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SCIENTIFIC ARTICLE

# *In vitro* evaluation of antimicrobial features of vasopressors

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Antimicrobial activities;
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**Abstract**
*Background:* Drugs administered as intravenous infusion may be contaminated during several stages of production or preparation. However studies focusing on antibacterial effects of vasopressor drugs are very rare. This study investigates the *in vitro* antimicrobial activity of the clinically used forms of vasopressors.
*Materials and methods: In vitro* antimicrobial activities of vasopressor drugs of different concentrations were investigated by using the micro dilution technique. Microorganisms used in the test were *Escherichia coli* ATCC 25922, *Yersinia pseudotuberculosis* ATCC 911, *Pseudomonas aeruginosa* ATCC 10145, *Listeria monocytogenes* ATCC 43251, *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 25923, *Bacillus cereus* 702 Roma, *Mycobacterium smegmatis* ATCC607, *Candida albicans* ATCC 60193, and *Saccharomyces cerevisiae* RSKK 251. Antibacterial assays were performed in Mueller-Hinton broth at pH 7.3 and antifungal assays were performed in buffered Yeast Nitrogen Base at pH 7.0.
*Results:* Two different dopamine preparations showed antimicrobial activity. No other study drug showed any antimicrobial activity.
*Conclusions:* In our opinion, dopamine's antibacterial effects may be advantageous for inhibiting the spread of bacterial contamination during the preparation of the infusion solutions. However, it is important that strict guidelines regarding the need for sterile equipment and deliverables be adhered to during all procedures performed in the intensive care units.


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## Introduction

Septic shock is the primary cause of death in critical care units. Shock states are primarily characterized by acute circulatory failure leading to tissue hypoperfusion, and potentially resulting in multi-organ failure. Observed hypotension can be the consequence of three major hemodynamic disorders: hypovolemia, vascular failure, and heart failure.[1] When appropriate fluid administration fails to restore adequate tissue perfusion and arterial pressure, vasopressors are usually necessary to increase mean systemic pressure, cardiac output, and oxygen delivery.[2]

*In vitro* studies focusing on catecholamine molecules demonstrated proliferation of bacteria.[3–5] A portion of catecholamines, which are used as vasopressor, are endogenously produced in the body. However, catecholamines used as vasopressor drugs are synthetically produced and infused for the treatment of cardiovascular failure which arises during septic shock. Dopamine, dobutamine, adrenaline and noradrenaline are most frequently used vasopressors prepared synthetically with supplemental chemicals having antioxidant and antimicrobial activity. Sodium metabisulfite, N-acetylcysteine and disodium edetate are the most frequently used antioxidant and antimicrobials for this purpose in drugs commonly found in medical markets (Table 1).

Considering several studies pointing catecholamine molecules' proliferating effect on bacteria, we investigated commercially prepared catecholamine products' *in vitro* effect on proliferation of several yeast and bacterial strains commonly encountered in septic shock.

## Materials and methods

Microorganisms used in tests were obtained from the Refik Saydam Hifzissihha Institute (Ankara, Turkey) and were as follows: *Escherichia coli* ATCC 25922, *Yersinia pseudotuberculosis* ATCC 911, *Pseudomonas auroginosa* ATCC 10145, *Listeria monocytogenes* ATCC 43251, *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 25923, *Bacillus cereus* 709 ROMA, *Mycobacterium smegmatis* ATCC607, *Candida albicans* ATCC 60193 and *Saccharomyces cerevisiae* ATCC 60193.

Antimicrobial effects of the drugs were tested quantitatively in appropriate broth media using the double dilution method, and the minimum inhibitory concentration (MIC) values in μg/mL were determined.[6,7] Antibacterial assays were performed in Mueller-Hinton broth (MH) (Difco, Detroit, MI) at pH 7.3 and antifungal assays were performed in buffered Yeast Nitrogen Base (YNB) (Difco, Detroit, MI) at pH 7.0. Each tested drug was prepared in 0.1 mL volumes of sterile MH and YNB broths in concentrations ranging from 5 μg/mL to 5 mg/mL for microdilution. One drop (0.02 mL) of microorganism's suspension (approximately $10^6$ microorganisms per mL) was added to the extract/broth dilutions. After incubation at 35 °C for 18–72 h, the media were examined for growth. MIC is defined as the lowest concentration of drug showing no growth of microorganism. The dilutions without visible growth were used to determine minimum bactericidal concentration (MBC) by spreading 100 μL of the sample across the surface of dried MH and YNB agar plates with sterile glass rods, and then incubating at 35 °C for 18 h. MBC of each extract is defined as the lowest concentration

