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# Effect of Nine Antimicrobial Agents Against Microoganisms Isolated from Food*

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**Abstract**

Microorganisms are widespread in the environment. Most of them are not dangerous to human health, but some produce toxins which are toxic, carcinogenic, mutagenic or teratogenic for humans and animals. Their fast reproduction leads to contamination in food and damage to material goods. Within this experimental research, the effects of nine laboratory- obtained antimicrobial agents of different chemical compositions and concentrations are determined against foodborne pathogenic microorganisms.

The following microorganisms were used in the test: *Escherichia coli* ATCC 8739, *Bacillus subtilis* ATCC 6633 и *Staphylococcus aureus* 6538, isolate FM2 (yeast from pickled peppers), isolate FM20 (yeast from pickle), isolate FM3 (mold from bread), isolate FM4 (mold from jam), isolate FM18 (mold from tangerine) and isolate FM15 (mold from meat). The experiment was based on the macrodilution method. The microorganisms were exposed to different compositions and different concentrations of components of antimicrobial agents (designated from No1 to -No9), and the minimum inhibitory concentration (MIC) was determined.

The results showed that all antimicrobial agents possess antimicrobial activity against the tested microorganisms, depending on the type of microorgasnisms (procaryiots or eucaryots) and the compositions of the antimicrobial agents. The lowest MIC of 3.125% for both procaryots and eucaryots was shown by antimicrobial agent No2 the antimicrobial agent with no or some weak activity for eucaryots was No4, and for procaryots agents No7 and No8. Each of the tested agents has a different potential impact on the growth and multiplication of the test organisms. These differences result from the composition of the laboratory-obtained antimicrobials and the resistance of the test microorganisms to them.

**Key words:** macrodilution method; microbial growth; inhibition; antimicrobial activity.

**Резюме**

Микроорганизмите са широко разпространени в околната среда. Повечето от тях не са опасни за човешкото здраве, но други произвеждат токсини, които са токсични, карциногенни, мутагенни или тератогенни за човека и животните. Тяхното бързо размножаване води до контаминация на храните и увреждане на стоките. В настоящата експериментална работа се определят ефектите на девет получени в лаборатория антимикробни агента с различен химичен състав и концентрация срещу патогенни микроорганизми, пренасяни с храната.

В теста са използвани следните микроорганизми: *Escherichia coli* ATCC 8739, *Bacillus subtilis* ATCC 6633 и *Staphylococcus aureus* 6538, изолат FM2 (дрожди от туршия от чушки), изолат FM20 (дрожди от туршия), изолат FM3 (плесен от хляб), изолат FM4 (плесен от конфитюр), изолат FM18 (плесен от мандарина) и изолат FM15 (плесен от месо). Експериментът се основава на метода на микроразрежданията. Върху микроорганизмите се въздейства с различни по състав и концентрация компоненти на антимикробните агенти (обозначени от №1 до №9) и е определена минималната инхибиторна концентрация (МИК).

Резултатите показаха, че антимикробните агенти притежават антимикробна активност срещу

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изследваните микроорганизми, в зависимост от вида микроорганизми (прокариоти или еукариоти) и състава на антимикробния агент. Най-ниска МИК, 3.125% при про- и еукариоти показа антимикробен агент №2, антимикробният агент без, или с много слаба активност към еукариоти беше №4, а към прокариоти – агенти №7 и №8. Всеки от изследваните агенти има различен потенциален принос към растежа и размножаването на тестовите организми. Тези разлики са резултат от състава на получените лабораторно антимикробни препарати и от резистентността на тестовите микроорганизми.

## Introduction

Food production, processing, distribution, and preparation have stimulated an increasing demand for food safety research in order to ensure a safer global food supply (Arvanitoyannis *et al.*, 2009). Antimicrobial agents are increasingly being incorporated into a wide variety of products for use in food industry for different hygiene measures. The lack of proper sanitation procedures can cost plant operators a lot of money. All food processing equipment surfaces are subject to adhesion of microorganisms, which can even survive after proper cleaning and sanitization (Silva *et al.,* 2010), and thus become a possible cause of diseases caused by contaminated food (Andrade and Macedo, 1996).

The number and the growth of the microorganisms must be controlled because of their fast way of reproduction and because of the conditions which are crucial for their survival indifferent types of mediums.

