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Antimicrobial activity of positively or negatively charged electro-aerosol solutions based on ammonium compounds

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Summary

Positively or negatively charged electroaerosols of quaternary ammonium salt solutions and uncharged aerosols were used for disinfection. Before disinfection, the premises were sprayed with Escherichia coli or Staphylococcus aureus bacterial cultures. The air was sampled at 0, 10, 30, and 60 minutes after disinfection. Our studies have shown that electro-aerosols made using Dezinfekt IV, which is based on ammonium compounds, are highly effective against S. aureus and E. coli. In addition, electro-aerosols with negative or positive charges were more efficient against Gr– and Gr+ bacteria in comparison to electro-aerosols without an electric charge. The particles with negative or positive charges had quite intensive interference, which leads to an equal distribution of the particles in the air, increased speed of movement of the particles and improved contact with bacteria.

Keywords: ammonium compounds, disinfection

Thermomechanical aerosol generators, disk aerosols, pneumatic electroaerosol devices (aerosol fractions are electrified with positive or negative charge) (10, 20), stream and ultrasonic sprayers as well as vapour generators (4) are used for disinfection of premises worldwide.

Aerosol disinfection with not-charged particles is suitable for air disinfection inside the building and particularly for the disinfection of horizontal surfaces. However it is not very much effective for the disinfection of vertical surfaces and hardly accessible places (for example, lower surfaces of various devices, piled trays, pipes and radiators). Electro-aerosol particles with any kind of charge precipitate and distribute on different types of surfaces more abundantly and evenly. It is already proved in practice that the fractions of electrified aerosol cover walls, ceiling and places which are more difficult to access 2-4 fold better compared to uncharged aerosol. The superficial encrustment with positively and negatively charged electroaerosol particles depends on concentration, size and dispersion speed of aerosol particles in the air. These parameters usually determine sedimentation and adhesion of particles on the surface of microorganisms (11). The concentration of aerosol particles in the air will directly influence the manner of distribution of them on the surfaces. Some authors indicate the fact (9), that electro-aerosol particles, which are electrified with negative charge, precipitate on a variety of surfaces 15-20% quicker and more evenly compared to uncharged particles. The recommended size of particles is between 10 and 20 μm. In order to get bigger particles circulating in the air, special ventilators are needed, because the formation of condensate starts otherwise.

The influence of antimicrobial disinfectant aerosol on the microorganisms might be ambivalent. Sprayed aerosol particles evaporate and vapours of disinfectants precipitate on the surface of the bacterium forming the capsule consisting of the disinfecting substance. After the precipitation on the bacterium surface the disinfecting substance penetrate inside the bacteria through its' capsule (8). The destruction of the cell surface is a typical mechanism of action for the quaternary ammonium compounds. Although the quaternary ammonium compounds, as antimicrobial and disinfecting substances, are often used (12, 13, 17, 20), there is no any information in the literature about their usage for disinfection in a form of electro-aerosol.

Escherichia coli and *Staphylococcus aureus* can be used just as control microorganizms for the evaluation of the disinfection quality, since both of them are common in the premises where animals are being held.

The purpose of this work is to evaluate the disinfecting activity of poly-disperse electro-aerosol of Dezinfektas IV solution with different charges and to compare this activity with the one of the aerosol without any charge.

Materials and methods

Disinfecting compound (Dezinfektas IV), which is used in the experiment, was made of alkyl dimethyl benzyl ammonium chloride (3.5%), didecyl dimethyl ammonium chloride (1.5%), chlorhexidine bigluconate (1.5%), nonionic emulsifier (1.5%), odorant eugenol (0.25%), ethyl alcohol 70% (up till 15%) and water. 3%, 5%, 10%, 20% and 30% of Dezinfektas IV solutions were used in the experiments. The disinfecting activity of Dezinfektas IV can be evaluated by testing the changes of the bacterial contamination in the air of the closed premises. Before the disinfection the premises were sprayed over with bacterial biomass, consistence made up 4 McF. Density of the biomass was determined with the help of colorimeter MCI-5 (Latvia). To spray over the premises the active biomass solutions of *E. coli* ATCC 25922 and *S. aureus* ATCC 25923 was used a general-purpose electro-aerosol device UEA-5 (9) with electro-aerosol atomizer UEP-2. The room disinfection was performed using 9-150 μm particle electro electro-aerosols with the help of electro-aerosol device UEA-5 and electroaerosol sprayer UEP-2. The electro--aerosol particles of the disinfectant were charged with positive and negative charges.