**Table 1** Study drugs and ingredients.

| Catecholamine | Ingredients |
|---|---|
| Epinephrine | *In 1 mL Ampoule:*<br>- Epinephrine 0.5 mg<br>- Sodium chloride 8.5 mg<br>- Metabisulfite 0.5 mg<br>- Water for injection |
| Norepinephrine | *In 4 mL Ampoule:*<br>- Norepinephrine bitartarate 8 mg (equivalent to 4 mg norepinephrine base)<br>- Sodium metabisulfite 4 mg<br>- Sodium chloride 34.35 mg<br>- Water for injection |
| Dobutamine | *In 20 mL Ampoule:*<br>- Dobutamine hydrochloride 280 mg (equivalent to 250 mg dobutamine base)<br>- Sodium metabisulfite 4.8 mg<br>- Water for injection |
| Dopamine | *In 5 mL Ampoule:*<br>- Dopamine hydrochloride 200 mg<br>- N-acetylcysteine 2 mg<br>- Disodium edetate 2 mg<br>- Water for injection |
| Dopamine | *In 5 mL Ampoule:*<br>- Dopamine hydrochloride 200 mg<br>- Sodium metabisulfite 50 mg |

that showed no growth of microorganism on agar plate. Fluconazole, Ampicillin and Streptomycin were used as standard antifungal and antibacterial drugs, respectively.

Ingredients of study drugs widely used as vasopressor in medical market are presented in Table 1.

## Results

None of the study drugs containing norepinephrine, epinephrine and dobutamine showed any antimicrobial activity (Table 2). However study drugs containing dopamine showed antimicrobial activity (Table 2), one of which showed no activity against yeast-like microorganisms. The solutions containing dopamine (125–1000 μg/mL), N-acetylcysteine and disodium edetate (1.25–10 μg/mL) showed bacteriostatic activity against Gram-positive and -negative microorganisms. Higher concentration solutions showed bactericidial activity against all microorganisms except *Y. pseudotuberculosis*, which is capsular. Solutions of different concentrations of dopamine (125–500 μg/mL), N-acetylcysteine and disodium edetate (1.25–5 μg/mL) resulted in similar MIC and MBC values for bacterial strains like Gram-positive bacillus *L. monocytogenes*, Gram-positive coccus *E. faecalis*, Gram-negative *M. smegmatis*, which contain mycolic acid in their cell wall. However, these values were markedly different for each solution. Solutions containing dopamine (125–250 μg/mL), N-acetylcysteine and disodium edetate (1.25–2.5 μg/mL) showed bacteriostatic activity against *E. coli*, *P. aeruginosa* and *S. aureus*. Solutions containing dopamine 2000 μg/mL, N-acetylcysteine and disodium edetate 20 μg/mL showed bactericidial activity against the same microorganisms. Solutions containing dopamine 1000 μg/mL, N-acetylcysteine and disodium edetate 10 μg/mL showed bacteriostatic and bactericidal activity against *B. cereus*.

Other drugs containing dopamine (125–500 μg/mL) and sodium metabisulfite (62.5–125 μg/mL) showed bactericidial activity against all microorganisms used in the test.

## Discussion

In this study, we have found that two different dopamine preparations out of all tested showed antimicrobial activity.

Drugs manufactured for intravenous use should be prepared and administered in sterile conditions. Infectious microorganisms can be introduced into the patient through contaminated containers, rubber diaphragm, needles and infusion sets. Anesthetic agents and vasopressors may be contaminated by microorganisms during the preparation of an infusion. For this reason, the antimicrobial effects of anesthetic agents and vasopressors have been deemed important, and they have been investigated in previous studies.[8] Notably, propofol is known to support the growth of microorganisms.[8] On the other hand, previous studies have shown that morphine sulphate, thiopental sodium, fentanyl citrate, dexmedetomidine and midazolam all have antimicrobial effects.[8–13] However, studies on the antimicrobial effects of vasopressors drugs, which are commonly used in intensive care units (ICU), are very few.

Studies demonstrating that catecholamines stimulate growth of microorganisms are increasing.[4,5,14,15] Among the causative factors are binding of catecholamines to transferrin and lactoferrin, enabling bacteria to acquire normally inaccessible ferric-iron[14] and possible α-adrenergic specific response system of some bacteria to recognize catecholamines.[15]

On the other hand, additives having antioxidant and antimicrobial properties are commonly added to commercial vasopressor formulas to prevent bacterial contamination.[16–19] However, studies investigating the *in vitro* antimicrobial activity of clinically used commercial forms of vasopressors are very few. Most commonly used additives in vasopressors are N-acetylcysteine and disodium edetate, which are known as potent antioxidants with antimicrobial properties.[16–19]

This study evaluates the antimicrobial properties of most commonly used vasopressors in the medical markets in different concentrations by the micro-dilution method.

Vasopressor drugs are administered as infusion through a preferably central, high caliber vein to ensure a steady state plasma concentration. We used the micro-dilution method to mimic different levels of concentrations since catecholamines interact over a wide dose-response range and exhibit multiple potencies.

Our study revealed antimicrobial activity of both of the dopamine preparates. No other study drug was able to inhibit microorganismal growth at any concentration. This finding could be explained by the high sodium metabisulfite concentration for one of the Dopamine preparate compared to norepinephrine, adrenaline and dobutamine preparates.