The choice of the most appropriate antimicrobial agents should be carefully taken considering the potential contaminants as well as the types of surfaces found in industries (Kunigk and Almeida, 2001). The ideal sanitizers should be approved by the competent authorities, have a wide spectrum of antimicrobial activity, be able to rapidly destruct microorganisms and be stable under several use conditions, and present low toxicity and corrosivity (Andrade *et al.,* 2008).

The food industry routinely employs a wide variety of chemical sanitizers, including quaternary ammonium, peracetic acid, sorbic acid, lactic acid and alcohols. Of all these recognized compounds, just a few sanitizers have a wide spectrum of activity (Block, 1991).

There are several antimicrobial agents that may be used alone or in combination. Some of these, such as chlorine-based and iodine-based products, are well established as being very useful in specific situations (eg, dilute bleach is recommended for cleaning up spillage of body fluids) (Boyce and Pittet, 2002) Chloroxylenol (parachlorometaxylenol or PCMX) is used as a preservative in cosmetics and other products, and is also used in antimicrobial soaps (Boyce and Pittet, 2002). Antimicrobial agents often block active metabolism and prevent the synthesis of macromolecules needed for reproduction.

The objective of this work was to evaluate the efficiency of nine laboratory- obtained antimicrobial agents of different chemical compositions and concentrations. The general aim was to employ different combinations of several already known antimicrobial agents as potential agents to sanitise external surfaces in the food industry.

## Materials and Methods

Within this experimental research, nine combinations of six different components were used in different concentrations (Table 1). Also, they were

**Table 1.** Laboratory obtained antimicrobial agents

| Agent No | Benzalkonium chloride | Tartaric acid | Benzoic acid | Ascorbic acid | Sorbic acid | Lactic acid |
|---|---|---|---|---|---|---|
| 1 | 3% | 9% | / | / | / | / |
| 2 | 3% | 9% | 5% | / | / | / |
| 3 | 3% | 9% | / | 5% | / | / |
| 4 | 3% | / | / | 5% | / | / |
| 5 | 3% | 9% | / | / | 1% | / |
| 6 | 3% | 9% | / | / | / | 1% |
| 7 | 3% | 9% | / | / | 1% | 1% |
| 8 | 3% | 9% | / | 5% | 1% | 1% |
| 9 | 3% | 9% | 5% | 5% | 1% | 1% |

employed in six dilutions (100%, 50%, 25%, 12. 5%, 6.25% and 3.125%), with 30 minutes of contact time at room temperature.

The microbiological efficiency of the sanitizers was measured using the macrodilution method. The basic principle of this assay is the broth macrodilution assay- a set of test tubes with different concentrations of antimicrobial agent with the same volumes are prepared. The tubes are inoculated with a microbial inoculum adjusted to 0.5McFarland standard. The solutions were tested based on their effectiveness against nine test microorganisms (Table 2).

and sorbic acid is also reported to inhibit the germination and outgrowth of bacterial spores (Sofos and Busta, 1981; Blocher and Busta, 1985).

Quaternary ammonium compounds (QACs) have antimicrobial activity against gram-negative and gram-positive bacteria as well as against some pathogenic species of fungi and protozoa (Ivankovic and Hrenovic, 2009).

Figures 1, 2, and 3 show the minimal inhibitory concentration mediated by different concentrations ranging from 3.125% to 100% of nine laboratory-obtained antimicrobial agents on the nine microorganisms. Different organisms have demonstrated different rankings for the inhibiting effects of the antimicrobial agents.

**Table 2.** Test microorganisms used in the experimental research

| Bacteria | Yeasts | Molds |
|---|---|---|
| Escherichia coli<br>ATCC 8739 | FM2<br>(yeast from pickled peppers) | FM3<br>(mold from bread) |
| Bacillus subtilis<br>ATCC 6633 | FM20<br>(yeast from pickle) | FM4<br>(mold from jam) |
| Staphylococcus aureus<br>ATCC 6538 | | FM15<br>(mold from meat) |
| | | FM18<br>(mold from tangerine) |

After contact time of 30 minutes for each microorganism, the inoculum of 100 μl from each tube was transferred in Petri plates with Mueller-Hinton agar (for bacteria) or Sabouraud-Dextrose agar (for yeasts and molds) and incubated for 24 hours at 35°C (for bacteria) or for 3-5 days at 25°C (for yeasts and molds).

The observation of cell growth was performed visually and the Minimal Inhibitory Concentration (MIC) of each combination against each test microorganism was determined. The minimum inhibitory concentration is the lowest concentration of this chemical food preservative that inhibits the visible growth of microorganisms after incubation. MICs are used as a research tool to determine the *in vitro* activity of new antimicrobials.