The evaluation of bacterial contamination of the air was performed using Krotov's device for bacterial sedimentation. An electric pump absorbs the air through the opening and passes it through the air flow measurer. It allows estimate the amount of air that passes by a Petri dish that is placed inside the device. The increased amount of colonies in the 1 m³ of the air is being calculated in accordance with the following formula: $h_b = (a \times 1000)/V$, where h_b – the number of the bacterium colonies in the 1 m³ of air, a – number of the bacterium colonies in Petri dish, V – the amount of air in litres, pumped through the device, 1000 – constant.

At the beginning of the experiment the measuring device was fixed so, that 30 litres of air were being pumped within a minute.

The air was sampled immediately after the bacteria have been sprayed (the pumping process took about 30 seconds). Dezinfektas IV solution (30 ml to m³ of air) respectively concentration was sprayed immediately after that. The air samples were collected immediately after the Dezinfektas IV solution was sprayed over the premises (the pumping process took about 30 seconds), then 10 minutes (the pumping process took about 60 seconds), 30 minutes (the pumping process took about 2 minutes) and one hour after the Desinfekta IV solution was sprayed over (the pumping process took about 4 minutes).

McConkey agar was used for the experiments with *E. coli*, and peptone meat agar containing 8.5% sodium chloride was used for the experiments with *S. aureus*. On the same day Petri dishes with the samples of tested air were placed into the thermostat for 24 hours with the temperature of 37°C. The results were evaluated by counting the number of grown bacterial colonies in Petri dish.

Results and discussion

Experiments with *Staphylococcus aureus*. A culture of *S. aureus* was sprayed into the hermetic room during the first experiment. Furthermore, the disinfection with 3% and 5% aerosol of Dezinfektas IV solution was performed in two experimental groups, while no disinfection was used after *S. aureus* was sprayed in a control group. Our results have shown, that after dispersion of 3% and 5% solution of Dezinfektas IV, counts of *S. aureus* dropped dramatically and were reduced by 99.8-100% and 99.7-100%, respectively. Because reduction of *S. aureus* counts in both solutions was comparable, only 3% solution of Dezinfektas IV was used in the second experiment.

The results from the second experiment are presented in tab. 1. *S. aureus* counts in the air were reduced gradually after spraying of microorganisms in a control group. Furthermore, comparable reduction of bacteria counts was registered in experimental groups with different charges (without charge, negative and positive) of Dezinfektas IV (tab. 1). It should be mentioned, that reductions of *S. aureus* counts continued for 10 minutes after disinfection and onwards. In all experimental groups where electroaerosol particles electrified with a negative or positive charge, or even not charged particles were used, high reduction (99.9--100%) of *S. aureus* counts were recorded ($P < 0.001$). In conclusion, the highest reduction of G+ bacteria *S. aureus* was registered when electroaerosol particles where electrified with negative charged or were not charged at all.

Experiments with *Escherichia coli*. A culture of *E. coli* was sprayed into the hermetic room during the first experiment. Furthermore, the disinfection with 5% aerosol of Dezinfektas IV solution was performed in experimental groups, while no disinfection was used after *E. coli* was sprayed in the control group. A higher concentration of disinfectant compared to the experiments with *S. aureus* was selected in the experiments with *E. coli* because *E. coli* are G– bacteria, which are more resistant to disinfection compared to G+ bacteria.