Sodium metabisulfite is an oxidizing agent active at low pH. While all the study drugs have effective pH ranges between 2.2 and 5.0, MH broth has a pH value of $7.3 \pm 0.1$ and buffered YNB used in our study has a pH value of 7.0 at 25 °C (unbuffered medium has a pH value of $5.4 \pm 0.2$ at 25 °C). Therefore we do not think that sodium metabisulfite may exhibit any antimicrobial activity at the neutral pH. Since human blood has a slightly alkaline pH value of 7.35–7.45, and most pathogenic bacteria prefer a narrow pH range of 6–8,[11] we think that this finding is concordant with real-life applications of the drugs.

The other oxidizing additive contained in tested drugs is N-acetylcysteine, which was shown to be a valuable mucolytic agent, capable to aid in antimicrobial treatment if combined with antibiotics. Examples where antimicrobial activity of N-acetylcysteine is seen are lysis of gastric basal mucosal layer, which enables *Helicobacter pylori* to escape from the acidic gastric secretions[20] and decreasing formation of biofilms by reducing production of extracellular polysaccharide matrix and promoting the disruption of mature biofilm.[21] Both of these activities are augmented by an acidic environment, and the slightly alkaline pH levels established in the study broth environments may have hindered N-acetylcysteine's antimicrobial activity. However, as for metabisulfite, we conclude that a slightly alkaline pH level is more concordant with real-life applications of these drugs.

It is important that strict guidelines regarding the need for sterile equipment and deliverables be adhered to during all procedures performed in the ICU. In some circumstances, vasopressor drugs may be contaminated with microorganisms that can then lead to infections.[8,9] Thus, the antimicrobial effect of vasopressor drugs in these types of settings

**Table 2** Antimicrobial activity of the compounds expressed as MIC value in 100 mL volume.

| Study drugs | Ingredient | Concentration (μg/mL) | MIC values (100 mL) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Ec | Yp | Pa | Li | Ef | Sa | Bc | Ms | Ca | Sc |
| Norepinephrine | Norepinephrine | 1000 | – | – | – | – | – | – | – | – | – | – |
| 4 mg/4 mL Ampoule | Sodium metabisulfite | 1000 | – | – | – | – | – | – | – | – | – | – |
| | Sodium chloride | 34.350 | – | – | – | – | – | – | – | – | – | – |
| Adrenalin 0.5 mg/1 mL | Epinephrine | 500 | – | – | – | – | – | – | – | – | – | – |
| Ampoule | Sodium metabisulfite | 500 | – | – | – | – | – | – | – | – | – | – |
| | Sodium chloride | 8500 | – | – | – | – | – | – | – | – | – | – |
| Dobutamine | Dobutamine | 12.500 | – | – | – | – | – | – | – | – | – | – |
| 250 mg/20 mL Ampoule | Sodium metabisulfite | 240 | – | – | – | – | – | – | – | – | – | – |
| Dopamin 200 mg/5 mL | Dopamine | 40.000 | 125 | 125 | 250 | 250 | 125 | 125 | 1000 | 250 | – | – |
| Ampoule | N-acetylcysteine | 400 | 1.25 | 1.25 | 2.50 | 2.50 | 1.25 | 1.25 | 10 | 2.50 | – | – |
| | Disodium edetate | 400 | 1.25 | 1.25 | 2.50 | 2.50 | 1.25 | 1.25 | 10 | 2.50 | – | – |
| Dopamine 200 mg/5 mL | Dopamine | 40.000 | 500 | 500 | 500 | 250 | 250 | 500 | 250 | 250 | 500 | 125 |
| Ampoule | Sodium metabisulfite | 10.000 | 125 | 125 | 125 | 62.5 | 62.5 | 125 | 62.5 | 62.5 | 125 | 62.5 |
| Ampicillin | | 10 | 2 | 32 | >128 | 2 | 2 | 2 | <1 | | | |
| Streptomycin | | 10 | | | | | | | | 4 | | |
| Fluconazole | | 5 | | | | | | | | | <8 | <8 |

Bc, *Bacillus cereus* 702 Roma; Ca, *Candida albicans* ATCC 60193; Ec, *Escherichia coli* ATCC 25922; Ef, *Enterococcus faecalis* ATCC 29212; Li, *Listeria monocytogenes* ATCC 43251; Ms, *Mycobacterium smegmatis* ATCC607; Pa, *Pseudomonas aeruginosa* ATCC 10145; Sa, *Staphylococcus aureus* ATCC 25923; Sc, *Saccharomyces cerevisiae* RSKK 251; Yp, *Yersinia pseudotuberculosis* ATCC 911.
(–): no activity.

is of paramount importance.[8,9] In our opinion, dopamine's antibacterial effects may be sufficient to inhibit contamination during the preparation of the infusion solutions.

We have shown that dopamine has antibacterial effects on some microorganisms frequently encountered in hospital settings. We suggest that dopamine preparations should be preferred in septic patients due to their antimicrobial activity against several yeast and bacterial strains commonly encountered in septic shock. However, translating such laboratory researches into recommendations requires delineation of the interactions between catecholamines, several molecules co-existing or co-secreted with them, and microorganisms in their expected environments.

## Conflicts of interest

The authors declare no conflicts of interest.

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