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The hybrid nanomaterial PVA/AgNps, as biologically active product with reserved space in the antimicrobial therapy

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ABSTRACT

Increased microbial resistance, the emerged pan-resistant strains and the lack of approved alternative for treatment of such pathogens are urgent issues that cannot be delayed according to microbiologists, pharmacists and doctors. One of the main advantages of the metal nanoparticles converted in their major deficiency. The fact that they cannot differentiate pathogen from not pathogen bacteria make them difficult, but applicable for targeted therapy. The most prescribed antibiotics, nowadays, are the same, aren't they? Although nanobiomaterials exert their active action by multiple not clarified yet mechanisms, it is clear that their nano-sizes give them an advantage over all known antibiotics so far. However, there are already reports of development of resistance or accurate indifference towards nanobiomaterials. The mechanisms of resistance to antibiotics and whether they are the same for nanobiomaterials will be examined. Can the antibiotics by using the nanobiomaterials harness the forces in the fight against life-threatening pathogens? What is the type of interaction between them and can it be successful?

Keywords: *antibiotics, antimicrobial resistance, hybrid nanomaterial, silver nanoparticles, PVA/AgNps.*

1. INTRODUCTION

Antibiotics are the only currently approved form of treating infections both in hospitals and in the community. Collectively named, these include antimicrobial agents for the treatment of both bacterial and fungal infections. They are also used in complications after viral infections. The increasing antibiotic resistance, the impotence of antibiotics against viral infections, as well as causing many side effects are some of the reasons increasingly for treatment to be prescribed alternative products instead antibiotics. Their application, however, is quite limited in severe invasive and systemic infections. Because of this, great hope is assigned to the developments related to the success of nanotechnologies when deciding the issues of antibiotic resistance.

We will dwell on a brief overview of the antibiotics and their mechanisms of resistance and will then demonstrate the established properties and fields of application of a well-studied physico-chemical, microbiological and cytological hybrid nanomaterial. This will answer the question whether such developments future alternatively to antibiotics in multi- and pan-resistant pathogens.

2. THE BASIC CHARACTERISTICS OF ANTIBIOTICS

Microorganisms have existed on Earth for more than 4 billion years and exhibit the greatest genetic and metabolic diversity. They are an essential component of the biosphere and serve an important role in the maintenance and sustainability of ecosystems. Killing microorganisms is relatively simple as long as it does not have to be done selectively. They can be killed by heat, radiation, strong acids, etc. To target them specifically, without damaging the host cells and tissues, is much more difficult. Antibiotics are molecules that stop microbes, bacteria, viruses and fungi from growing, or kill them outright.

The era of antibiotics began with Paul Ehrlich, who first coined the term "magic bullet", chemical substance for the treatment of bacterial infections. He discovered the first antibiotic in 1910, salvarsan, for the treatment of syphilis. Ehrlich is a follower of Alexander Fleming, who discovered penicillin in 1928.

Antibiotics that stop the bacteria from growing are bacteriostatic, exemplified tetracycline and antibiotics that cause the death of bacterial cells are bactericidal as penicillin. However, the difference is not exact and depends on the amount of the drug, the type of bacteria and the growth phase. Antimicrobial drugs work better against actively growing bacteria than persistent agents or spores. Antibiotics can have a wide and narrow range of action. For example, ciprofloxacin, a broad spectrum antibiotic, active against Gram (+) bacteria, Gram (-) bacteria; such as vancomycin, which has a relatively narrow range and can be used mainly against Gram (+) microorganisms. Fidaxomicin has an even narrower range, and can be used only for Clostridium difficile. Antibiotics act by inhibiting certain vital bacterial processes of the bacterial cells or metabolism. On this basis, we can divide them into five main categories:

1. Cell wall inhibitors, such as: penicillin and vancomycin;
2. Inhibitors of nucleic acid synthesis, such as: fluoroquinolones that inhibits DNA synthesis and rifampin that inhibits RNA synthesis;
3. Protein synthesis inhibitors, such as: aminoglycoside;

4. Anti-metabolites, such as: the sulfa drugs;

5. Antibiotics that can damage the membrane of the cell, such as: polymyxin B and daptomycin.

2.1. Beta-Lactam Antibiotics.

They are bactericidal by inhibiting the construction of the bacterial cell wall by interfering with peptidoglycan synthesis. Bacterial elements that are affected by beta-lactam antibiotics are called penicillin-binding proteins (PBPs). There are various types of PBPs, differing in their function, the amount and affinity to beta-lactams. These antibiotics are active mostly against dividing bacteria, and are not effective against germs without a cell wall containing peptidoglycan (mycoplasma, chlamydia, and rickettsia). Many beta-lactam antibiotics are unstable in acid and decompose with gastric juices. Absorption of beta-lactams in the gastrointestinal tract is limited. The majority of the beta-lactams is obtained only in a parenteral formulation. Esterification of the original drug is sometimes done to facilitate absorption; these esterified beta-lactams should be administered with food. Beta-lactam antibiotics act primarily in the extracellular space. The penetration through biological barriers is different and can sometimes change with a higher dose. Intracellular penetration of beta-lactam antibiotics is good, but they quickly leave this space and do not act on intracellular microorganisms. The majority of beta-lactam antibiotics are removed by the kidneys, but there are exceptions (ceftriaxone, oxacillin, cefoperazone). The half-life of beta-lactam antibiotics is very short and ranges from half an hour (cephalothin, penicillin) to 2-2.5 hours. Ceftriaxone has exclusive half time (8 hours), which allows for an administration once a day [1].

The effect of the beta-lactams depends on the "time above MIC". The aim of the dosing is to maintain the level of an antibiotic at the site of infection above the MIC as long as possible. The maximum concentration is not very important. In mild infections, the level of a drug is sufficient and exceeds the MIC with 40-50% of the dosing interval [2].

Beta-lactams perform only short or no post antibiotic effects, except carbapenems. Beta-lactam antibiotics are not toxic and have a minimum concentration dependent on adverse effects. The degree of dosing is extremely high, especially penicillin's. The most important side effects are allergic reactions of varying intensity (mainly caused by penicillins), phlebitis, during intravenous administration (hyperosmolar solutions) infiltrates, and local pain, during intramuscular administration, and thrombocytopenia (mainly during cephalosporin treatment). Broad spectrum antibiotics lead to dysmicrobia including pseudomembranous colitis.

Beta-lactam antibiotics can be used in lactating or pregnant women and in newborns.

Beta-lactam antibiotics are mostly used for the treatment of acute infections profoundea well tissue or for the treatment of sepsis. Some drugs are also appropriate for the prophylaxis surgically.

More frequent dosing is recommended to reach a powerful effect. Enlarged doses of the individual, where it is indispensable for the penetration site of the infection, has been problematic.

2.1.1. Penicillins.

The set can be divided into four subgroups:

1. Natural penicillins, which have narrow spectrum containing S.pyogenes and other streptococci, pneumococci, meningococci, gram-positive rods (*L.monocytogenes*, corynebacteria), spirochetes (*Treponema* spp., *Leptospira* spp., *Borrelia* spp.), and some anaerobes (*Actinomyces*, peptostreptococci, clostridial spp.).

