

ANTIMICROBIAL ACTIVITY OF GARLIC (*A. SATIVUM* L.) AGAINST *SALMONELLA ENTERITIDIS* AND *STAPHYLOCOCCUS* *AUREUS**

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SUMMARY: *Worldwide emergence of antimicrobial resistance among zoonotic pathogens imposes a need for an alternative approach to antibacterial therapy. The aim of this study was to evaluate activity of raw garlic extract against locally isolated serotypes of Salmonella Enteritidis and Staphylococcus aureus. For this purpose, standard antibiogram method was used. Results showed high efficacy of full garlic concentration, except in the case of one isolate of S.aureus, and varying efficacy of half extract doses, which was higher for S. aureus than for S. Enteritidis.*

Key words: *Allium sativum*, antimicrobial activity, *Salmonella Enteritidis*, *Staphylococcus aureus*

INTRODUCTION

Bacteria from genus *Salmonella* represent an important zoonotic pathogen of economic significance in both humans and animals. *Salmonella Enteritidis* (*S. Enteritidis*) and *Salmonella Typhimurium* are the serovars most frequently associated with human illness. Human cases of *S. Enteritidis* infection are most commonly associated with the consumption of contaminated eggs and poultry meat, and usually

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result in gastrointestinal illness of varying severity (EFSA and ECDC, 2013b). Invasive forms of the disease, such as bacteremia and meningitis may occur in a smaller subset of patients, while the state of asymptomatic carrier (convalescent or chronic) may be frequently present in both humans and animals (D'Aoust, 1991; EFSA and ECDC, 2013b).

Staphylococcus aureus (*S. aureus*) is an ubiquitous organism that has been recognized as one of the leading causes of foodborne bacterial intoxications worldwide. It is also responsible for a diverse spectrum of human and animal diseases ranging from minor skin infections to life threatening conditions, such as pneumonia and meningitis (Normanno et al., 2007; Sasidharan et al., 2011).

Although mild and self-limiting in many cases, infections caused by these two pathogens may also turn dubious, or even fatal, in the absence of efficient antibiotic therapy. Worldwide, number of reports on antimicrobial resistance in both human and animal bacteria is increasing, making it an important public health concern (Normanno et al., 2007). The resistance develops as a consequence of extensive prophylactic and therapeutic usage of antimicrobial drugs, often in subtherapeutic concentrations, and due to their administration as growth promoters in food animal production, where large number of animals is being exposed irrespectively of their health (De Oliveira et al., 2005; Yildirim et al., 2011). Consequently, antimicrobial-resistant bacteria in food animals may threaten the efficacy of human drugs if antimicrobial-resistant bacteria or antimicrobial-resistance genes become incorporated into human bacterial populations (De Oliveira et al., 2005).

Results of studies on complementary and alternative medicine practices suggest that introduction of plant extracts, such as garlic extract, in antimicrobial therapy may significantly decrease this emerging burden of drug resistance (Ćirković et al., 2012).

Garlic (*Allium sativum* L.) is a hardy annual monocotyledon plant, and one of the oldest cultivated vegetable crops. The earliest record of the medical properties of garlic and directions for its use come from historian Herodotus and Hippocrates, „the father of western medicine“ (Jones and Mann, 1963; Rabinowich and Brewster, 1990). It is a well known fact that garlic has been used to treat infections for centuries (Onyeagba et al., 2006). Today, garlic's antidiabetic, antibiotic and perhaps anticancer effects are receiving a worldwide recognition due to the abundance of scientific literature supporting these effects. It is also believed that garlic has hepatoprotective, and antioxidant effect (Lalošević i sar., 2013).

The aim of this study was to evaluate antimicrobial activity of two crude garlic extract concentrations against locally isolated serotypes of *S. Enteritidis* and *S. aureus*.

MATERIAL AND METHOD

In the experimental work of the study selected strains of *S. aureus* and *S. Enteritidis*, originating from human swab, were included. All strains were isolated and identified in the Center for Microbiology, of the Institute of Public Health of Vojvodina in Novi Sad. In this scientific work, 9 strains of *S. aureus* and 13 strains of *S. Enteritidis* were used.

The sensitivity of strains to crude garlic extract was tested by the disk diffusion technique according to Kirby-Bauer on Muller-Hinton agar (pH 7.3 ± 0.1 at 25°C). After autoclaving, the prepared substrate was poured into Petri dishes thickness 4 mm. An overnight culture of the bacterial suspension was prepared in physiological saline, concentration of 6×10^8 cfu ml⁻¹ (the 2th scale per McFarland (Klement et al, 1990.)). Inoculation of bacterial strains on Muller-Hinton agar was performed with sterile swab. Thirty minutes after application of the bacterial suspension, filter disks size 6 mm, immersed in a crude extract of garlic and distilled water, were placed on the surface of the substrate at regular intervals. The crude extract of winter garlic (*Allium sativum* var. *vilgare*) was obtained by the mechanical pressing of cloves, after previous immersion in 75% alcohol and rinse with water for surface disinfection. Concentrated extract, and 50% solution of the extract, were used for testing, while sterile distilled water was used as control.

