$, $r = 0.9903$ for *Salmonella* spp. isolate 4 at pH 7 and pH 6, respectively.

Initial inoculum concentrations of *Salmonella* spp. in biofilm forms are calculated according to the correlation lines and given in Table 1.

It has been reported that MBIC values are greater than MIC values against the strains isolated from infections and this situation leads to problems in the treatment of infections (Spoering and Lewis, 2001; Toté et al., 2010). Papavasileiou et al. (2010) investigated the susceptibility of biofilm and planktonic forms of *Salmonella* Typhimurium isolated from kids with gastroenteritis and reported that $MIC_{50}$ of the biofilm forms were higher than $MIC_{50}$ of the planktonic counterparts. They also mentioned that the $MIC_{90}$ values of the biofilm forms were over the susceptibility value of all antimicrobial agents tested. Olson et al. (2002) studied the antimicrobial susceptibility of some gram-negative and gram-positive microorganisms and determined that the MIC and minimum biofilm eradication concentration (**MBEC**) values were >1,024 μg/mL for *Salmonella* Typhimurium and emphasized that MIC values were lower or equal to MBEC values for all bacterial isolates.

Wong et al. (2010) used the Calgary biofilm method to compare the susceptibility of a 3-d-old biofilm and planktonic salmonellae to disinfectants at different exposure times, and they concluded that at concentra-



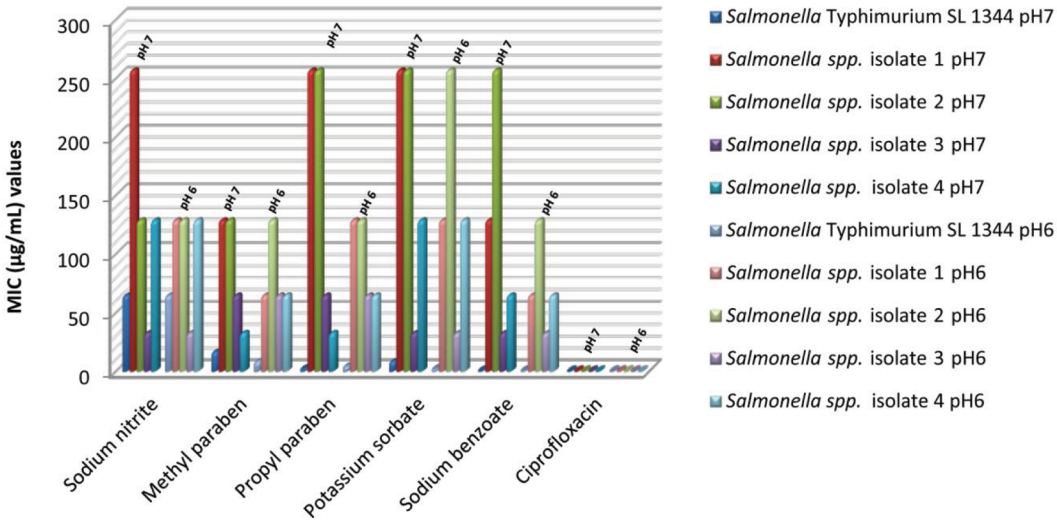


**Figure 2.** Minimum inhibitory concentration (MIC; μg/mL) values of preservatives and ciprofloxacin. Color version available in the online PDF.

**Table 1.** Inoculum concentrations of biofilm forms

| Strain | pH | Time (min) | cfu/glass beads |
|---|---|---|---|
| *Salmonella* Typhimurium SL1344 | 7 | 477 ± 4.16 | $4.0 \times 10^3$ |
| | 6 | 501 ± 3.61 | $3.0 \times 10^3$ |
| *Salmonella* spp. isolate 1 | 7 | 487 ± 2.65 | $0.7 \times 10^4$ |
| | 6 | 498 ± 2.65 | $1.0 \times 10^4$ |
| *Salmonella* spp. isolate 2 | 7 | 661 ± 1.00 | $0.9 \times 10^1$ |
| | 6 | 672 ± 3.61 | $4.0 \times 10^1$ |
| *Salmonella* spp. isolate 3 | 7 | 623 ± 2.65 | $1.7 \times 10^3$ |
| | 6 | 606 ± 3.61 | $0.9 \times 10^4$ |
| *Salmonella* spp. isolate 4 | 7 | 571 ± 2.00 | $1.4 \times 10^3$ |
| | 6 | 610 ± 6.25 | $2.0 \times 10^3$ |