2. Anti-staphylococcal penicillins. They are resistant to staphylococcal beta-lactamases, but not to other beta-lactamases produced by microbes. They have a more narrow spectrum of activity than the natural penicillins; methicillin, nafcillin, oxacillin, cloxacillin, dicloxacillin

3. Aminopenicillins. The aminopenicillins were the first penicillins, discovered to be active against gram-negative bacteria (Escherichia coli, Salmonella enterica, Shigella sp., Proteus mirabilis, Helicobacter pylori, or Haemophilus influenzae) and they are acid-resistant so are administered orally. These agents are susceptible to inactivation by beta-lactamases. They are more effective than natural penicillin against enterococci and listeriae.

4. Extended Spectrum Penicillins include both alpha-carboxypenicillins (carbenicillin and ticarcillin) and acylaminopenicillins (piperacillin, azlocillin, and mezlocillin). These agents have similar spectrums of activity as the aminopenicillins but with additional activity against Pseudomonas aeruginosa. They are also susceptible to inactivation by beta-lactamase.

The beta-lactam inhibitors are structurally similar to the beta- lactam antibiotics, but they do not show any antibacterial activity. When administered along with penicillin in a patient with infection, due to beta- lactamse producing organisms, they prevent the degradation of the penicillin by this enzyme. These beta-lactams inhibitors are clavulanic acid, sulbactam and tazobactam. They are bound to the susceptible beta- lactamases, especially penicillinase, and prevent the hydrolysis of penicillin [2].

The mechanism of action of cephalosporins is similar to penicillins, since they are bound to the specific cephalosporin binding proteins present on the bacterial surface. Like the penicillin's they inhibit the bacterial cell wall synthesis by inhibiting the transpeptidation of the peptidoglycans. These activate the autolysin enzyme, present in the bacterial cell wall, which results in the lyses of bacteria. Hence, like the penicillins, cephalosporins are also a bactericidal drug. The introduction of different side chains in the cephalosporins nucleus results in compounds with varying a pharmacokinetic profile and a different antibacterial activity [1].

2.1.2. Cefalosporins.

For ease, they have been divided into "generations" that mostly correlate with their spectrum of activity, with some exceptions.These drugs were isolated from fungi and were known, early in their history, to be effective for microorganisms that produced beta-lactamases. Like penicillins, the cephalosporins tied to penicillin-binding proteins and inhibit the building of peptidoglycan and therefore damage cell wall synthesis. They are concentration-dependent on antibiotics and optimum results are get if they have levels above the MIC (minimal inhibitory concentration) of the organism at all times. Cephalosporins unlike the penicillins, have no activity against enterococci, and with the exception of cefoxitin and cefotetan, these drugs do not have activity versus B. fragilis.

First generation cephalosporins was marketed for their capability to treat S. aureus which had get resistant to penicillin. In addition to gram positive organisms, but, the spectrum of cephalothin includes some gram-negative organisms, especially E. coli and Klebsiella spp.. The spectrum involves most organisms killed by penicillin (Streptococcus pyogenes, viridans streptococci, and S. pneumoniae).

Cefazolin, due to its gram-positive and gram-negative spectra, is one of the most commonly used antibiotics. Cefazolin is often used to treat skin infections. It is often used in place of penicillin in the event of patients with a history of penicillin allergy. Because of its relatively long half life and ease of management, cefazolin is usually used as a prophylactic agent to prevent surgical infections. In this situation the antibiotic is administered foregoing to surgery.

The second generation cephalosporins includes the real cephalosporins (cefamandole, cefuroxime), as well as the cephamycins (cefoxitin, cefotetan). The cephamycins, chemically and pharmacologically similar to the cephalosporins, are produced from the bacterium Streptomyces, as opposed to the true cephalosporins which are produced from the fungus Cephalosporium acremonium. Cefoxitin and cefotetan have an more methoxy group in the beta-lactam ring . This makes these drugs poor sensitive to the beta-lactamase produced by anaerobes like B. fragilis. For this why they can be used to treat abdominal infections including situations in which B. fragilis is a major ingredient of the infecting flora. Notwithstanding the fact that these agents have a broader gram-negative spectrum, including more activity against most E. coli, Klebsiella spp., and Proteus spp., their activity on staphylococci and streptococci is reduced. The real second generation cephalosporins (cefamandole and cefuroxime) have activity against a wider spectrum of Enterobacteriaceae than the first generation cephalosporins, and they also have activity on H. influenzae. They are used to treat intra-abdominal, gynecological infections, foot infections in diabetics and other blended aerobic-anaerobic infections. Attention, resistance to cefoxitin take up fast in places where this drug used to be given regularly.

The third generation cephalosporins presents a major progress in the treatment of meningitis. As opposed to previous generations of cephalosporins, these drugs have the capacity to pass the blood–brain barrier. This effect in their rroutine use as a first-line agent for bacterial meningitis of they have activity on S. pneumoniae, H. influenzae and N. meningitidis. Given their long plasma half-life (5–8 h), Ceftriaxone can be given as a single daily dose for many infections (the exception being meningitis where it is usually given at maximum doses: 2 g IV q12h for adults). Cefotaxime also has good CSF penetration but must be given every 4 h for best action in meningitis.

Ceftriaxone, ceftazidime and cefotaxime all they are not optimal medications for treating Pseudomonas aeruginosa. A modification of the side chain to making ceftazidime effect in more activity against P. aeruginosa . Unfortunately, this variation results in poor affinity for the penicillin-binding proteins of staphylococcus, issue in poor activity on staphylococcus. Since of its wide spectrum of activity, ceftazidime has been used often for cure of infections in neutropenic patients – mostly people by acute leukemia actively receiving chemotherapy.

Cefoperazone also has activity against P.aeruginosa and have unique feature to prevalent excretion via the bile (its use in hepatobiliary tract infections and in renal failure). Cefoperazone is available in a compound with beta-lactamase inhibitor - cefoperazone/sulbactam, that can be appropriate against Acinetobacter spp.

Fourth generation cephalosporins have a broad spectrum of activity. They are not a substrate for some powerful beta-lactamases. Yet, their activity on staphylococci is not better than with cephalothin and activity on P.aeruginosa is comparable to ceftazidime, cefepim - these drugs are used in nosocomial infections of unknown origin where covering the broad spectrum of pathogens is necessary.

Ceftaroline is a new cephalosporin with activity against Methicillin-resistant Staphylococcus aureus and resistant S. pneumoniae . It shows efficacy in the cure of complicated skin and soft tissues infections where MRSA is a probably pathogen and in community-acquired pneumonia where resistant S. pneumoniae is a potential pathogen.

2.1.3. Carbapenems.

The carbapenems are beta-lactam agents but differ from penicillins by the exchange of a carbon atom for a sulfur atom and the supplement of a double bond to the ring of the penicillin nucleus. Meropenem, imipenem, ertapenem, and doripenem are most commonly used in practice members of this group. Carbapenems are produced from Streptomyces cattleya and proposal a broader spectrum of activity than more other beta-lactam drugs. Such as other beta-lactams, the carbapenems tie up to penicillin-binding proteins, destroy bacterial cell wall synthesis and dispatch susceptible microorganisms. They are not a substrate for to hydrolysis by most beta-lactamases.

