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Antimicrobial activity of wine in relation to bacterial resistance to medicinal antibiotics

Nataša Boban[1], Marija Tonkić[2], Mia Grga[3], Ana Marija Milat[3], Ivana Mudnić[3] and Mladen Boban[3]

[1]Department of Clinical Epidemiology and Infection Control, University Hospital Split and University of Split School of Medicine, Spinčićeva 1, 21 000 Split, Croatia
[2] Clinical Microbiology and Parasitology, University Hospital Split and University of Split School of Medicine, Spinčićeva 1, 21 000 Split, Croatia
[3]Department of Pharmacology, University of Split School of Medicine, Šoltanska 2, 21 000 Split, Croatia

*corresponding author: mladen.boban@mefst.hr

ABSTRACT

Although antimicrobial properties of wine have been extensively studied, antimicrobial effects of wine in relation to bacterial resistance to medicinal antibiotics have not been examined. Therefore, our aim was to determine whether bacterial resistance to antibiotics can be related to their resistance to red wine as an unspecific antimicrobial medium. The organisms studied were *Salmonella enteritidis* (ATCC 13076), *Escherichia coli* (ATCC 25922), and two clinical isolates which exhibited different resistance to antibiotics, ESBL - producing *Escherichia coli* UR 3612 and *Salmonella enteritidis* KK 962. The time-kill curves method was used. The minimal incubation time of the bacterial suspension with wine, necessary for prevention of bacterial growth, was 3 and 20 min for *E. coli* ATCC and ESBL *E. coli* respectively. This was associated with susceptibility testing in which *E. coli* ATCC proved highly sensitive in contrast to ESBL-producing *E. coli*, which exhibited resistance to a spectrum of antimicrobial drugs of different classes regarding their principal mechanism of action. In the case of *S. enteritidis* strains, they were similar in their susceptibility against test antibiotics and time-kill curves following exposure to wine. Bacterial resistance to wine is closely associated with bacterial resistance to antimicrobial drugs. The exact mechanisms of antimicrobial activity of wine are still a matter of debate. However, wine might be less susceptible to bacterial resistance development and may include mechanisms different from those of medicinal antibiotics. The present study represents an initial contribution to this important subject which has been practically unexplored.

KEYWORDS

wine, antibiotics, bacterial resistance, time-kill curves

INTRODUCTION

Because of their potent antimicrobial activity, wine and numerous wine-based mixtures with other plant-derived antimicrobials have been investigated as a part of non-antibiotic strategies for tackling foodborne and medical pathogens (Friedman, 2014). Although the exact mechanisms and relative contribution of wine components to the antimicrobial activity of wine are still a matter of debate, it is possible that they are different to those of standard medicinal antibiotics (Pojer *et al.,* 2013; Cushnie and Lamb, 2005; Cisowska *et al.*, 2011). Hence, wine might be less susceptible to the development of bacterial resistance.

In contrast to numerous studies demonstrating the effectiveness of using different phytochemicals and plant extracts against resistant bacteria (Friedman, 2015), the antimicrobial effects of wine in relation to bacterial resistance to antibiotics is largely unexplored.

Therefore, the present study was undertaken to examine whether resistance to medicinal antibiotics of standard strains and clinical isolates of *E. coli* and *S. enteritidis* is related to their resistance to wine as an unspecific antimicrobial medium.

MATERIALS AND METHODS

1. Microbial strains

The organisms studied included *Escherichia coli*, American Type Culture Collection (ATCC) 25922, *Salmonella enteritidis*, ATCC 13076, and two clinical isolates which exhibited different resistance to some antibiotics, extended-spectrum beta-lactamase(ESBL)-producing*Escherichiacoli* UR 3612 and *Salmonella enteritidis* KK 962.

2. Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was carried out with the VITEK 2 – compact system using the AST – GN27 cards according to the manufacturer's instructions (bioMérieux, Marcy l'Etoile, France).

Results were expressed as susceptible (S) or resistant (R) according to the European Committee on Antimicrobial Susceptibility Testing – EUCAST criteria (EUCAST, 2018).