tions and times, all the planktonic cells were eliminated and there were still sufficient numbers of viable cells in the biofilm. They have reported a need for susceptibility assessment of biofilm forms of the microorganisms to disinfectants.

Rodrigues et al. (2010) have studied with sodium hypochlorite, benzalkonium chloride, hydrogen peroxide, and triclosan and showed that sodium hypochlorite had the lowest and triclosan had the highest MBEC values and *S. enterica* biofilm was not eradicated even at the maximum concentrations of the disinfectants.

Joseph et al. (2001) studied hypochlorite and iodophor with the biofilm form of 2 salmonellae isolates and reported that biofilm cells of salmonellae are much more resistant to sanitizers compared with planktonic counterparts.

However, no data have been reported that emphasized the difference between MIC and MBIC values of food preservatives.

The methods used to determine the MBIC or MBEC values of the antimicrobial agents are not standardized. The absence of a standardized susceptibility testing method for biofilm forms of the microorganisms have led the researchers use different techniques to determine the effective concentrations of the antimicrobial agents to treat biofilm infections. 2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-5-[(phenylamino) carbonyl]-2H-tetrazolium hydroxide reduction assay, dry weight measurements or radioisotope assays, novel methods like Calgary biofilm device assay are some of the different methods to determine the susceptibility of biofilm and planktonic forms of the microorganisms to various antimicrobial agents (Hawser and Douglas, 1994; Ceri et al., 1999; Kuhn et al., 2002; Kaynak Onurdağ et al., 2010). However, these methods cannot detect the inoculum of the bacteria in biofilm. Pantanella et al. (2008) described a novel method (BioTimer assay) to count staphylococci in the biofilm niche and determined the MBEC and MBIC values of the antimicrobial agents. Kaynak Onurdağ et al. (2010) modified the BioTimer method for *Candida* strains and detected the susceptibilities of *Candida* spp. to antifungal agents. As a result of their study, it is deduced that susceptibility testing should be done according to the planktonic and biofilm forms of the strains but the values may change due to the inoculum of the strain. It was concluded that BioTimer assay that can determine the initial inoculum of the fungi/glass bead is a reliable method (Kaynak Onurdağ et al., 2010).

The aims of our study were i) to determine the MIC and MBIC of selected preservatives against planktonic and biofilm forms of *Salmonella* spp. isolated from