2.1.4. Mechanisms of resistance to beta-lactams.

The beta-lactamase are one of the most commonly used antibiotics because of their ready availability and relatively low cost. The beta-lactam ring is important for the activity of these antibiotics which results in the inactivation of a set of transpeptidases that catalyze the final cross-linking reactions of peptidoglycan synthesis in bacteria. The effectiveness of these antibiotics relies on their ability to reach the penicillin-binding protein (PBP) intact and their ability to bind to the PBPs. The completeness of chemical structure is basic for antibiotic activity. Unfortunately, several type of enzymes give resistance by targeting and slash chemical bonds that are hydrolysis inclined. For example, it is the amidases that slash the β-lactam ring of the penicillin and cephalosporin classes of drugs. Resistance to beta-lactams in many bacteria is usually due to the hydrolysis of the antibiotic by a beta-lactamase or the modification of PBPs or cellular permeability.

There are two basic classes of β-lactamases found on the molecular mechanism of hydrolysis of the β-lactam ring: (1) Ser-beta-lactamase, that work through the action of a Ser nucleophile active site and (2) metallo-beta-lactamases that activate water through a Zn_2+ center.

2.1.5. Glycopeptides.

Glycopeptide antibiotics are a class of drugs, consisting of glycosylated cyclic or polycyclic peptides. Clinically helpful glycopeptides include vancomycin, teicoplanin, telavancin and dalbavancin. Vancomycin is a isolated in 1956 from Streptomyces

orientalis. It is active drug gram-positive bacteria including those resistant. Teicoplanin is a glycopeptide with a comparable spectrum of activity to vancomycin, but to be a little more active *in vitro* than vancomycin against staphylococci and streptococci.

Glycopeptide repressed bacterial cell wall building and show concentration-dependent bactericidal activity. They inhibit peptidoglycan polymerase and transpeptidation reactions by making a complex with the terminal d -alanyl- d -alanine portion of the peptide precursor units. The spectrum of activity of vancomycin includes all Staphylococcus spp., Corynebacterium spp., Clostridium difficile, Enterococcus spp. , Streptococcus spp.

The medicaments are not absorbed from the gastrointestinal tract. Influx in biological barriers is bad. Glycopeptides carry out post antibiotic effect of about 2 hours. Crucial antibiotics for the treatment of heavy gram-positive infections as sepsis, endocarditis, nosocomial pneumonia.

Adverse effects of vancomycin include fever, ague, rash and phlebitis at the site of infusion. Redness due to histamine release "red man syndrome" often get after fast intravenous administration. Vancomycin can be administered orally if when we treat colitis caused by Clostridium difficile.

Dalbavancin and telavancin are structurally similar to vancomycin. Like vancomycin, telavancin suppress bacterial cell wall synthesis and destroy bacterial membranes by depolarization.

The high-level resistance to vancomycin is encoded by the vanA gene that results in the production of VanA, a novel D-Ala-D-Ala ligase resulting in the rebuilding of the peptidoglycan side chain to express D-alanyl-D-lactate type which has less affinity for glycopeptides [3]. There are also other proteins in this gene cluster that are necessary for resistance, including VanH and VanX, as well as VanB which confers moderate levels of resistance to vancomycin and susceptibility to teicoplanin. Vancomycin gained clinical importance because it was traditionally reserved as a last resort treatment for resistant infections, especially of methicillin-resistant Staphylococcus aureus (MRSA). The emergency of vancomycin-resistant organisms has deprived the usefulness of this drug.

Aminoglycosides are a molecule composed an amino group and a sugar part (glycoside). These drugs are made naturally generally by bacteria of the genus Streptomyces (such as Streptomycin, Kanamycin, Neomycin, Spectinomycin) and Micromonospora (such as Gentamicin, Amikacin, Sisomicin, Netilmicin, Isepamicin). Aminoglycosides have very fast and strong bactericidal effect on bacteria. They action in some sites of bacterial cell (outer membrane, ribosomes). They tie to the aminoacyl site of the 16S ribosomal RNA at the 30S ribosomal subunit. This binding be obstructive to reading of the genetic code resulting in depression of protein synthesis. The spectrum of activity of these drugs includes a broad spectrum of aerobic gram-negative bacilli, including Enterobacteriaceae, Pseudomonas spp., Haemophilus influenzae, more staphylococci, any mycobacterium and methicillin-sensitive Staphylococcus aureus. Activities for Burkholderia cepacia, Stenotrophomonas maltophilia, and anaerobic bacteria are commonly bad or absent.

Aminoglycosides are badly absorbed from the gastrointestinal tract. They are highly polar cations and their distribution is normally limited to the extracellular fluid compartment. They are mainly excreted by the kidney by glomerular filtration. The serum half-life is 2–3 h in patients with regular renal function and prolonged in patients with impaired kidney function. They are removed effectively by continuous hemofiltration vs hemodialysis.

The main toxicities are nephrotoxicity and ototoxicity. The distribution of nephrotoxicity depends of the particular patient population and attendant risk factors. The incidence has been reported as high as 20%. The classic start becomes after a treatment of 6–10 days. Manifest ototoxicity usually gets in 2–10% of patients. Vestibular or cochlear failure is showed as ototoxicity. Vestibular toxicity may include lightheadedness, dizziness, ataxia, retch and vomiting. Ergo, careful supervise is crucial to avoid potential adverse occasions. Most often, the toxicities are reversible. Other significant side effect is neuromuscular blockade that can becomes in predispose patients (hypomagnesemia, myasthenia gravis, hypocalcemia) or in patients being treated with nicotinic acetylcholine receptor agonists or other medications interfering with neuromuscular transmission.

The most common uses of aminoglycosides include empiric therapy for sepsis, intra-abdominal infections, complicated urinary tract infections, and osteomyelitis secondary to aerobic gram-negative bacilli. Aminoglycosides are frequently used in combination with a beta-lactam agent.

Gentamicin and tobramycin have comparable spectrums with the exception that gentamicin is few more active against Serratia spp. and tobramycin a little more active against Pseudomonas spp. They are mostly used for severe infections such as pneumonia and sepsis caused by gram-negative microorganisms. Amikacin is used to cure serious gram-negative infections including many strains of Pseudomonas, Enterobacter, Serratia and Proteus. This drug is resistant to many enzymes that inactivate gentamicin and tobramycin. Multi-drug-resistant Mycobacterium tuberculosis is normally sensitive to amikacin.

Resistance: There are numerous mechanisms through which bacteria may develop resistance to aminoglycosides. These include: - Inactivation of the aminoglycosides by enzymes like acetyl transferase, phosphotrans-ferase and adenyl transferases present in the bacterial cells. The microorganisms alter their bacterial cell surface and prevent the drugs to diffuse across the bacterial cell wall.

One of the ways to overcome this kind of resistance is to combine the β lactam drug with the aminoglycosides. The former will weaken the bacterial cell wall, enable the aminoglycosides drug to enter the microorganism, and inhibit their protein sythesis.

