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INVESTIGATION OF THE BIOCIDAL EFFECT OF ELECTROCHEMICALLY ACTIVATED AQUEOUS SODIUM CHLORIDE SOLUTION ON STAPHYLOCOCCUS AUREUS

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ABSTRACT

Studies were carried out to determine the sensitivity of Staphylococcus aureus to electrochemically activated 3% aqueous sodium chloride solution (anolyte) in different concentrations – 100 %, 50 %, 25 % and 12.5 %. As a control was used the disinfectant Virkon S, applied at final concentrations of 1 %, 0.5 %, 0.25 % and 0.125 %. Two referent strains of S. aureus were used – ATCC and Kowan.

It had been found that the anolyte in concentrations of 50 and 100 % inactivates the cells of S. aureus ATCC in suspension at a density of 106 cells/ml within 5 min. After 10 minutes of impact and lower concentrations (25 and 12.5 %) had a bactericidal effect. The anolyte in all tested concentration (12.5 to 100 %) had a bactericidal effect on the cells of S. aureus Kowan in suspension with concentration of 106 cells/ml in 10-minutes. Shorter intervals tested (2 min and 5min) were not sufficient for achieving bactericidal action even at a concentration of anolyte 50 and 100 %, while after 10 min and even smaller concentrations (25 and 12.5 %) had such action. S. aureus ATCC showed slightly higher sensitivity to anolyte and Virkon S compared to the other tested strain Kowan. The effect of the control disinfectant Virkon S on the tested staphylococcal strains was completely analogous to that of the anolyte.

Key words: electrochemically activated solution of sodium chloride, anolyte, Virkon S, Staphylococcus aureus, antibacterial activity.

Introduction

One of the biggest problems in modern medicine is the increasing resistance of pathogenic bacteria to antimicrobial, as well as to disinfectants. This is a prerequisite to search for new effective antimicrobials, which while not be dangerous for patients and the environment. One possibility in this respect provides the technology for the electrochemical processing of water and the preparation of electrochemically activated aqueous solution (catholyte and anolyte). These can be used for disinfection of water and of other objects, as well as for the treatment of bacterial and viral diseases (Atanasov et al., 2014; Karadzhov et al., 2014; Ignatov et al., 2015). In Bulgaria Gluhchev et al. (2015) found a significant inhibitory effect of anolyte on Escherichia coli, as well as on the development of Classical swine fever in cell culture. In laboratory conditions Tasheva et al. (2010) found antimicrobial activity of electrochemically activated aqueous solutions (anolytes) of alkaline and alkaline earth metal salts on field strains of Candida albicans.

The activated water obtained by electrolysis acquire completely new properties and becomes effective acting ecologically clean disinfectant and means for prevention of many diseases. The fields of application of activated water are constantly increasing. The resulting solutions are also called "live" (catholyte) and "dead" (anolyte) water. Catholyte is an alkaline solution with pH between 10.7 and 11.1, with a low redox potential (RP) smaller than -400mV when is fresh and + 200 mV when is stored for several days. It has considerable detergent and washing properties. Anolyte has acidic to neutral pH (between 6.8 and 7.3), high RP (above + 900 mV when is fresh)

and a wide range of disinfecting properties. Its high redox potential determines its bactericidal action. Caroline with its alkaline properties proved an effective tool against free radicals in the body (Act Beauty, 2015; RADICAL WATERS, 2016). It was found that the activated water is not toxic and not dangerous both for external and internal use (Korodetski, 2011).

It turns out that such a water has occurred first in nature. In the bowels of the earth in the mineral springs it has layers with a large difference in the electrode potentials, which act as anode and cathode in an underground electrolyser (eg. copper or zinc and potassium and nickel, and zinc, in this case attaches electrons and copper accept them). Thus, the crust itself produces activated ingredients. Most mineral waters actually proved natural activated solutions and their healing effect is due more to this feature, rather than their mineral composition. This explains the fact that the mineral water at the very headspring has a much greater impact than bottled such, because the activated solutions over time lose its healing power (Korodetski, 2011).

Since staphylococci are one of the most spread bacteria and some of the most common causes of purulent infections with different location in animals and humans, while these are among the most resistant to antimicrobials Gram-positive bacteria, in this work we aim to investigate the effect of the anolyte on suspensions of Staphylococcus aureus.

Materials and methods

Anolyte (activated water). Tested was the effect of the anolyte containing Cl-, prepared by electrochemical activation of distilled water 3 % NaCl, applied in various final concentrations from 12.5 to 100 %.

Control. Virkon S was used in final concentrations of 1 % to 0.125 %.

Microorganisms. In the study were used suspensions with concentrations 106 cells/ml of two reference strains of Staphylococcus aureus (ATCC and Kowan).

Nutrient media. Culture media from Scharlau - Antisel, Bulgaria were used - agar of Mueller Hinton for the preparation of 24-hour cultures of the bacterial strains, Mueller Hinton broth, as well as Chapman Stone agar to determine the effect of the tested solutions for antimicrobial activity on S. aureus.