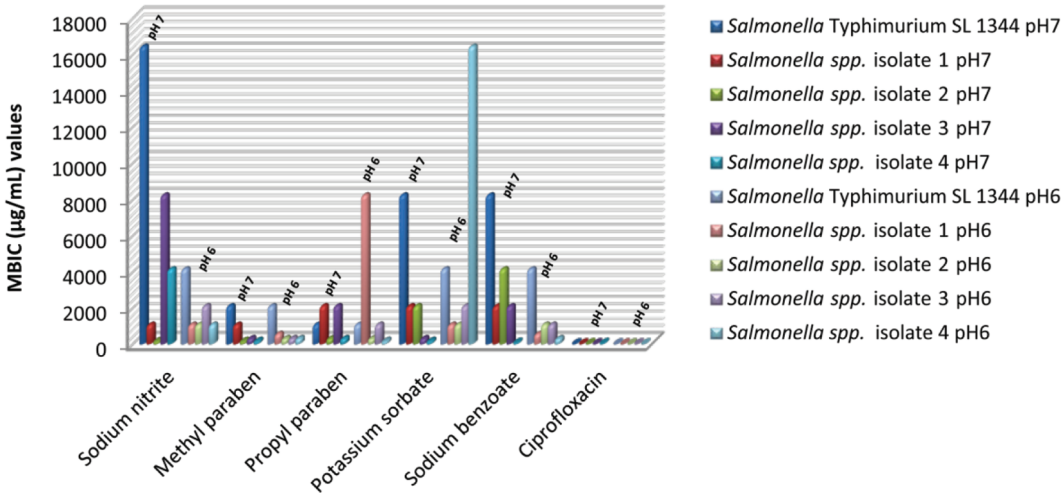


**Figure 3.** Minimum biofilm inhibitory concentrations (MBIC; µg/mL) values of preservatives and ciprofloxacin. Color version available in the online PDF.

chicken samples and *Salmonella* Typhimurium SL1344 standard strain, ii) to show the differences in the susceptibility patterns of same strains versus the planktonic and biofilm forms to the same preservative agent, and iii) to determine and compare antimicrobial and antibiofilm effects of selected food preservatives against *Salmonella* spp. Sodium nitrite, methylparaben, propylparaben, potassium sorbate, sodium benzoate, and as an antimicrobial agent, ciprofloxacin, were used. The MIC value of ciprofloxacin was within the limit values mentioned in CLSI M100-S18 (CLSI, 2008).

Initial inoculum concentrations of salmonellae in biofilm per glass beads were determined to be lower than initial inoculum concentration of the planktonic forms. Nevertheless, the MBIC values were greater than the MIC values of all the preservatives against all the salmonellae strains.

Efficacy of the preservatives against microorganisms varies, depending on pH conditions (Stanojevic et al., 2009; Çakır and Mehmetoğlu, 2013). In our study, 2 pH conditions were used even in the microdilution method and the BioTimer method. However, the MIC and MBIC values were equal or one dilution part different from each other; therefore, pH conditions did not affect the inhibitory values of the preservatives.

As a result of our study, we determined greater MBIC values than the MIC values of the preservatives even if the inoculum concentrations of bacteria in biofilm were determined as lower than the inoculum of the planktonic counterparts. This result verified that familiar resistance seen for antimicrobial agents and disinfectants is also necessary for preservatives in a biofilm community. In view of the resistance of bacterial biofilm to antimicrobial agents, new strategies should be implemented for the control of biofilm. In conclusion, increasing the amount of preservatives is not necessary due to the toxic doses in humans. Designing new compounds or inhibiting biofilm formation by changing the packaging properties of food seems to be a more appropriate option.

## REFERENCES


Abdi-Ali, A., M. Mohammadi-Mehr, and Y. A. Alaei. 2006. Bactericidal activity of various antibiotics against biofilm-producing *Pseudomonas aeruginosa.* Int. J. Antimicrob. Agents 27:196–200.

Amorena, B., E. Gracia, M. Monzon, J. Leiva, C. Oteiza, M. Perez, J. L. Alabart, and J. Hernandez-Yago. 1999. Antibiotic susceptibility assay for *Staphylococcus aureus* in biofilms developed in vitro. J. Antimicrob. Chemother. 44:43–55.

Balaban, N. 2008. Introduction. Pages 1–12 in Control of Biofilm Infections by Signal Manipulation. N. Balaban, D. Ren, M. Givskov, and T. B. Rasmussen, ed. Springer, Berlin, Germany.

Bayhan Öktem, A., F. Kaynak Onurdağ, B. Er, and B. Demirhan. 2009. A research of *Salmonella spp.* in egg and egg products and survival of *Salmonella* in different temperatures. Turkish J. Pharm. Sci. 6:147–154.

Çakır, R., and A. Ç. Mehmetoğlu. 2013. Sorbic and benzoic acid in non-preservative-added food products in Turkey. Food Addit. Contam. B Surveill. 6:47–54.