Mutation and subsequent alteration of the 30 S ribosomal sub unit prevent the attachment of the drug to the ribosome.

Natural resistance to aminoglycosides to penetrate the cytoplasmic membranes can be seen in the facultative and other anaerobic bacteria.

2.1.6. Tetracyclines.

The tetracyclines are broad-spectrum antibiotics discovered in the late 1940s following the isolation of chlortetracycline from Streptomyces aureofaciens. These drugs were used the first broad-spectrum antibiotics of bacterial infections in humans. Tetracyclines consist of a hydro naphthacene nucleus with four fused rings. Though they have a very wide spectrum of antibacterial activity, their present clinical use is mainly for the

treatment of intracellular organisms like Mycoplasma, Chlamydia and Rickettsia. They are also used as alternative drugs for the cure of syphilis in the case of penicillin allergy.

Tetracyclines tie to the 30s subunit of the bacterial ribosome and block the binding of the aminoacyl-tRNA to the ribosome complex stopping the amplification of peptides in protein synthesis. Tetracyclines are commonly bacteriostatic antibiotics as they do not kill the bacteria but only stop their growth. Resistance to tetracyclines can get in a diversity of ways including reduced transport into the bacterial cells or enhanced efflux out of the bacterium, protection of the bacterial ribosomes and enzyme modifications.

Tetracyclines have activity for a wide spectrum of both gram-positive organisms (including Streptococcus spp. and Staphylococcus spp.) as well as gram-negative organisms (not including Pseudomonas spp.). They also have fine activity for some anaerobes. The progress of resistance by S. pneumoniae has restricted its value in treating pneumonia. Likewise, when these agents have a wide spectrum of activity for gram negative organisms, the evolution of resistance except its use as a first-line agent for urinary tract infections. The tetracyclines, mainly doxycycline, are yet loose used to treat intracellular pathogens, primarily rickettsial diseases.

Tigecycline has an extended spectrum that includes methicillin-resistant staphylococci (MRS) and vancomycin-resistant enterococci (VRE). It also has a wide gram-negative spectrum including many organisms that are resistant to another tetracyclines but both Proteus and Pseudomonas species are resistant. Tigecycline is approved for cure of skin and soft tissue infections and is frequently used to treat MRS or VRE. It does not good concentration in the urine, and ergo is not an chosen drug for urinary tract infections.

The most common adverse events for tetracyclines are retch, vomiting and/or diarrhea. These adverse events can often be regulate by administration with food or reduce the dosage. Application of tetracyclines is contraindicated in children under than 8 years elderly and in pregnant women because it binds calcium and can lead to discoloration of teeth, as well as enamel and bone complications. These antibiotics have been associated with liver toxicity, especially in pregnant women. Photosensitization is related with application of tetracyclines, and vestibular reactions including daze and vertigo have been seen, especially with high doses of doxycycline.

Mild to moderate infections caused by anaerobes: acne, actinomycosis, some pelvic inflammatory diseases. The resistance to these agents occurs mainly through three mechanisms [4], namely: - Efflux of the antibiotics; -Ribosome protection; - Modification of the antibiotic. These tetracycline resistance determinants are widespread in different microorganisms [5]. The efflux of the drug occurs through exporting protein from the major facilitator superfamily (MFS). These exported proteins are membrane-associated proteins, which are coded by the tet efflux genes and the exported tetracycline from the cell. The exported tetracycline reduces the intracellular drug concentration, and thus, protects the ribosomes within the cell. The tetracycline efflux proteins have amino acid and protein structure similarities with other efflux proteins, which are involved in multiple-drug resistance, quaternary ammonium resistance, and chloramphenicol and quinolone resistance. The gram-negative efflux genes are widely distributed and normally associated with large plasmids, most of which are conjugative. The ribosome protection occurs through ribosome protection proteins that protect the ribosomes from an action with the tetracyclines [6]. The ribosome protection proteins are cytoplasmic proteins that bind to the ribosome and cause an alteration in the ribosomal conformation, which prevents the tetracycline from binding to the ribosome, without altering or stopping the protein synthesis. They confer resistance mainly to doxycycline and minocycline and confer a wider spectrum of resistance to the tetracyclines than is seen with bacteria that carry the tetracycline efflux proteins.

The modification of the antibiotic on the other hand occurs through enzymatic alteration of the drugs. Some of these genes are coded by the tet(X) genes.

2.2. Macrolides.

Macrolides are a group of drugs with a macrocyclic lactone nucleus attached to sugar groups. They are produced from Streptomyces erythreus. Azithromycin and clarithromycin are make from constructional change of erythromycin and result in better spectrum of activity, absorption and administration. Clarithromycin has the same 14-membered lactone ring as erythromycin exchanging the hydroxyl group by a methoxy-group at position "6". Azithromycin is a 15-membered lactone ring exchanging the carbonyl group of erythromycin by methyl-substituted nitrogen at position "nine A".

These antibiotics tie up specifically to the 50S part of the bacterial ribosome and inhibit bacterial protein synthesis.

Erythromycin is active for Streptococcus pyogenes and penicillin susceptible Streptococcus pneumoniae . The better part of the strains of groups B, C, F, and G streptococci are sensitive to erythromycin. Erythromycin is effective for Bordetella pertussis, Mycoplasma pneumoniae, Treponema pallidum, Legionella pneumophila, Ureaplasma urealyticum, several strains of Rickettsia, Chlamydophila species and MSSA. Generally, both azithromycin and clarithromycin supply more gram-negative activity vs erythromycin including Escherichia coli, Helicobacter pylori, Campylobacter jejuni. Erythromycin is separated primarily in the bile; only 2–5% is separated in the urine.

The spectrum of activity of azithromycin is much wide than erythromycin. H. influenzae is sensitive to azithromycin, until it is partly resistant to clarithromycin and fully resistant to erythromycin. It look that azithromycin is most active fort Legionella spp. collated with erythromycin and clarithromycin. Azithromycin is excreted in the bile and dosage adjustment is not necessary for patients with impaired renal function.

Clarithromycin is a derivative of erythromycin and it changes in the body to make a metabolite that keep antibacterial activity, but has remodel pharmacokinetic qualities. The activities of this drug and its metabolite are like to that of erythromycin, when concentrations necessary to inhibit legionella and chlamydiae are usually lower.

There has been pretense of very increase penetration in pneumonic sites, useful cooperation between the original compound and the metabolite, and other minor priority. It is questionable if these translate into significantly better curative efficacy, but clarithromycin is more absorbed and less given to cause ventral disadvantage than previously macrolides. It has been

successfully used in concord regimens for the cure of infections with Helicobacter pylori and several mycobacteria, especially those of the M. avium group.

2.3. Lincosamides.

The original lincosamide, lincomycin, a naturally gets up product of Streptomyces lincolnensis, has been replaced by clindamycin, which have better antibacterial activity.

Lincosamides interrupt on the process of peptide elongation in a mode that has not been exactly determine. The ribosomal binding place is probably like to that of erythromycin, because resistance to erythromycin bred by methylation of the ribosomal solder site effects lincosamides as well.