Scaffold. Twice increasing dilutions of the anolyte were prepared in sterile distilled water, as the obtained concentrations were respectively 100 % anolyte, 50 %, 25 % and 12.5 % in an amounts of 9 ml. To each of them was added a suspension of tested microorganism with concentration of 107 cells/ml in an amount of 1 ml, whereby it was achieved a final concentration of 106 cells/ml.

Enclosed were the following controls - sterile distilled water (without anolyte) with the same content of studies bacterial strain, as well as 100% anolyte without microorganisms.

After various time intervals for the influence of the anolyte (2 min, 5 min and 10 min) cultures were made from each of the samples in Mueller Hinton broth, which were cultured at 37 °C for 24–48 h under aerobic conditions. At cases of establishment of growth in liquid media, subcultures were made on the selective medium Chapman Stone agar to check for growth of the tested microorganisms.

Microscopic studies of preparations stained by Gram were made of materials from cultures in liquid and on solid media.

Results

The summarized results of the conducted researches are presented in Tables 1–4 and some of them – and in Figures 1–3.

Table 1: Growth (amount of colonies) of *S. aureus* ATCC with concentration 10^6 cells/ml after various intervals of exposure of anolyte, applied in different concentrations

Concentration of the anolyte in %	Exposure time - min		
	2	5	10
100	Single	0	0
50	Single	0	0
25	Single	Single	0
12.5	Single	Single	0
Control without anolyte	Many	Many	Many
Control (anolyte without bacteria)	0	0	0

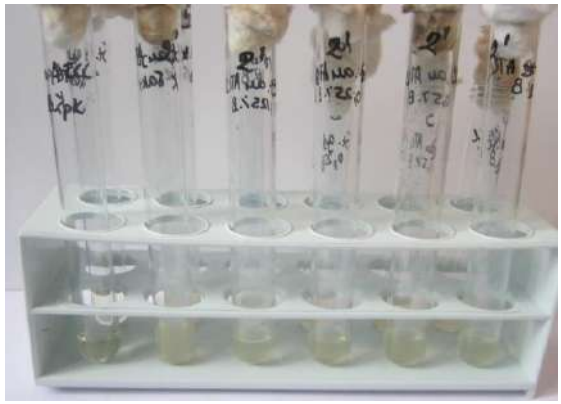

Figure 1: Growth in Mueller Hinton broth after impact of the anolyte, applied in different concentrations (100 %, 50 %, 25 % and 12.5) with a duration of 2 min on suspensions of *S. aureus* ATCC with concentration 10^6 cells/ml

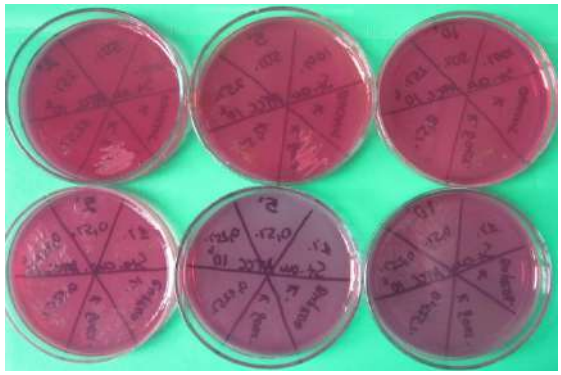

Figure 2: Growth of *S. aureus* ATCC after impact of the anolyte (above), applied in different concentrations (100 %, 50 %, 25 % and 12.5) and of Virkon S (below) at a concentration of 1 % to 0.125 % with duration of 2 min, 5 min and 10 min on suspensions with concentration10^6 cells/ml.

Table 2: Growth (amount of colonies) of *S. aureus* Kowan at concentration10^6 cells/ml after various intervals of exposure of anolyte, applied in different concentrations

Concentration of the anolyte in %	Exposure time - min		
	2	5	10
100	Many	Many	0
50	Many	Many	0
25	Many	Many	0
12.5	Many	Many	0
Control without anolyte	Many	Many	Many
Control (anolyte without bacteria)	0	0	0

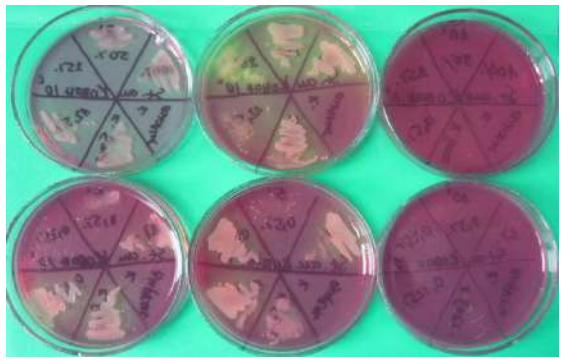

Figure 3: Growth of *S. aureus* Kowan after impact of the anolyte (above), applied in different concentrations (100 %, 50 %, 25 % and 12.5) and of Virkon S (below) at concentration of 1 % to 0.125 % with duration of 2 min, 5 min and 10 min on suspensions with concentration of 10^6 cells/ml.