**Results and Discussion**

In the production of food it is crucial that proper measures are taken to ensure the safety and stability of the product during its whole shelf-life. The most common classical preservative agents are the weak organic acids, for example, acetic, lactic, benzoic and sorbic acids. These molecules inhibit the outgrowth of both bacterial and fungal cells,

Figures 1, 2 and 3 also show the effective MIC concentration of each laboratory- obtained antimicrobial agent against all test microorganisms. The results showed that *S. aureus* ATCC 6538 is the most sensitive microorganism of all the tested microorganisms in this study. Among the nine microorganisms tested, the antimicrobial agent designated as No2 (a combination of benzalkonium chloride, tartaric acid and benzoic acid) highly inhibited the growth of all the microorganisms.

The efficiency of the laboratory-obtained antimicrobial agents also depends on the Gram reaction of bacteria. Gram-positive bacteria are more sensitive than Gram- negative to the chemicals, except *B. subtilis* ATCC 6633 because of the nature of sporogenesis. The cell wall of Gram-positive bacteria is composed of peptidoglycan, which is an essential polymer and interference with its synthesis or structure leads to loss of cell shapes and integrity, followed by bacterial death (Willey *et al.,* 2011). The Gram-negative bacteria are known to possess lipopolysaccharide on the outer membrane.

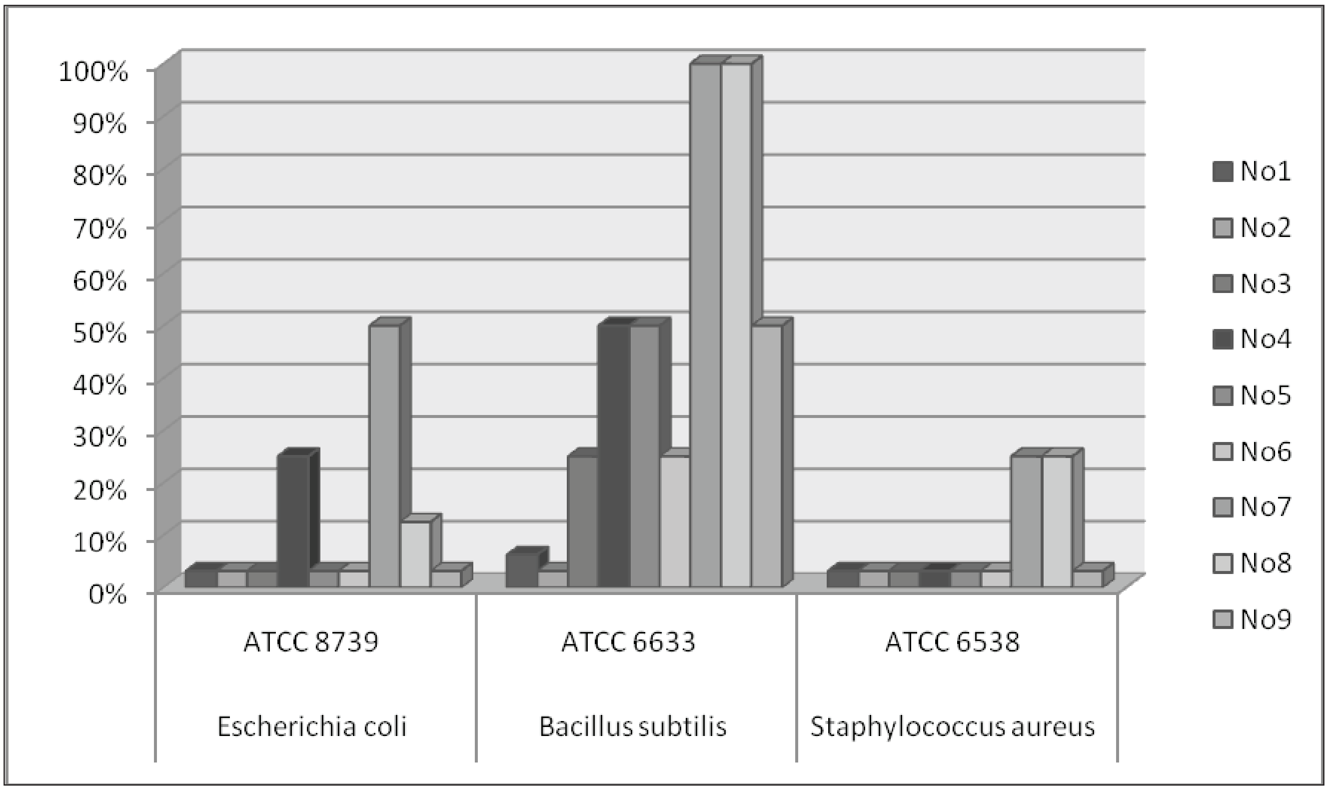


**Fig. 1.** Efficiency of antimicrobial agents at different concentrations over a 30-min exposure at 25°C against tested bacteria