Clindamycin has good antistaphylococcal and antistreptococcal activity and owns also demonstrated therapeutically beneficial in the treatment of infections due to Bacteroides fragilis and several another anaerobes. Enterobacteria and P. aeruginosa are not sensitive to this drug. Clindamycin shows various activity against parasitic protozoa and has been useful in the treatment in toxoplasmosis, malaria, and babesiosis.

Patients cured with clindamycin usually experience diarrhoea caused by a clostridial toxin, which sometimes evolves into a potentially crucial pseudomembranous colitis. Other drugs, especially ampicillin and broad-spectrum cephalosporins, may too cause this adverse effect, but the spread of toxin-associated colitis looks to be few higher following clindamycin therapy.

2.4. Mechanisms of resistance of Macrolides, Lincosamides and Streptogramines.

Three different mechanisms of the acquired Macrolide, Lincosamide and Streptogramin (MLS) resistance have been found in gram-positive bacteria [7]. These include the following:

- Post-transcriptional modifications of the 23S rRNA by the adenine-N6-methyltransferase, which alters a site in 23S rRNA common to the binding of the MLS antibiotics, also confers a cross-resistance to the MLS antibiotics (MLS-resistant phenotype), and remains the most frequent mechanism of resistance. In general, the genes that encode these methylases have been designated erm (erythromycin ribosome methylation).
- The efflux proteins that pump these antibiotics out of the cell or the cellular membrane, keep the intracellular concentrations low and the ribosomes free from an antibiotic, and they have become more frequent in gram-positive populations and often coded by mef, msr, and vga genes.
- Hydrolytic enzymes, that hydrolyze streptogramin B, or modify the antibiotic by adding an acetyl group (acetyltransferases) to streptogramin A, are also described. They confer resistance to structurally related drugs.

2.5. Fluoroquinolones.

Fluoroquinolones have bactericidal activity however are not as powerful as beta-lactams and aminoglycosides. They hinder on DNA metabolism in the bacterial cell. They are active primarily at gram-negative bacteria but the recent members are effective for gram-positive bacteria, intracellular pathogens and several anaerobes.

Nalidixic acid was the first quinolone present in 1962. Constructive alteration has accrued in an extended spectrum and better pharmacokinetics profile. The fluorinated 4-quinolones, such as ciprofloxacin, norfloxacin, levofloxacin, moxifloxacin are effective for treatment of a broad range of infectious diseases.

First generation (nalidixic acid, cinoxacin, oxolinic acid) with narrow antibacterial spectrum after oral administration are only helpful for cure of urinary tract infections. The fluoroquinolones with broad spectrum proposal therapeutic serum and tissue levels. Fluoroquinolones are potential against Enterobacteriaceae, as well as other gram-negative organisms such as Neisseria, Haemophilus, Brucella, Branhamella, Legionella, Shigella, Salmonella, Vibrio, Yersinia and Campylobacter. Ciprofloxacin and levofloxacin are the only quinolones potent against Pseudomonas aeruginosa. Quinolones are useful for infections caused by Mycobacterium tuberculosis, M. kansasii and M. fortuitum. Levofloxacin, moxifloxacin and gemifloxacin own the best activity for gram-positive organisms, including Staphylococcus aureus, S. epidermidis and S. pneumoniae. Inappropriate use of quinolones has issue in rising resistance in opposition to E. coli, P. aeruginosa and S. aureus. Moxifloxacin supplies small range against some of the anaerobic pathogens, including Bacteroides fragilis and oral anaerobes.

The fluoroquinolones are widely distributed in body fluids and tissues. They are effective for treatment of sexually transmitted diseases, prostatitis, urinary tract, enteritis, gynecologic, lower respiratory tract, soft tissue infections.

Patients must be advised of possible drug interactions with nonsteroidal anti-inflammatory agents, cations and warfarin. In the children population, the application of fluoroquinolones is restricted to treatment of life-endangering infections and lower respiratory infections in cystic fibrosis.

Common adverse effects include daze, nausea, vomiting, insomnia and headache. Another less common side effect includes elevation of hepatic enzymes, skin rashes, hypoglycemia, eosinophilia and leukopenia. QTc prolongation has been reported with moxifloxacin. Torsades de pointes have been reported with levofloxacin and ciprofloxacin. Quinolones are limited prescribed for pediatric population due to reports of joint and cartilage damage in young animal studies. Tendinitis, including rupture of the Achilles tendon, has been observed in adults treated with quinolones together with nonsteroidal anti-inflammatory agents.

Fluoroquinolones are one of the most useful classes of antimicrobial drugs since of their extended spectrum and pharmacokinetic profile. Inappropriate uses of these drugs have issue in increased resistance and clinicians must rational use these agents for proper indications.

2.5.1. Mechanisms of bacterial resistance to quinolones.

Mechanisms of bacterial resistance to quinolones as described by Hooper (1999) fall into two principal categories as follows: 1. alterations in drug target enzymes and 2. alterations that limit the permeability of the drug to the target. The target enzymes are most commonly altered in domains near the enzyme active sites, and in some cases reduced drug-binding affinity. In gram-negative organisms, DNA gyrase seems to be the primary target for all quinolones. In gram-positive organisms, topoisomerase IV or DNA gyrase is the primary target depending on the fluoroquinolones considered. In almost all instances, amino acid substitutions within the quinolone resistance-determining region (QRDR) involve the replacement of a hydroxyl group with a bulky hydrophobic residue. Mutations in gyrA induce changes in the binding-site conformation and/or charge that may be important for quinolone–DNA gyrase interaction [8]. Changes in the cell

envelope of gram-negative bacteria, particularly in the outer membrane, have been associated with decreased uptake and increased resistance to fluoroquinolones, and this has not been demonstrated in gram-positive bacteria.

2.6. Chloramphenicol.

Chloramphenicol is fully absorbed after oral consumption. It have even spread in the human body and easy crosses diffusion barriers like as the blood-brain barrier. Regardless of these good properties, application of this drug is seldom indicated (CNS infections) since of the risk of bone marrow harm. Two kinds of bone marrow depression can happen: first - a dose- dependent, toxic, reversible form showed during therapy and, second - a very often fatal shape that may happens after a response time of weeks and is not dose-dependent. Expected to high tissue penetrability, the risk of bone marrow depression must too be taken into description after local use.

Chloramphenicol has a wide spectrum of activity. It is bacteriostatic, but is very effective against staphylococci, streptococci, salmonellae, H. influenzae and others. Rarely it causes aplastic anemia, that its use is mostly limited to life-threatening illness (meningitis, typhoid fever) and to topical use. Chloramphenicol hinders on bacterial ribosome task by inhibiting the 50S ribosomal peptidyl transferase, as a result of that stopping peptide elongation. Chloramphenicol inhibits the metabolism of warfarin, phenytoin and theophylline. Chloramphenicol should not be writing a prescription for pregnant women and it is not preferable for neonate and suckling's child: the liver in child's cannot metabolize drug chloramphenicol good and the chloramphenicol collects in tissues make up so-called gray baby syndrome.