3. Testing of antimicrobial activity of wine

The sensitivity of the test bacterial strains to wine was determined by time-kill curves as described in a previous study (Boban *et al.*, 2010). Briefly, the bacteria were grown overnight in blood agar with 5 % sheep's blood (Bio-Rad, Hempstead, UK) at 35 °C, and then suspended in Suspension Medium (bioMerieux, Marcy l'Etoile, France) to a density of 10^7 colony forming units (CFU)/ml (turbidity of 0.5 McFarland opacity standard). After that, aliquots of 200 µL of bacterial suspension were added to 3.8 ml of wine, yielding an initial concentration of 10^5 to 10^6 CFU/ml. The suspension was then mixed with a vortex-mixer and at fixed times inoculated in duplicate with a calibrated loop (0.01 ml) to the blood agar. Plates were incubated for 24 hours at 35 °C at ambient atmosphere. Bacterial growth was determined as the number of colonies seen with the naked eye, and the results of the duplicate plates were averaged. The lower limit of detection was defined as no visible growth of bacteria on the plates.

Data are averages of at least three independent experiments and are expressed as a mean value.

The red wine used in this study was Vinagra, Refosco variety, Bric winary, Šmarje, Slovenia 12.9 vol % ethanol. Biochemical properties and methods of analysis of the wine used in this study have been described in an earlier study (Boban *et al.*, 2010).

RESULTS AND DISCUSSION

The minimal incubation time of the bacterial suspension with wine, necessary for prevention of bacterial growth, was 3 and 20 min for *E. coli* ATCC and ESBL *E. coli* respectively (Figure 1A). This notable difference in the time-kill curves between the two *E. coli* strains can be clearly linked to a clinically relevant difference in their susceptibility to a spectrum of antimicrobial drugs. Table 1 shows minimal inhibitory concentrations (MIC) in µg/mL for antimicrobial drugs to which ESBL *E. coli* was intermediately or fully resistant relative to ATCC *E. coli,* which proved sensitive to all test antibiotics.

In the case of *S. enteritidis* strains, they were both susceptible with practically the same MIC values for all tested antibiotics (clinical isolate *S. enteritidis* KK 962 showed slightly higher MIC values for piperacillin, tigecycline and chloramphenicol; data not shown). This was reflected in the similar time-kill curves (4 and 5 min for *S. enteritidis* ATCC 13076 and *S. enteritidis* KK 962 respectively (Figure 1B)).

TABLE 1. Results of susceptibility and minimal inhibitory concentrations (MIC) of different antimicrobial drugs against standard *Escherichia coli* American Type Culture Collection (ATCC) 25922 and multi-resistant, extended-spectrum beta-lactamase (ESBL) - producing clinical isolate *E. coli* UR 3612.

Antimicrobial drug	*Escherichia coli* ATCC 25922 MIC (μg/ml)	*Escherichia coli* ESBL UR 3612 MIC (μg/ml)
Ampicillin	4 (S)	≥ 32 (R)
Piperacillin	≤ 4 (S)	≥ 128 (R)
Ticarcillin	≤ 8 (S)	≥ 128 (R)
Cefuroxime	4 (S)	≥ 64 (R)
Cefixime	≤ 0.25(S)	≥ 4 (R)
Ceftriaxone	≤ 1 (S)	≥ 64 (R)
Ceftazidime	≤ 1 (S)	16 (R)
Cefepime	≤ 1 (S)	2 (I)
Aztreonam	≤ 1 (S)	4 (I)
Tobramycin	≤ 1 (S)	4 (R)
Gentamicin	≤ 1 (S)	≥ 16 (R)
Ciprofloxacin	≤ 0.25(S)	≥ 4 (R)
Moxifloxacin	≤ 0.25(S)	≥ 8 (R)
Levofloxacin	≤ 0.12(S)	4 (R)
Nitrofurantoin	≤ 16 (S)	32 (I)
Trimethoprim/ Sulfamethoxazole	≤ 20 (S)	40 (I)

*S = susceptible; I = intermediate; R = resistant.