Ceri, H., M. E. Olson, C. Stremick, R. R. Read, D. Morck, and A. Buret. 1999. The Calgary biofilm device: New technology for rapid determination of antibiotic susceptibilities of bacterial biofilms. J. Clin. Microbiol. 37:1771–1776.

CLSI (Clinical and Laboratory Standards Institute). 2008. (formerly NCCLS). Performance standards for antimicrobial susceptibility testing 6th informational supplement. CLSI M100–S18. Wayne, PA.

European Parliament and the Council of the European Union. 1998. Directive No. 98/72/CE of European Parliament and Council of 15 October 1998. Accessed Apr. 18, 2013. http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:1998:295:0018:0030:EN:PDF.

Fazlara, A., and M. Ekhtelat. 2012. The disinfectant effects of benzalkonium chloride on some important foodborne pathogens. Amer-Eurasian J. Agric. Environ. Sci. 12:23–29.

Ganesh Kumar, C., and S. K. Anand. 1998. Significance of microbial biofilms in food industry: A review. Int. J. Food Microbiol. 42:9–27.

Hawser, S. P., and L. J. Douglas. 1994. Biofilm formation by *Candida* species on the surface of catheter materials in vitro. Infect. Immun. 62:915–921.

ISO (International Organization for Standardization). 2002. Microbiology of Food and Animal Feeding Stuffs, Horizontal Method for the Detection of *Salmonella* spp., ICS 07.100.30. International Organization for Standardization, Geneva, Switzerland.

Joseph, B., S. K. Otta, I. Karunasagar, and I. Karunasagar. 2001. Biofilm formation by *Salmonella spp.* on food contact surfaces and their sensitivity to sanitizers. Int. J. Food Microbiol. 64:367–372.

Kaynak Onurdağ, F., B. Er, S. Özgen, B. Demirhan, U. Abbasoğlu, and A. Bayhan Öktem. 2011. Antimicrobial susceptibilities of *Salmonella* spp. isolated from chicken meat samples. J. Food Sci. Eng. 1:135–140.

Kaynak Onurdağ, F., S. Özgen, U. Abbasoğlu, and İ. S. Gürcan. 2010. Comparison of two different methods for the investigation of in vitro susceptibilities of planktonic and biofilm forming *Candida* species to antifungal agents. Bulletin Microbiol. 44:619–631.

Küçükçetin, A., B. Filk, and M. Demir. 2008. Determination of sodium benzoate, potassium sorbate, nitrate and nitrite in some commercial dairy products. Gıda 33:159–164.

Kuhn, D. M., T. George, J. Chandra, P. K. Mukherjee, and M. A. Ghannoum. 2002. Antifungal susceptibility of *Candida* biofilms: Unique efficacy of amphotericin B lipid formulations and echinocandins. Antimicrob. Agents Chemother. 46:1773–1780.

Lianou, A., and K. P. Koutsoumanis. 2012. Strain variability of the biofilm-forming ability of *Salmonella enterica* under various environmental conditions. Int. J. Food Microbiol. 160:171–178.

Olson, M. E., H. Ceri, D. W. Morck, A. G. Buret, and R. R. Read. 2002. Biofilm bacteria: Formation and comparative susceptibility to antibiotics. Can. J. Vet. Res. 66:86–92.

Pace, J. L., M. E. Rupp, and R. G. Finch. 2006. Candida. Pages 171–184 in Biofilms, Infection and Antimicrobial Therapy. S. Hawser and K. Islam, ed. CRC Press, Boca Raton, FL.

Pantanella, F., P. Valenti, A. Frioni, and T. Natalizi. 2008. BioTimer assay, a new method for counting *Staphylococcus* spp. in biofilm without sample manipulation applied to evaluate antibiotic susceptibility of biofilm. J. Microbiol. Methods 75:478–484.