2.6.1. Resistance to chloramphenicol.

Resistance to the chloramphenicol is generally due to inactivation of the antibiotic by a chloramphenicol acetyltransferase [9]. Various enzymes have been described and they are coded for by the cat genes found in gram negative and gram-positive bacteria and usually show little homology [10]. Sometimes decreased outer membrane permeability or active efflux is responsible for the resistance in gram-negative bacteria [11].

2.7. Trimethoprim /Sulfamethoxazole (SXT).

Sulfonamides are antimicrobial agents founded on a sulfonamide group attached to a benzene ring. The medicines were discovered by screening paint for activity for streptococci exercise an animal model. Sulfonamides are active opposed to microbes that cannot use outer folate and must synthesize it from para-aminobenzoic acid. This drug is a para-aminobenzoic acid analog and be in the service as an antagonist of folate with comparatively small toxicity to humans.

Although sulfonamides have a wide spectrum of activity in opposition to gram-positive organisms and helpful to cure certain filamentous bacteria (Nocardia). Generally their primary use now is in the cure or prophylaxis of urinary tract infection caused by gram-negative microorganisms or in the cure or prophylaxis to prevent Toxoplasma gondii or Pneumocystis jiroveci disease.

2.7.1. Resistance to sulfonamides.

Resistance in sulfonamides is commonly mediated by alternative, drug-resistant forms of dihydropteroate synthase (DHPS). Sulfonamide resistance in gram negative bacilli generally arises from the acquisition of either of the two genes sul1 and sul2, encoding forms of dihydropteroate synthase that are not inhibited by the drug [12]. The sul1 gene is normally found linked to other resistance genes in class 1 integrons, while sul2 is usually located on small nonconjugative plasmids or large transmissible multi-resistance plasmids. Trimethoprim is an analog of dihydrofolic acid, an essential component in the synthesis of amino acid and nucleotides that competitively inhibits the enzyme dihydrofolate reductase (DHFR). Trimethoprim resistance is caused by a number of mechanisms [13] including: - overproduction of the host DHFR; - mutations in the structural gene for DHFR; -acquisition of a gene (dfr) encoding a resistant DHFR enzyme which is the most resistant mechanism in clinical isolates. At least 15 DHFR enzyme types are known based on their properties and sequence homology [14].

2.8. Multidrug Resistance.

Multiple-drug resistance become that microorganisms are resistant to more than one antibiotic. Due to improper use and antibiotic overuse, multidrug resistance at the moment is the rule than the exception among resistant bacteria. This problem frequently is a consequence of by genetic movable elements such as transposons, plasmids and integrons [15]. Integrons are mobile DNA elements with the capacity to catch genes, especially those encoding antibiotic resistance, by site specific recombination, and they own an integrase gene, a close by recombination site and a promoter [16]. Integrons look to have a crucial role in the distribution of multidrug resistance in gram-negative bacteria yet integrons in gram-positive bacteria have too been reported [15]. The most of genes encode antibiotic disinfectant resistance, including resistance to penicillins, cephalosporins, aminoglycosides, tetracycline, erythromycin, trimethoprim and chloramphenicol.

3. BASIC CHARACTERISTIC OF ANTIFUNGALS

Only a limited number of antifungals can be used to treat systemic fungal infections. Basic classes antifungal drugs in clinical use, according to their effect on the fungal cell are: a) Azoles; b) Polyenes; c) 5-flucytosine; d) Echinocandins [17].

According their mechanism of action the most important antifungal drugs are divided into four different classes [18].

3.1. Polyene macrolides.

They cause disruption of membrane functions (Nystatin, with its liposomal formulation for intravenous administration; amphotericin B [AmB] and its lipid derivatives). They bind to ergosterol to form pores in the cell membrane. In high concentrations, they inhibit cell-membrane enzyme responsible for chitin synthesis.

Mode of resistance to the polyenes is the significant infringement of the lipid composition of the cytoplasmic membrane (eg reduced ergosterol content as a result of missing D (8,7) isomerase).

3.2. Azole derivatives.

They inhibit lanosterol 14a-demethylase, a key enzyme in the ergosterol biosynthesis (ketoconazole, fluconazole, itraconazole, voriconazole, posaconazole and ravuconazole). They induce accumulation of toxic sterol intermediates that cause cell

membrane stress. The sterol synthesis takes place in the endoplasmic reticulum.

Resistance to azoles develops as a result of several mechanisms:

Modified efflux: when Candida is associated with genes for drug resistance in Candida (CDR).

Change in the target enzyme lanosterol demethylase: increased expression of the target enzyme for azoles, discovered in C.glabrata.

Change in genes ERG3: altered sterol D (5,6) desaturase.

Modified influx: a modified composition of the sterols in the cytoplasmic membrane, which affects the acceptance of drug in the cell. This changes the asymmetry and fluidity of the membrane, which leads to reduced drug intake.

Frequency of the resistance mechanisms at azoles:

at 85% of the resistant isolates have increased expression of efflux pumps (frequency of expression of CDR genes and MDR1 gene is similar);

substitution of amino acids in the target enzyme lanosterol-demethylase is at 58% -65%;

increased expression of genes encoding enzymes was found at 35% -42% of the isolates;

Multiple mechanism of resistance is at 75% of the resistant isolates.

It has not been established yet resistance to posaconazole.

3.3. 5-fluorocytosines.

They are inhibitors of the synthesis of DNA and RNA (flucytosine).

There are described two mechanisms of resistance. In the first case as a result of mutation has a reduced activity of a cytosine permease or deaminase which results in reduced acceptance or conversion of the drug. The second mechanism is due to the loss of activity of uracil phosphoryl transferase, an enzyme responsible for the conversion of 5-fluoridylic to 5-fluorouridylic acid [18].

3.4. Echinocandins.

They are inhibitors of the 1,3-b-glucan synthesis (caspofungin and micafungin), and thus damage the integrity of the cell wall.

4. PROPERTIES AND POSSIBILITIES FOR APPLICATION OF THE HYBRID MATERIAL WITH SILVER NANOPARTICLES (PVA/AgNps)

4.1. Bio nanotechnology.

Nanotechnology is a rapidly growing field with applications in science and technology for the production of new materials with dimensions from the nanoscale. The word "nano" is used to refer to one billionth of a meter or 10-9. The term "nanotechnology" was introduced by Professor Norio Taniguchi from Tokyo Science University in 1974, to be able accurately to describe the production of materials with dimensions in the order of nanometer. The nanoparticles are clusters of atoms in the size range 1-100 nm. "Nano" is a Greek word, a synonym of small, in the sense of extremely small. The use of nanoparticles is stimulated in our century, once were detected owned by them chemical, optical and mechanical properties. Bionanotechnology occurred as integration between biotechnology and nanotechnology to explore the biosynthetic and environmentally friendly technologies for the synthesis of nanomaterials. Nanotechnology has an especially pressing and beneficial use in the field of medicine. It may be used to provide advanced biomedical research tools [19].