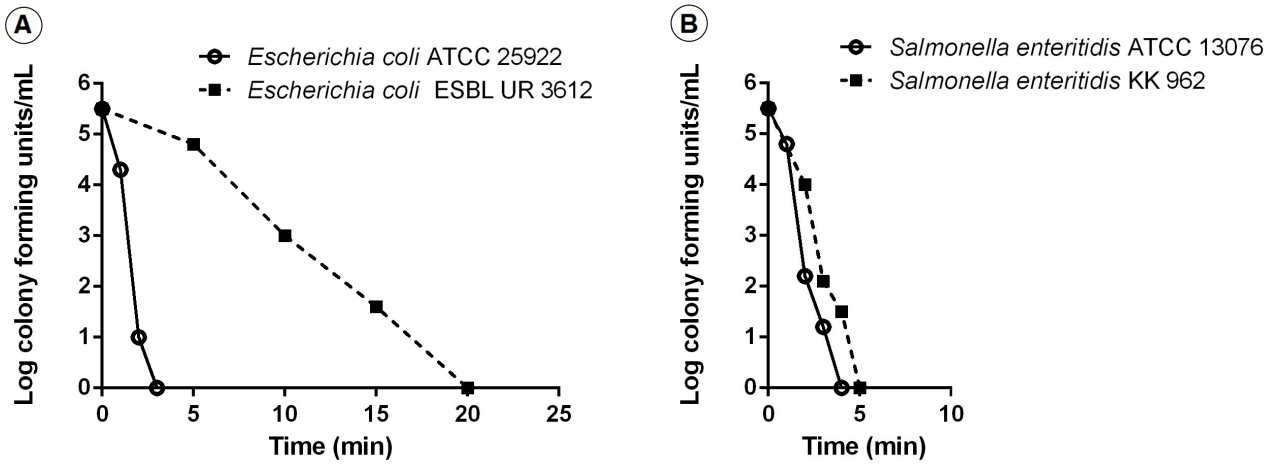

FIGURE 1. Time-kill curves for *Escherichia coli* ATCC 25922 and multi-resistant, extended-spectrum beta-lactamase - producing clinical isolate *Escherichia coli* UR 3612 (A) and *Salmonella enteritidis* ATCC 13076 and clinical isolate *Salmonella enteritidis* KK 926 (B) following incubation with red wine.
Data are averages of at least three independent experiments.

The fact that wine affected the same bacterial species of differing origin in different ways does not support the notion that wine may not kill bacteria, but just cause their unspecific aggregation due to the polyphenolic compounds contained in wine (Cushnie and Lamb, 2005). On the other hand, the inhibition of growth in the nutrient agar of the bacteria which were previously inoculated in wine, has been described to be just a transient phenomenon; namely, after exposure to a harsh antimicrobial medium such as wine, bacteria may turn into a viable, but non-cultivable, state, thereby preserving potential to restore colony-forming activity when incubated

in a favourable growing environment (Millet and Lonvaud-Funel, 2000). Whatever the mechanism of the loss of colony-forming activity observed in this study, it does not alter the key finding that bacterial sensitivity to wine is associated with the susceptibility of bacteria to medicinal antibiotics.

Moreover, we believe that the method in which test strains are exposed to whole wine, in which physical-chemical properties and the matrix are not disturbed, provide the most realistic insight into the "true" effects of wine. ESBL producing *E. coli* as the most resistant bacteria to wine was also resistant to a wide range of antimicrobial drugs which belong to different classes depending on their principal mechanism of action. Among known basic mechanisms of bacterial resistance, multidrug efflux systems capable of pumping a broad spectrum of unrelated antibiotics and biocides (Poole, 2005) are likely to be involved.

However, the antimicrobial activity of wine and its constituents involves many modes of action (Cisowska *et al.,* 2011; Cho *et al.,* 2014; Cushnie and Lamb, 2005; Daglia *et al.,* 2007). The relationship between bacterial multidrug resistance and various mechanisms of antibacterial action of wine and its constituents is practically unexplored.

In an attempt to distinguish the roles of polyphenols, pH, ethanol and other wine components, in our previous work we examined the antimicrobial effects of intact wine in comparison to those of phenols-stripped wine, dealcoholized wine, ethanol and low pH applied separately and in combination (Boban *et al.,* 2010). It was not possible to relate the antibacterial activity of the samples to their total phenolic and resveratrol content, ethanol content, or pH. It was concluded that the antimicrobial activity of complex solutions, such as an intact wine, cannot be exclusively attributed to its phenolic or nonphenolic constituents, nor can the antimicrobial activity of wine be predicted on the basis of its particular components.

We hope that this simple yet straightforward study may be inspiring for further studies, thus deepening insight into this complex matter.

CONCLUSION

The key finding of this study is that bacterial resistance to wine appears to be closely associated with bacterial resistance to antimicrobial drugs. This indicates that bacterial resistance to wine and medicinal antibiotics share, at least in part, the same mechanisms.

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