The results have demonstrated that after dispersion of 5% solution of Dezinfektas IV, *E. coli* counts were reduced by 85.8-91.5% ($P < 0.05$). In order to increase the efficiency of disinfection, the concentration of Dezinfektas IV was increased up to 10% and 20% in the second experiment. Highly significant (99.9%) decline compared to control group ($P < 0.001$) was observed, 10 minutes after disinfection with 10% or 20% solution of Dezinfektas IV in experimental groups with particles carrying different electric charge (negative, positive) as well as without charge. However, efficiency was reduced to 96.1-97.2% one hour

Tab. 1. Efficiency of Dezinfektas IV against *Staphylococcus aureus*

Mean (standard deviation) of colonies of *S. aureus* in 1 m^3 of air before disinfection	Concentration of Dezinfektas IV and charge of particles	Mean (standard deviation) of colonies of *S. aureus* in 1 m^3 of air after disinfection			
		0 minutes	10 minutes	30 minutes	60 minutes
1 061 400 (8533)	3% (without charge)	89 (22)	11 (11)	0	8 (8)
1 049 000 (20 866)	3% (negative charge)	22 (22)	33 (33)	6 (6)	5 (3)
1 115 400 (39 300)	3% (positive charge)	9 (22)	378 (11)	44 (11)	103 (19)
1 060 644 (50 044)	Control	–	432 322 (15 978)	146 128 (13 305)	25 656 (6697)

Tab. 2. Efficiency of Dezinfektas IV against *Escherichia coli*

Mean (standard deviation) of colonies of *E. coli* in 1 m^3 of air before disinfection	Concentration of Dezinfektas IV and charge of particles	Mean (standart deviation) of colonies of *E. coli* in 1 m^3 of air after disinfection			
		0 minutes	10 minutes	30 minutes	60 minutes
1 436 400 (218 833)	30% (without charge)	778 (78)	144 (28)	61 (53)	25 (12)
1 297 222 (7622)	30% (negative charge)	0	11 (11)	17 (8)	81 (19)
1 264 800 (6866)	30% (positive charge)	67 (0)	0	6 (6)	14 (10)
1 223 000 (35 900)	Control	–	620 945 (8928)	102 117 (13742)	44 167 (2501)

after disinfection. The concentration of Dezinfektas IV used for disinfection against *E. coli* was increased up to 30% in the third experiment.

The results from the third experiment are presented in tab. 2. The concentration of *E.coli* in the air after spraying of microorganisms fluctuated, however, high concentrations persisted throughout the experiment in control group (tab. 2). Disinfection with 30% of Dezinfektas IV solution resulted in 99.3-100% efficiency against *E. coli* bacteria compared to the controls ($P < 0.001$). The highest efficiency was registered in groups where particles with negative and positive electric charge were used. Ten minutes, 30 minutes and 60 minutes after disinfection a 99.9% decline of *E. coli* counts was recorded compared to the control group, respectively ($P < 0.001$).

The disinfection of air and horizontal surfaces using aerosols is quite common and effective. It was suggested, that electro-aerosol particles with negative charge precipitate and distribute on different types of surfaces and places with restricted access (11). Our studies have demonstrated, that electro-aerosols of Dezinfektas IV solution, which is based on ammonium compounds, are highly efficient against *S. aureus* and *E. coli*. In addition, electro-aerosols with negative or positive charge where more efficient against Gr– and Gr+ bacteria in comparison to electro-aerosols without electric charge.

The charge of the bacterial cell wall is created by carboxyl, phosphorile and amino groups. The degree of dissociation of these anionic and cationic complexes depend on the electrolytes, surrounding bacteria, and pH of the environment (8). The majority of bacteria possess negative charge of various degree, because the number of groups of carboxyl and phosporyl is higher compared to the number of the amino groups (1, 2, 15). Furthermore, the positive charge posses only a few bacteria, i.e. *Stenotrophomonas maltophilia* and *Streptococcus thermophilus* (14, 15). Gr+ bacterium wall has negative charge and is hydrophilic, because thr wall has teichoic acid. Bacteria cell wall contains 2-5% of lipids. The main cell wall components are mucopeptides. The external layer of Gr– bacteria is formed from lipopolisaccharide (LPS) and proteins which are responsible for formation of the high negative charge thus making bacteria resistant to the influence of cationic complexes (16, 19). There are two forms of LPS: hydrophobic (A form) and hydrophilic-electrostatic (B form) (5, 17, 18). For instance, wall capsule of *Sallmonella* contains 22-25% of lipids, when *E. coli* capsule contains 40% of lipids. The speed of penetration of different substances into cytoplasm membrane is reduced due to the binding of quaternary ammonium compounds to the lipids of the bacteria wall. In addition, the amount of active substances, which usually get into the contact with cytoplasm membrane, significantly increases. Quaternary ammonium compounds are free from the contact with peptidoglucans, which the basic component of Gr+ bacteria walls. The active substance of quaternary ammonium compounds without any obstacles penetrate trough the bacteria wall and interacts with cytoplasmic membrane without any destruction of their integrity.