4.2. The metal nanoparticles.

The metal nanoparticles are the most promising, because they showed among others good biomedical properties corresponding to the large surface area relative to their volume. It is known that they have an antiviral activities against various viruses. Viral infections poses significant global health challenges, especially in view of the fact that the emergence of resistant viral strains and the adverse side effects associated with prolonged use of antiviral drugs. Since metals may attack a broad range of targets in the virus there is a lower possibility to develop resistance as compared to conventional antiviral therapeutics. It is established that there is also an antiviral activity of various metal nanoparticles to the human immunodeficiency virus (HIV-1), herpes virus, respiratory syncytial virus, influenza virus, hepatitis virus, and other [20, 21, 22].

4.3. Biological properties of the silver.

Many scientific publications in the literature contain facts concerning the antibacterial properties of silver [23, 24, 25, 26]. Knowledge of the antimicrobial activity of silver dates back to early history. In 344 BCE Aristotle advised his pupil Alexander the Great to store boiled water in silver vessels to prevent disease by source water [26]. Literary studies by Clement, J.L. and Jarrett P.S. (1994) take us back to a time when it was already known that silver objects exhibit a healing effect. Herodotus described how the king of Persia stored boiled water in silver vessels among his provisioning. The first modern description of this fact was given by Raulin in 1869, who observed that Aspergillus niger can not grow in silver vessels. He was somewhat overshadowed by the Swiss botanist von Ngeli, who introduced the term "oligodynamic" ("oligos", small + "dynamic", force) to describe any metal that exhibits bactericidal properties with minimal concentration in the range of 10-9 to 10-6 mol / l. [27]. Von Ngeli explored silver, for which this is especially true, although copper and tin also have oligodynamic activity. For centuries, silver is used in the treatment of burns and chronic wounds because silver cations are effective against a wide spectrum of Gram positive and Gram negative bacteria, fungi, protozoa and viruses. The antimicrobial properties of silver have been associated with the quantitative content of silver and the rate of release. Silver as metal is inert, but reacts with moisture in the skin and the wound fluid, wherein is ionized. Ionized silver is highly reactive and binds to tissue proteins. Ionic silver is reactive to bacteria and to some extent to fungi and viruses [28]. Silver in its oxidation states (Ag^0, Ag^+, Ag^{2+} and Ag^{3+}) has long been known as having an inhibitory effect on many micro-organisms normally present in the medical and industrial processes [25].

4.4. Silver ions.

Precipitation and incorporation of silver ions in various products is very attractive approach to prevent biofilm formation due to the strong antiseptic, strong bactericidal effect and

anticipated lower activity to animal tissues due to their natural origin [25].

Historically, the use of silver nitrate for treatment is started as a liquid and is known by various names such as "Lunar caustic" in English, "Lapis infernale" in Latin and "Pierre infernale" in French [26]. In 1700 the silver nitrate is used for the treatment of venereal diseases, fistulas of the salivary glands and bones and perianal abscesses. In the nineteenth century the granulation tissue is removed using silver nitrate, which allowed epithelialization and facilitate the formation of a crust on the surface of wounds. Various concentrations of silver nitrate were used in the treatment of fresh burns. In 1881 Carl S.F. Crede cured ophthalmia neonatorum using eye drops with silver nitrate. His son, C. Crede developed impregnated with silver dressings that are used in skin grafting. In the 40s following the introduction of penicillin, the use of silver for the treatment of bacterial infection is reduced. It starts once again to be used in the 60s, when Moyer introduced 0.5% silver nitrate for the treatment of burns. He suggested that this solution will not prevent epidermal proliferation and at the same time possesses antibacterial properties against Staphylococcus aureus, Pseudomonas aeruginosa and Escherichia coli.

The silver nitrate was combined with sulphonamides and used in 1968 as component of the silver sulfadiazine cream. It was accepted as an antimicrobial agent with a broad spectrum for usage by wound treatments. The silver sulfadiazine is effective against pathogen bacteria as E.coli, S.aureus, Klebsiella sp., Pseudomonas sp.. It demonstrates also some antifungal and antiviral properties. As a result of more often appearance of antimicrobial resistant bacteria and the restricted choice of antimicrobials, the clinicians pay attention to the silver wound bandages with different amount of silver [27]. Impregnated with silver dressings are increasingly frequent use in coating burns and traumatic injuries in humans and animals. The silver sulfadiazine (AgSD) binds to cellular components, including DNA and induces destruction of membrane. Reaches bacterial inhibition by binding to the base pairs in the DNA helix and inhibits transcription. Similarly, he connects also the phage DNA [24].

According to literary study of Clement, JL and PSJarrett (1994) silver salts (I) lead to the formation of high concentrations of Ag + in solution which are precipitated from chlorides and proteins by effecting also astringent effect. This principle applies in caustic pencils with silver nitrate for cauterization of wounds and the removal of granulation tissue. This effect is unwanted for an antibacterial agent, which directed the efforts towards preparation of colloids, including silver proteinate designed mainly for local use. They were, however, subsequently replaced by other antibiotics. Although Ag+ is locally astringent, it is not toxic to animals. Most of the data were collected right from the Romans. The excessive content of silver components or long-term treatment with silver (I) may cause argyria. This is a darkening of the skin or the tissue, which is essentially irreversible, but can not be harmful. Actually research in many cases can not establish the cellular effects in contact with silver. It was found that Canadian women take 7.1 mg of it daily with food, with no apparent ill effects. Acute oral toxicity parameters are from 2 to 30g.

Today main therapeutic use of silver (I) is in the local prophylaxis in new born [26].

The silver nitrate was used in the past in high concentrations, but when it is introduced in 1965 is used in lower concentrations. Control tests on the compresses containing 0.5% silver nitrate in severely burned patients show its effectiveness, as well as its role in the prevention of infection by *Pseudomonas aeruginosa* and accompanying septicemia. The use of $AgNO^3$ was accompanied with lower mortality as other topical antibiotics that had problems at same time. Partial problem of silver nitrate is that hypotonic solution causes electrolyte changes that are treated with extra calcium, sodium and potassium. This prompted Fox to investigate sulfadiazin- insoluble silver salt, which dissolves only slightly in biological fluids. The intention was to maintain low concentrations of Ag^+ chlorides do not precipitate easily and to preserve the antibacterial activity. Control tests confirm its suitability for use and today it is the drug of choice for treatment. Sulfadiazina is a sulfonamide and of itself is an antibiotic. Although an antagonist of sulfonamide is para aminobenzoic acid, silver sulfadiazine (AgSu) is not inactivated. Further evidence that silver is the active component comes from the observation that sulfadiazin at itself is ineffective at concentrations that AgSu inhibit bacterial growth. Indeed sulfadiazinat shows specific synergism in combination with sub-inhibitory levels of AgSu, suggesting that the two agents kill bacteria by various mechanisms. Moreover, Ag resistant bacteria isolated from treated with AgSu burns, are not necessarily resistant to Su, on the contrary. The sensitivity to the silver sulfadiazine did not correlate in any way with sensitivity or resistance to other antibiotics. Strains resistant to sulfadiazine (sodium) or multidrug-resistant strains (including strains possessing R factors) were sensitive to AgSu.

4.5. Colloidal silver.

The dispersion of small particles of silver in water, also known as colloidal silver is usually used as a disinfectant. Colloidal silver can also serve as a means for ensuring of drinking water which remained drinkable and clean even after a long period of time. It was believed that a more stable colloidal silver solutions can be prepared by using smaller particles. However, raised the suspicion, that the final colloidal silver solution can regain their molecular state [25].