Table 3: Growth (amount of colonies) of *S. aureus* ATCC with concentration 10^6 cells/ml after different intervals of impact of Virkon S, applied in different concentrations

Concentration of Virkon S in %	Exposure time - min		
	2	5	10
1	Single	0	0
0.5	Single	0	0
0.25	Single	0	0
0.125	Single	0	0
Control without Virkon S	Many	Many	Many
Control (Virkon S without bacteria)	0	0	0

Table 4: Growth (amount of colonies) of *S. aureus* Kowan with concentration 10^6 cells/ml after different intervals of impact of Virkon S, applied in different concentrations

Concentration of Virkon S in %	Exposure time - min		
	2	5	10
1	Many	Many	0
0,5	Many	Many	0
0,25	Many	Many	0
0,125	Many	Many	0
Control without Virkon S	Many	Many	Many
Control (Virkon S without bacteria)	0	0	0

The data in Table 1 and Figures 1 and 2 show that the anolyte in concentrations 50 and 100 % inactivated the cells of S. aureus ATCC in suspension with density of 106 cells/ml within 5 min. After 10 minutes of impact and lower concentrations (25 and 12.5%) have a bactericidal effect.

From the results presented in Table 2 and Figure 3 it is seen that in all tested concentration (12.5 to 100 %) the anolyte has a bactericidal effect on cells of S. aureus Kowan in suspension with concentration 106 cells/ml in 10-minute interval. The shorter intervals tested (2 min and 5 min) were not sufficient for achieving the bactericidal action even at a concentration of anolyte 50 and 100 %, while after 10 min and even smaller concentrations (25 and 12.5 %) had such action.

The results obtained when testing control disinfectant Virkon S on both used staphylococcal strains were similar to the anolyte. They can be seen in Tables 3 and 4 and in Figures 2 and 3. In all tested concentration Virkon S inactivated S. aureus ATCC within 5 min, but not for 2 min, while the other strain Kowan showed smaller sensitivity in tested concentrations of 106 cells/ml and died within 10 min.

Discussion

The results obtained by the current research show that the anolyte can be used as a reliable disinfection agent in the presence of staphylococci in amounts of 106 cells/ml in an aqueous medium. Due to differences in the sensitivity of the strains for a certain effect is required exposure not less than 10 min. In the presence of proteins slower action of the anolyte would be expected, as well as the use of higher concentrations of 50 % or most preferably 100 %. The effectiveness of anolyte is completely analogous to that of disinfectant Virkon S. Significant advantages, however, are the ecological safety of anolyte and the low price.

In some of the tubes with growth was proved that it is not due to bacteria studied, but because of entrance and development of spores of bacilli. However, for complete accuracy taking into account the time required for achieving the bactericidal action of the tested substances, it is preferable to use liquid media with subsequent subcultures on solid selective media, as this avoids completely some residual effect of the anolyte and Virkon S after direct inoculation on solid medium after the detected period of time before being absorbed into the agar.

S. aureus ATCC showed a slightly higher susceptibility to anolyte and Virkon S compared with the other tested strain Kowan. The resistance of staphylococci, however, turns out to be higher than that of P. aeruginosa, which under the same conditions are killed within 2 min (Popova et al., 2016), while staphylococci - for 5-10 min. In previous our studies (Popova et al., 2016) have established experimentally high antibacterial activity of the freshly prepared anolyte, which in a concentration of 100 %, 50 % and 25 % kills for a short time (2 minutes) suspensions and of other Gram-negative bacteria: Salmonella enterica, Escherichia coli and Pseudomonas aeruginosa with concentration of 106 cells/ml and suspensions of Salmonella enterica with concentrations of 108 cells/ml. Tasheva et al. (2010) found antimicrobial action of electrochemically activated aqueous solutions (analytes) of alkaline and alkaline earth metal salts on field strains of Candida albicans, while in two of the solutions had been observed suppress the growth of fungi on the 15th minute from the start of their action. Obviously for inactivation of Gram-positive microorganisms such as staphylococci and Candida albicans is needed more prolonged exposure of the anolyte in comparison with Gram-negative bacteria. What is the period of retention of the antibacterial activity, however, is not known, although it is important from a practical point of view? This requires further research.

Conclusions

The anolyte at concentrations of 50 and 100 % inactivates the cells of S. aureus ATCC in suspension with density of 106 cells/ml within 5 min. Smaller concentrations (25 and 12.5 %) have a bactericidal effect after 10-minute exposure.

In all tested concentration (12.5 to 100 %) the anolyte has a bactericidal effect on the cells of S. aureus Kowan in suspension with concentration of 106 cells/ml in 10-minute intervals. Shorter intervals tested (2 min and 5 min) are not sufficient for achieving the bactericidal action even of the anolyte with concentrations of 50 and 100 %.

The effect of the control disinfectant Virkon S on both used staphylococcal strains is similar to that of the anolyte. In all tested concentration Virkon S inactivates S. aureus ATCC within 5 min. S. aureus Kowan shows less sensitivity in the tested concentration of 106 cells/ml and dies within 10 min.

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