Negative charge of bacteria can be reduced or sometimes might be reversed to a positive charge using a special pharmacological substances (6, 7). The change of charge can be caused by the charged electro-aerosols of cationic substances solutions.

In the literature there is contradictory information regarding different bacteria wall charge. Some authors have pointed, that the electric charge has no influence on the bacterial interactions with the different materials (3, 14). However, the others have stated, that negative charge (18) of particles might increase adhesive properties of bacterial wall. The majority of authors have agreed, that adhesion of bacterium is reduced when particles carry on negative charge and adhesion increases in case of reduction of negative charge or appearance of the articles with positive charge. It is predicted by the rule of heterogenous charges, that two different charges must attract each other. However, it might be exceptions. For instance, bacterium and throat epithelium cells usually have the same charge (16). Our results support the hypothesis regarding the role of the negative charge of bacteria, because negatively charged electro-aerosol solution had slightly better contact with Gr– or Gr+ bacteria compared to positively charged electro-aerosol.

Finally, it can be suggested that electro-aerosols with any charge are more effective in presence of bacteria in the air. While evaluating the results main attention has been paid to the efficiency achieved one hour after disinfection. It was estimated, that the growth of Gr– *E. coli* was reduced by the disinfectant with negatively charged particles, while Gr+ *S. aureus* growth was suppressed by negatively and without charged disinfectant particles. The particles with negative or positive charge have quite intensive interference, which leads to more equal distribution of the particles in the air, to increased speed of particles movement and to improved concact with bacteria. This is in concert with results (18) that surfaces of the different objects usually have a marginal negative or positive charge, which improves activity and sedimentation of electro-aerosol particles with a different charge and inactivation of microorganisms.

Our preliminary results on the disinfection of vertical surfaces using Dezinfekt IV electro-aerosols have demonstrated 78.1% efficiency.

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VICCA J., MAES D., JONKER L., DE KRUIF A., HAESENBROUCK F.: Skuteczność tylozyny stosowanej jako dodatek do paszy w leczeniu i zwalczaniu zakażeń wywołanych przez *Mycoplasma hyopneumoniae*. (Efficacy of in-feed medication with tylosin for the treatment and control of *Mycoplasma hyopneumoniae* infewctions*).* Vet. Rec. 156, 606-610, 2005 (19)

Skuteczność tylozyny w enzootycznym zapaleniu płuc oceniono u odsadzonych prosiąt zakażonych eksperymentalnie dotchawicowo *Mycoplasma hyopneumoniae*. Antybiotyk stosowano jako dodatek do karmy. Dawka zakaźna wynosiła 7×10^7/7ml podłoża hodowlanego. Grupa 10 prosiąt otrzymywała antybiotyk przez 21 dni, począwszy od 12. dnia po zakażeniu w ilości 100 mg/kg karmy. Grupa 10 nieleczonych prosiąt stanowiła kontrolę. Trzecia grupa licząca 10 prosiąt otrzymała w iniekcji jałowe podłoże hodowlane stosowane do namnażania *M. hyopneumoniae*. Wszystkie prosięta obserwowano przez 33 dni po zakażeniu, a po tym okresie poddano eutanazji i oceniono nasilenie oraz charakter zmian w płucach, wykonano posiewy oraz test immunofluorescencji na obecność *M. hyopneumoniae*. Wystąpienie zaburzeń ze strony układu oddechowego i nasilenie zmian w płucach były istotnie wyższe u prosiąt zakażonych eksperymentalnie w porównaniu z kontrolą. Leczenie w sposób istotny obniżało nasilenie zmian chorobowych w płucach. Po 33 dniach po zakażeniu serokonwersja wystąpiła u 80% zakażonych i nieleczonych prosiąt, u 70% zakażonych i leczonych zwierząt. Serokonwersja nie wystąpiła u prosiąt niezakażonych.

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