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Disinfectants Effect On Microbial Cell.

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ABSTRACT

The literature review focuses on the main chemical disinfectants used in animal husbandry and poultry breeding. The article considers chlorine-containing and iodine-containing preparations, formaldehyde, alkalis, hydrogen peroxide, surfactants. The mechanism of the listed chemicals effect and the changes occurring in a microbial cell are described.

Keywords: animal husbandry, poultry breeding, bacterial safety, microbial cell, disinfection, chemical disinfection, bactericides.

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REVIEW

Bacterial safety is one of the key factors in the effective prevention of infectious diseases in animals and birds, which plays an essential role [1].

Disinfection is defined as a set of measures aimed at the destruction of infectious agents to prevent the spread of infection [1,2, 38]. The main purpose of these measures is to break the epizootic chain by affecting its most important link – the factors of transmission of the pathogen from the source of infection to the susceptible organism [4; 5, 6].

In this regard, effective and safe disinfectants are necessary to prevent or at least reduce the accumulation of microflora in livestock and poultry houses [7, 8].

Pathogenic bacteria resistance to the effects of disinfectants depends on the characteristics of the chemical used (concentration, duration, etc.), and to a large extent on differences in the ultrastructural organization of bacteria [9].

Resistance of bacterial cells depends on the type of microorganisms, features of the structure and permeability of cell walls, the stage of their development, the number of lipids that protect them from the adverse effects of many chemical factors [32]. Spore shape or formation of capsule in bacteria contribute to the increasing resistance of microbial cells to the action of chemical agents, while vegetative forms of cells are harmful and has low toxicity chemicals.

The essence of the impact of bactericides on microorganisms is reduced to various kinds of reactions between the organism and the chemical substance. However, the destruction of the pathogen from a chemical disinfectant is primarily associated with the reactions that occur between the disinfectant and the protein of the microorganism.

Chemical disinfectant, located in the solution, while contacting the microbial cell, either is adsorbed by it, or penetrates it, and then to some degree it combines to the substances that make up the cell. The rate of penetration of the chemical is affected by the greater or lesser capacity for dissociation: the sooner and more completely is the chemical dissociated, the faster it penetrates into the cytoplasm and the greater is its destructive effect [10].

Disinfectants are different in their chemical nature, and penetrating into the cells, they have a different selective effect. Observations of many authors explain the mechanism of action of disinfectants on microorganisms in different ways. Studies have shown that certain groups of the base disinfectants have different effects on microbial cell [2; 12; 13, 14]. Thus, oxidants (chlorine, chlorine products, hydrogen peroxide) react with proteins of the cell, causing oxidation reaction. Mineral acids and alkalis, acting together with hydrogen and hydroxyl ions, cause hydrolysis. Phenolic drugs cause a coagulation reaction of the cell proteins. This schematic concept of the functioning mechanism does not explain all the complex ways of affecting the microbial cell, for example, the influence of disinfectants on enzymatic activity (breathing, nutrition, growth, etc.).

The analysis of the functioning mechanisms of disinfectants on microbial cell is a necessary condition for the development and improvement of new disinfection modes [9].

The study of Pavlova I.B. in 1966-1999 and Kulikovskii A.V. in 1969-1989 of ultrathin sections of cells of pathogenic bacteria under the influence of disinfectants using the method of transmission electron microscopy was the basis of these studies. This method allows studying the influence of preparations on certain structures of the isolated bacterial cell.

Electronic microscopic analysis of pathogenic microorganisms for animals and birds allowed to get a clear idea about their structure. Thus, according to I.B. Pavlova et al., gram-negative microorganisms (pathogens of colibacillosis, salmonellosis, brucellosis, etc.) have a three-layer cell wall, a cytoplasmic membrane located beneath it, a granular cytoplasm and a thin-fibrillar nucleotide. These bacteria fission by simple splitting. It has been established that the cell wall of gram-negative microorganisms comprises 6-9% of

the dry cell mass and consists of a large number of lipoproteins (up to 80%), 20-40% of them are lipo-saccharides and phospholipids.