Papavasileiou, K., E. Papavasileiou, A. Tseleni-Kotsovili, S. Bersimis, C. Nicolaou, A. Ioannidis, and S. Chatzipanagiotou. 2010. Comparative antimicrobial susceptibility of biofilm versus planktonic forms of *Salmonella enterica* strains isolated from children with gastroenteritis. Eur. J. Clin. Microbiol. Infect. Dis. 29:1401–1405.

Rodrigues, D., N. Cerca, P. Teixeira, R. Oliveira, H. Ceri, and J. Azeredo. 2011. *Listeria monocytogenes* and *Salmonella enterica* Enteritidis biofilms susceptibility to different disinfectants and stress response and virulence gene expression of surviving cells. Microb. Drug Resist. 17:181–189.

Rodrigues, D., P. Teixeira, R. Oliveira, H. Ceri, and J. Azeredo. 2010. Minimum biofilm eradication concentration (MBEC) of different antimicrobials on *Listeria monocytogenes* and *Salmonella enterica* biofilms. Page 176 in Book of Abstracts ISOPOL XVII International Symposium on Problems of Listeriosis, Porto, Portugal, May 5–8. Orgal Impressores, Porto, Portugal.

Russell, A. D. 1991. Mechanisms of bacterial resistance to non-antibiotics: Food additives and food and pharmaceutical preservatives. J. Appl. Bacteriol. 71:191–201.

Soni, M. G., I. G. Carabin, and G. A. Burdock. 2005. Safety assessment of esters of p-hydroxybenzoic acid (parabens). Food Chem. Toxicol. 43:985–1015.

Spoering, A. L., and K. Lewis. 2001. Biofilms and planktonic cells of *Pseudomonas aeruginosa* have similar resistance to killing by antimicrobials. J. Bacteriol. 183:6746–6751.

Stanojevic, D., L. Comic, O. Stefanovic, and S. I. Solujic-Sukdolak. 2009. Antimicrobial effects of sodium benzoate, sodium nitrite and potassium sorbate and their synergistic action in vitro. Bulgarian J. Agric. Sci. 15:307–311.

Steenackers, H., K. Hermans, J. Vanderleyden, and S. C. J. De Keersmaecker. 2012. *Salmonella* biofilms: An overview on occurrence, structure, regulation and eradication. Food Res. Int. 45:502–531.

TFC (Turkish Food Codex). 2011a. Food additives regulation. Official Journal of Turkish Republic: 29.12.2011–28157 (3rd repeated).

TFC (Turkish Food Codex). 2011b. Microbiological criteria regulation. Official Journal of Turkish Republic: 29.12.2011–28157 (3rd repeated).

Toté, K., T. Horemans, D. V. Berghe, L. Maes, and P. Cos. 2010. Inhibitory effect of biocides on the viable masses and matrices of *Staphylococcus aureus* and *Pseudomonas aeruginosa* biofilms. Appl. Environ. Microbiol. 76:3135–3142.

Van Houdt, R., and C. W. Michiels. 2010. Biofilm formation and the food industry, a focus on the bacterial outer surface. J. Appl. Microbiol. 109:1117–1131.

Wang, H., K. Ye, X. Wei, J. Cao, X. Xu, and G. Zhou. 2013a. Occurrence, antimicrobial resistance and biofilm formation of *Salmonella* isolates from a chicken slaughter plant in China. Food Contr. 33:378–384.

Wang, H., K. P. Ye, Q. Q. Zhang, Y. Dong, X. L. Xu, and G. H. Zhou. 2013b. Biofilm formation of meat-borne *Salmonella enterica* and inhibition by the cell-free supernatant from *Pseudomonas aeruginosa*. Food Contr. 32:650–658.

Wong, H. S., K. M. Townsend, S. G. Fenwick, R. D. Trengove, and R. M. O'Handley. 2010. Comparative susceptibility of planktonic and 3-day-old *Salmonella* Typhimurium biofilms to disinfectants. J. Appl. Microbiol. 108:2222–2228.