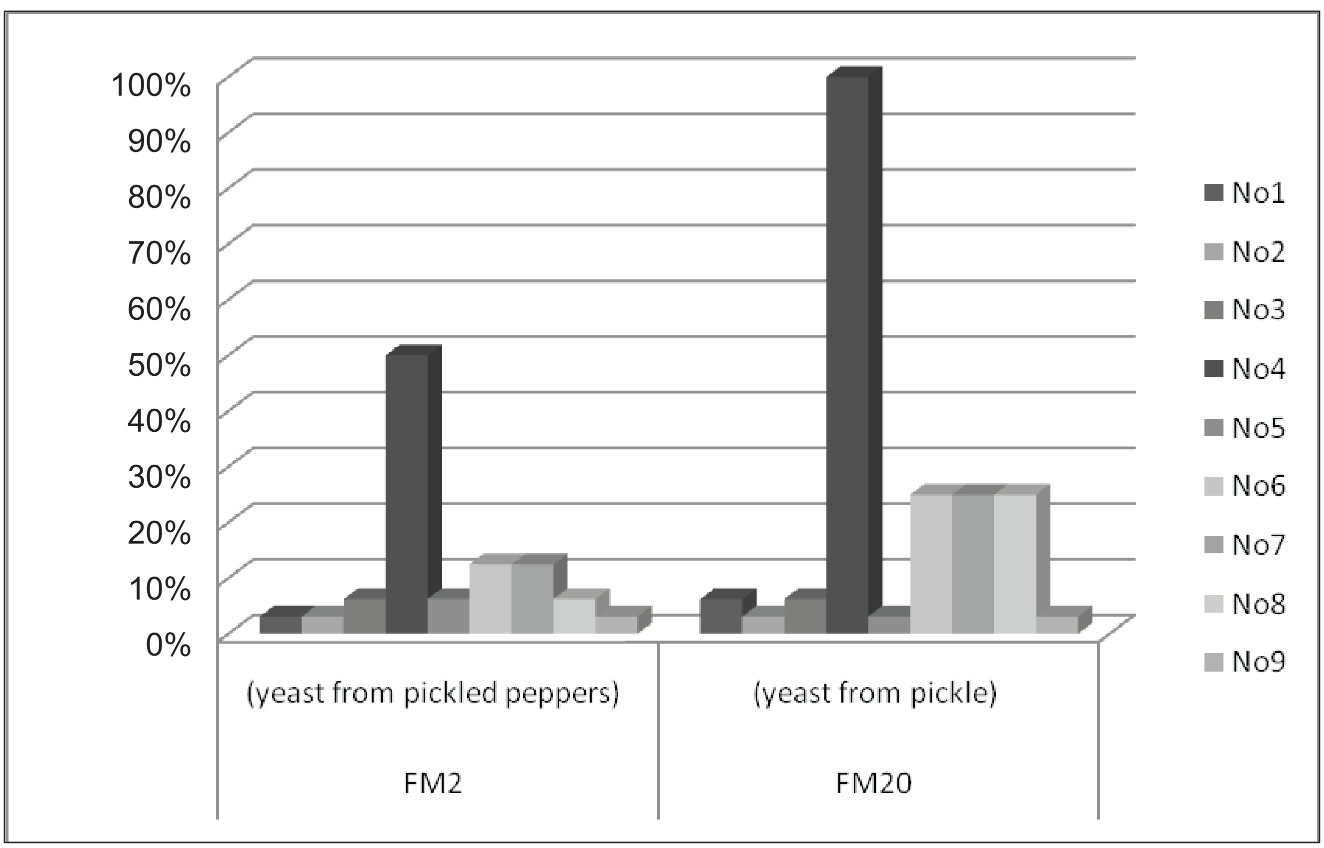


**Fig. 2.** Efficiency of antimicrobial agents at different concentrations over a 30-min exposure at 25°C against tested yeasts