Gram-positive microorganisms (staphylococci, streptococci, pathogens of listeriosis, swine erysipelas, etc.) usually have a homogeneous, thicker cell wall than gram-negative ones. It is possible to observe the capsule element on the surface. There are well-developed complexes of membrane structures in the bacteria cytoplasm, and the cells fission by forming a transverse partition. The cell wall of gram-positive microorganisms comprises 20 % of the dry cell mass and consist mainly of mucopeptides (up to 50 %)[35].

Cytoplasmic membrane consists of two protein layers, and a bimolecular layer of lipids between them. Proteins comprise 60-65%, lipids - 30-35%, and carbohydrates - 2%. Lipoprotein complex reaches 90% of all the chemical compounds that make up the membrane. The membrane is important in maintaining the osmotic barrier, protein synthesis, cell fission, toxigenesis and other vital processes in microbial cells [9].

Bacteria spores are of particular importance. It is known that they are resistant to physical and chemical factors and can survive for a long time in the environment [14; 15]. Nine morphological stages in the formation of spores (for example, Bac. cereus) have been studied and described. Mature spore has exosporium, multi-layered spore membrane, outer membrane, cortex and protoplast enclosed in spore-plasmatic membrane. It has been established that the spore shell of *Bac. cereus* comprises 50% of the total volume of spores, and it consists of about 3% of ash, 3% of phosphorus, 3% of lipids and from 35 to 80 % of protein.It is established that the spore shell of you. Cereus reaches 50% of the total spore volume, and consists of about 3% of ash, 3% of phosphorus, 3% of lipids and about 35 to 80% of protein.

Tuberculosis mycobacterium are paid special attention among the microorganism. In terms of resistance to disinfectants, they are superior gram-negative and gram-positive bacteria and concede in this respect only spores. High resistance of the tuberculosis pathogens in the environment is explained by the high content of lipids in the cell walls of mycobacteria [9].

Chlorine substances - active substances with a broad spectrum of bactericidal and virucidal action, well dissolved in water, but they are corrosive to the treated surfaces and lose their active propertiesquickly during storage and use, therefore, as a rule, they are used only once [12;16; 17; 18]. Chlorine-containing preparations include: chlorine, chlorine lime, chloramine, hypochlorites and other. They are strong oxidizers. Oxidation is one of the most important chemical methods of damaging effects on microbial cells. When chlorine comes into contact with the moisture contained in the microbial cell, hydrochloric and chloric acids are formed. Oxygen released thus oxidizes cell components [3,19; 37].

Iodine is known as one of the most common disinfectants. Among all the iodine compounds, iodine complex with a carrier is the most widely used for disinfection, for example, a complex with polyvinylpyrrolidone or ethoxylated non-ionic detergents, which may be presented as a reservoir of constantly released molecular iodine. The exact mechanism of the antimicrobial activity of iodine has not been studied yet. It is assumed that it reacts with amino acids and fatty acids, destroying cell structures and enzymes [3]. Iodine preparations have an pronounced antibacterial, antiviral and antifungal effect, but do not have sufficient activity against bacteria spores [20].

Aldehydes are widely used for disinfection of livestock and poultry premises [33, 39]. The main known representative of aldehydes is formaldehyde with pronounced antimicrobial properties, including activity against all types of microorganisms due to alkylation of amino and sulfhydryl groups of proteins and suppression of the synthesis of the latter.

More than half a century since the first discoveries until today, most modern farms continue to use formaldehyde for the vast majority of disinfection-related activities. Of course, it is difficult to overestimate the disinfectant power of formalin. It is a really powerful and effective method of disinfection [21; 22].

Formaldehyde solutions have a detrimental effect on the spore forms of microbes, as well as on non-spore-forming microorganisms, viruses and fungi. Anthrax spores when exposed to 1% formaldehyde solution perish after 24 hours, 3% - after 5 hours, 5% - after 3 hours. [22, 34].

The mechanism of action of alkalis largely depends on the object and the properties of the environment in which the object is located. Protoplasm of living cells under the influence of alkalis is undergoing significant changes due to the increase in pH of the medium are hydrolyzed proteins formed colloidal particles, fats are saponified and carbohydrates are splintered. Thus, the bactericidal activity of alkalis depends on the group of ions, for example, most of the sodium hydroxide ions interact with the cell membrane, and due to the fact that the membrane contains 22% of lipids [9], here occurs fat saponification take place here, which manifests itself in the destruction of the cell wall [23].