The incubation period was 20 hours at $37 \pm 1^{\circ}\text{C}$. After that, measuring of the inhibition zones, in mm, was carried out. Performing the procedure by disk-diffusion method and reading the results was performed according to the recommendation and guidance made by Clinical and Laboratory Standards Institute (CLSI 2010). The experiment was set up in three replication.

Statistical analysis was performed using software package Statistica 10. Interspecies differences in susceptibility to garlic were investigated with t-test, for level of significance of 95% ($p < 0.05$ was considered significant). Differences in mean inhibition zone values were compared using Duncan test.

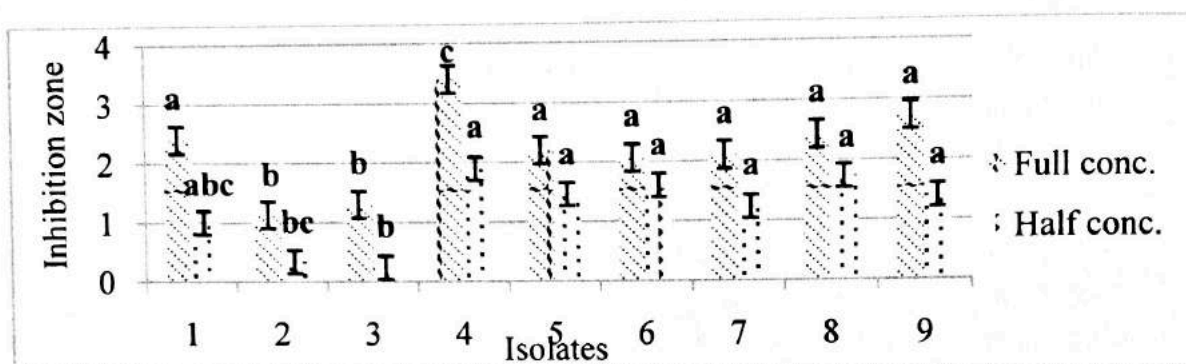
RESULTS

Antibacterial activity of crude garlic extract was observed in all clinical isolates of *S. aureus* and *S. Enteritidis* from the study. For both 100% and 50% extract concentrations, highest average inhibition zone was found in *S. aureus* than in *S. Enteritidis* (Table 1), but the observed difference could not be considered statistically significant (p -value was well above 0,05). However, significant differences were observed when sensitivity to crude garlic extract was compared between isolates of the same species/serotype (Graph 1 and Graph 2) which appeared more prominent in the case *S. aureus*.

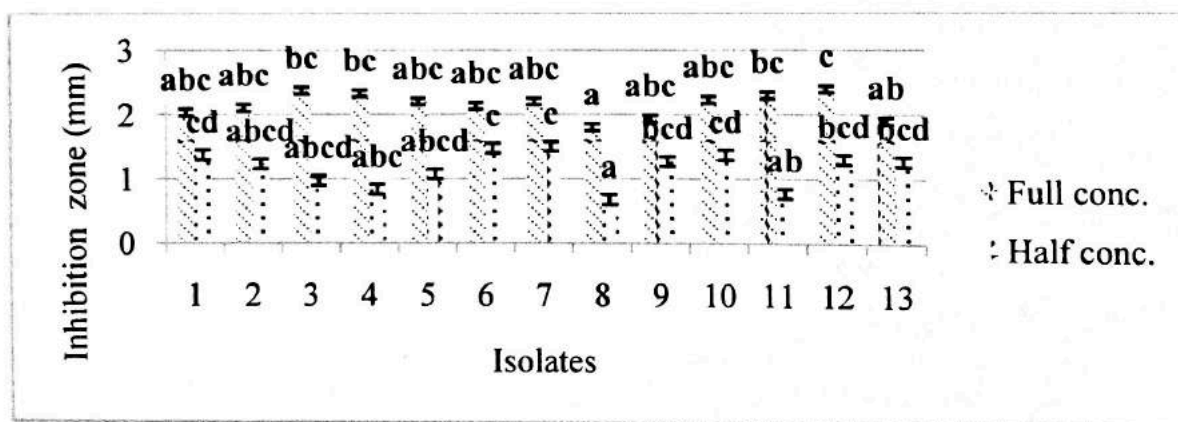
Table 1: Average inhibition zones, given for the 100% and 50% concentration of garlic extract

	Extract concentration	Inhibition zone of bacteria (mm)		p-Value
		<i>S. aureus</i>	<i>S. Enteritidis</i>	
Average	100%	21.6	21.4	0.940
	50%	12.1	11.6	0.815