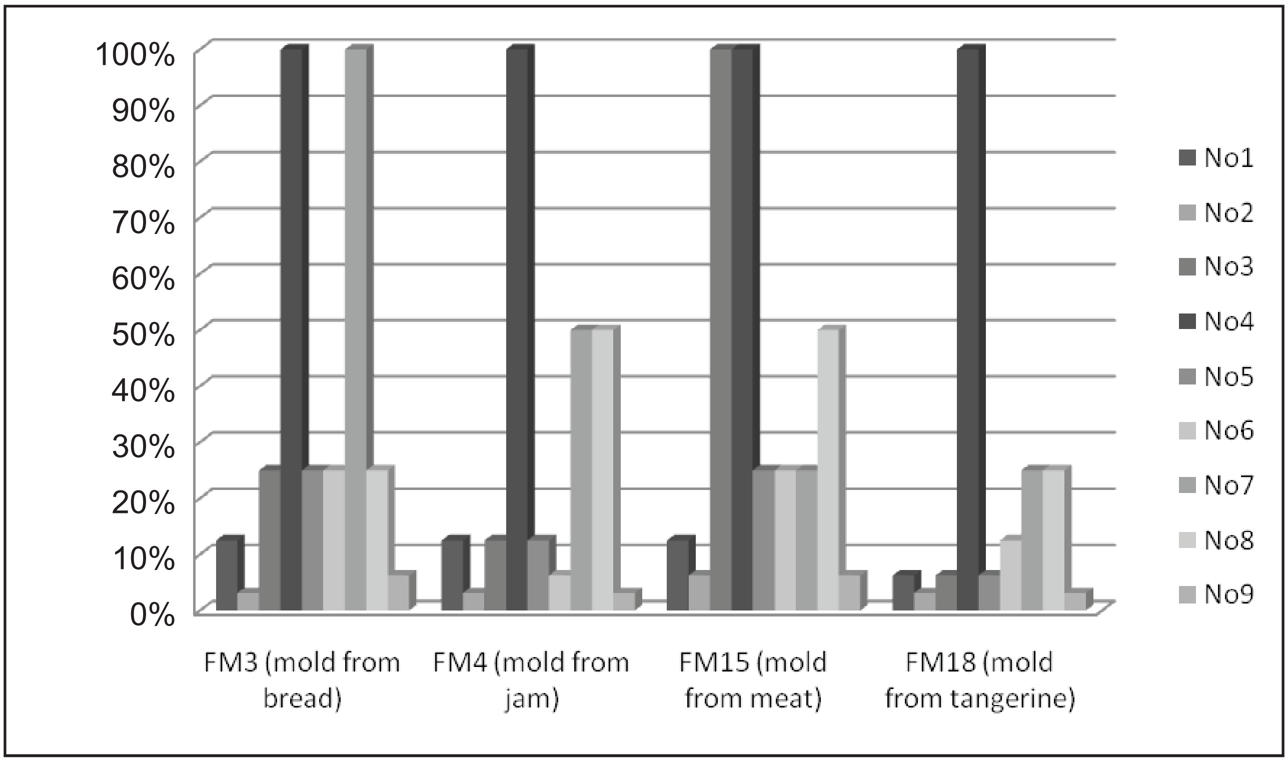


**Fig. 3.** Efficiency of antimicrobial agents at different concentrations over a 30-min exposure at 25°C against tested molds

The resistance to these compounds displayed by the tested yeasts and fungi includes enzymes that allow compound degradation, and changes to the cell envelope that minimize the diffusional entry of the preservative into the cell (Piper, 2011).

The antimicrobial agent designated as No2 was the most effective against the tested molds. These results correspond with the results of Korukluoglu *et al.* (2006). They showed that benzalkonium chloride, one of the components in the compounds tested here, was most effective against all tested yeasts and molds at a concentration of 1%. Ozyurt (2000) announced that *A. niger* was killed with QAC disinfectant at a concentration of 1% in < 2 min.

## Conclusions

Each of the tested agents has a different potential impact on the growth and multiplication of the test microorganisms. These differences result from the composition of the laboratory-obtained antimicrobials and the resistance of the test microorganisms to them.

The lowest MIC for both bacteria and eukaryots was observed for antimicrobial agent No2 (a combination of benzalkonium chloride, tartaric acid and benzoic acid) with a strength of 3.125%.

It can be recommended that the above compounds be used for disinfection of food equipment in order to achieve safe food production. Therefore, by employing such combinations of preservation treatments the required level of protection will be achieved.

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