In the harmful effects of acids on the bacterial cell E. coli and St. aureus surface structures break down, and the content of the cell is released. The penetration of H ions of the acid into the cell is implementing by the type of diffusion of the ion binds to the cell wall, but this binding capacity decreases with decreasing pH, in this regard, autolytic capacity of the cell activates. This leads to a "smearing" of the ribosomes of the cytoplasm [23].

Oxygen-containing products, in particular hydrogen peroxide, are strong oxidizing agents which form free radicals that damage lipid cell membranes, DNA and other important components of microbial cells. Despite the production of catalase by many microorganisms, which protects cells from exposure to hydrogen peroxide by decomposition into water and oxygen, the concentrations of H_2O_2 used in disinfection allow in most cases to overcome this resistance mechanism. However, in its high concentrations in the framework of such positive qualities as a wide range of activity, including bacteria spores, the ability to dissolve biological substances, odourless, rapid decomposition into non - toxic products in the external environment, there are some negative qualities-high tissue toxicity (II class) with a pronounced local irritant effect. When using peroxide, it is necessary to follow the instructions for their use clearly, since they are very aggressive at high concentrations. [3; 25].

While the study of the ultrastructure of *S. typhimurium* cells exposed to hydrogen peroxide it has been found that the substance causes significant destruction of the outer membrane of the cell wall, cytoplasmic membrane and ribosomes. The effect of hydrogen peroxide on *S. aureus* in the first minutes causes an increase in the cell volume, due to the active supply of activated oxygen and water, leading to hydration and an increase in the volume of the bacterial cell. With further contact of hydrogen peroxide with bacterial cells, there is a local violation of the integrity of the cell wall, cytoplasmic membrane and ribosomes. The main mechanism for the action of hydrogen peroxide on gram-negative and gram-positive bacteria, leading to inactivation, is the impact of activated oxygen, which, interacting with lipids and lipoproteins, induces the formation of toxic peroxides, causing oxidation and destruction of the structure of membranes and cell proteins [9; 36].

The natural response of the bacterial population to the impact of abiotic factors is the destruction of intercellular matrix and covers in colonies, violation of the integrity of cell walls, which leads to heteromorphism of cells with manifestations of L-transformation and the formation of stable or unstable L-forms. The study of structural and functional changes in bacterial cells and biochemical properties contributes to the deliberate development of new antibacterial products, as well as scientific validation of their use [9].

Currently, the most effective antibacterial properties have the substances that cause destruction of cell surface structures, as well as substances that violate the structure of ribosomes and DNA. These substances can be attributed primarily to the composition based on surface active agents (surfactants), which literally "undress" bacterial cells in the population, making them the most vulnerable to any impacts. Further impact on the population of bacterial cells depends on the effective beginning in the composition of the disinfectant, concentration, time of action, etc.

Distinctive properties of surfactantsis their minimum aggressiveness and toxicity, and rather expressed bactericidal, virucidal and fungicidal activity [26; 27; 28; 29].

Surfactants are divided into cationic, anionic, ampholytic and non-ionic. Only cationic and ampholytic [3] are used as self-disinfectants. Cationic surfactants are quaternary ammonium compounds (QAC). QAC impact on susceptible bacterial cells takes place in several stages: adsorption of QAC molecules to components of the cell wall and penetration through it; the interaction with phospholipids of the cytoplasmic membrane

followed by its disruption; release of intracellular low-molecular substances; protein and nucleic acid degradation; cell wall lysis caused by autolytic enzymes [30]. Sub-bactericidal concentrations of QAC cause less profound changes in the structure of the cytoplasmic membrane macromolecules, which manifests itself in violation of its functions (increased permeability, changes in osmotic pressure, disruption of transport through the membrane of molecules and ions, inhibition of metabolic processes and biological oxidation, inhibition of cell division regulated by mesosomes).

The QAC effect on mycobacteria is limited to inhibition of growth, on spores - inhibition the development of sprouting spores, but not the process of germination. Even a high concentration of the QAC do not have the sporicidal effect, although it can be achieved using this group of disinfectants at high temperature [31].

Thus, the analysis of the literature data helps us to conclude that the nature of the impact of different groups of chemicals on microbial cells is different, submicroscopic structure and functions of the cell are destroyed: autolysis, lysis, coagulation, denaturation, saponification and other changes take place.

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