Graph 1: Comparison of susceptibility of isolates within the *S. aureus* species



Graph 2: Comparison of susceptibility of isolates within the *S. Enteritidis* serotype



DISCUSSION

According to the European Food Safety Authority (EFSA) and European Centre for Disease Prevention and Control (ECDC) (2013a), a statistically significant decrease in the number of human salmonellosis cases was observed in the European Union (EU) over the period 2008-2011. On the other hand, studies around the world report an increase in antimicrobial resistance of this pathogen, with resistance prevalence varying between and within countries as well as between *Salmonella* serotypes (De Oliveira et al., 2005). Serotype with the lowest antimicrobial resistance seems to be *S. Enteritidis*, despite the fact that it is being most frequently isolated from samples. Along with the prevalence, varies the pattern of antimicrobial resistance. Fortunately, resistance to the critically important antimicrobials for human

medicine, cefotaxime (a third-generation cephalosporin) and ciprofloxacin (a fluoroquinolone) is relatively low (EFSA and ECDC, 2013b). In Serbia, resistant strains of *S. Enteritidis* have been confirmed in both human and animal samples (Stošić et al., 2006; Petrović et al., 2008; Kozoderović et al., 2012).

In staphylococci, the methicillin-resistance is considered to be the most significant form of antimicrobial resistance, because it is conferred by the presence of *mecA* gene, which encodes for production of an altered penicillin binding protein that has a low affinity for all beta-lactam antimicrobials (penicillins, cephalosporins, carbapenems), making methicillin-resistant bacteria insensitive to this broad range of antibiotics (Weese and van Duijkeren, 2010).

Until recently, human isolates of methicillin-resistant *S. aureus* (MRSA) were usually associated with hospital-acquired infections; currently, it became evident that MRSA may also represent a challenge as both community-acquired infections and colonizing zoonotic agent in companion and food producing animals (Normanno et al., 2007; Weese and van Duijkeren, 2010). Although recent data showed decreasing or stabilizing MRSA percentages in most European countries, they exceeded 25 % in eight states (EFSA and ECDC, 2013b) making MRSA a lingering human public health priority and a perfect candidate for alternative medical approaches.

Garlic has been described as one of the most significant plants of complementary and alternative medicine. It holds up the highest concentration of sulfur compounds such as allicin and other biologically active components. These are responsible not only for its pungent smell but also for its medicinal value (Kemper, 2000). The thiosulfate allicin has confirmed anti-microbial properties, but other non-sulphur constituents, like proteins, saponins and phenolic compounds, may also contribute to its anti-microbial activity (Corzo-Martinez et al., 2007). The mechanism of antibacterial activity of garlic has not been fully elucidated. According to Feldberg et al. (1988) allicin from garlic extract, directly or indirectly blocks the synthesis of bacterial RNA, without significant effect on DNA and protein synthesis. Therefore, it is considered that the primary tool of allicin is the blocking of RNA formation.

In our previous work, antimicrobial activity of garlic extract was established against different species of zoonotic pathogenic bacteria, such as: *Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella typhimurium*, *S. Enteritidis*, *S. bovis* and *S. aureus* (Vlajić et al., 2013). The experiment, however, included only one isolate of both *S. Enteritidis* and *S. aureus*, in contrast to the present study, where 9 isolates of *S. aureus* and 13 isolates of *S. Enteritidis* were examined. Current results showed a slightly higher sensitivity of *S. aureus* isolates in comparison to *S. Enteritidis* isolates; furthermore, variations in sensitivity between isolates were distinctively higher within *S. aureus* than within *S. Enteritidis* group. Higher sensitivity of *S. aureus* was also observed by other Serbian authors (Ćirković et al., 2012), although their research was of the quantitative nature. Antimicrobial efficacy of garlic against *Salmonella* and *Staphylococcus*, including the resistant strains, has

been observed by authors around the world, in both *in vitro* (Shokrzadeh et al., 2006; Daka and Awole, 2009; Belguith et al., 2010) and *in vivo* (Tsao et al., 2003; Bakri and Douglas, 2005; Uzodike and Igwe., 2005; Adebolu et al., 2011) studies. All these studies reported a concentration/dose-dependent antimicrobial efficacy of garlic compounds, which is in concordance with our results.

Synergistic action of garlic, which was observed in combination with some antibiotics (Tsao et al., 2003; Ćirković et al., 2012), and suggestion (Ankri and Mirelman, 1999) that development of resistance to beta-lactam antibiotics is 1000-fold easier than development of resistance to allicin from garlic, additionally contribute to existing antibacterial potential of garlic.

CONCLUSION

Both *S. aureus* and *S. Enteritidis* human isolates from our study proved susceptible to the crude garlic extract indicating its therapeutic potential. Average inhibition zones were slightly higher for *S. aureus* isolates, which also exhibited reciprocal differences in susceptibility levels. Correlation between crude garlic extract concentrations and diameter of inhibition zones was observed.

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