For colloidal silver states that there are many unidentified and/or uncertain claims for protection against respiratory diseases, and also it can be used to treat a variety of diseases, including skin cancer.

4.6. Silver zeolite.

Zeolite is aluminosilicate powder which has the ability to bind more than 40% of its weight other substances of a certain size of the molecules while omitting others. There is a high cation-exchange with calcium, magnesium, sodium and potassium; sorptional properties to ammonium and many organic compounds; sorptional properties to certain radioactive elements; cesium 134, cesium 137, strontium 90; adsorption to heavy metals - lead, zinc, chromium, etc .; have the ability to separate gas mixtures to the principle of the molecular sieve; dries gases,

taking excess moisture. In Bulgaria - the Eastern Rhodopes is natural deposits of zeolite, which contains minerals Klinoptionolit. Zeolite, which is produced in Village Beli Plast near Kardzhali is one of the cleanest in the world. Thus Bulgaria has been exporting worldwide. The silver zeolite is a component of medical and dental products. Ag + are released slowly and partially in which they provide anti-bacterial activity. Activation of silver zeolite mainly requires air and is discussed in its composition to include active oxygen species such as superoxide. The silver zeolite is active also against anaerobic oral bacteria, which indicates a large potential [24].

4.7. Silver nanoparticles.

There are publications for lethal action of silver nanoparticles against bacteria, fungi, viruses and parasites [22, 23, 24, 25, 26, 29]. They impact on the target object by multiple mechanisms, but a way of killing cells is the formation of 'pores' on their membranes [30, 31]. Due to the high internal osmotic pressure, the cytoplasm content is expelled through the tunnel, resulting in an empty bacterial cell envelope [32].

The improvement of the properties by decreasing the size of the particles is attributed to the increase in the number of active sites on the surface of the material. Nanomaterial with a radius of 10 nm has a higher percentage of active sites on the surface of the particles, rather than a material having a radius of 10μm. Larger particles have more inner active sites, which remain actually intact under chemical reactions, or external forces. Compared with a suspension of colloidal silver, the smaller particles have more surface active sites for interaction of liquid-solid phase and therefore are more likely to remain dispersed [25].

Incorporation of silver nanoparticles in different polymer structures allows control over their size and shape, as well as appropriate stabilization due to the possibility for rapid oxidation and aggregation in solution.

Nano-silver serves as a reservoir for supplying dissolved silver ions, which has a strong bactericidal effect. Ionic silver is toxic to bacteria and somewhat for fungi and viruses, making it a very effective biocide. Regardless of the shape of the silver essential characteristic is the concentration of released silver ions.

Silver nanoparticles are produced by chemical or physical methods, and because of their small size can potentially pass through biological membranes and reach more and different organs and tissues in the body. It can be summarized that the toxicity of nanosilver is greater than silver as a whole [33, 34]. The silver is significantly more toxic than other heavy metals when they are in the form of nanoparticles [33, 34, 35] and the silver is significantly more toxic than other heavy metals when they are in the form of nanoparticles [35, 36]. Determining the toxicity of nanosilver factors are particle size, shape and concentration [33]. Silver nanoparticles with sizes <10 nm can pass through the cell wall [38]. Numerous researchers believe that silver particles with different sizes have a different toxicity [34, 37, 38].

The mechanism of nanosilver toxicity

The mechanism of nanosilver toxicity is still unknown. However, all forms of silver can release silver ions. Silver cations interact with multiple target cell mechanism [39]. On the one hand the positively charged silver cations associated with negatively charged components of the bacterial cell - cell wall and membrane, which induce structural changes and cell lysis.

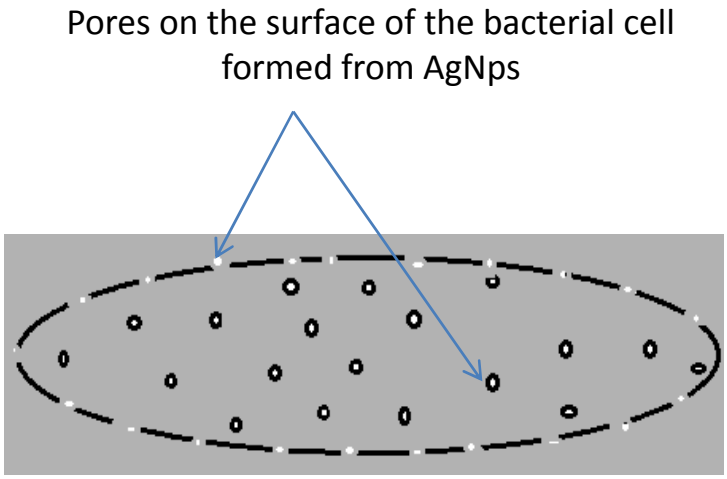

Figure 1. Mechanism of nanosilver toxicity

Another mechanism of action is penetration of the silver cations inside of the bacterial cell binding to the negatively charged proteins, enzymes, DNA or RNA, to interfere with electron transport, cell division and cell replication. Mechanisms of toxicity to bacteria cited to in the literature are:

DNA loses its ability to replicate [40, 41].

Deactivation of proteins essential for ATP [42].

Deactivation of membrane bound proteins, leading to structural changes and cell death [30].

Inhibition of respiratory enzymes to accelerate propagation of the oxygen species, and thus damage or kill cells [33]

Molecular mechanism: the increase of silver ions (even at very low concentrations), which can pass the cell membrane, deplete the cell wall proteins and already penetrated into the cell results in a loss of energy and cell death [43].

Nano-silver demonstrates activity against fungi by attaching in an analogous mechanism with the negatively charged parts [30]. Dimorphic transition of C. albicans from yeasts to the micellar form is considered to be responsible for pathogenicity. Silver nanoparticles inhibit the extension and the formation of mycelium. They can damage the yeast cells by attacking their membranes, and thus distort the membrane potential.

The silver nanoparticles stirred membrane lipid bilayer, causing outpouring of ions and other materials, and also formation of pores and distribution of the electric potential of the membrane.

At TEM is found that they cause the formation of holes in the cell walls and pores in the cytoplasmic membrane [31]

Ag- nanoparticles cause disturbances in the normal process of budding, which correlates with damage to the membrane [31].

Thus, following attachment of the silver nanoparticles (AgNps) to the cell membrane, they enter in the fungi, forming space with a small molecular weight in the center of the fungus attached to the respiratory chain and eventually stop the cell division, which results in cell death [44].

All information about the silver, its forms and routes of administration, presented above is to enable us to meet with its properties and to highlight the advantages that give small dimensions of the silver nanoparticles. Stabilized in a

biocompatible material such as polyvinylchloride, they demonstrate greater activity and may have greater relevance.

5.PVA/AgNps HYBRID MATERIAL

5.1. Synthesis of PVA/AgNps hybrid material.

PVA/AgNps hybrid materials was prepared by adding a silver salt (AgNO3), the precursor for silver ions, to the PVA solution thus leading to coordination of silver ions with hydroxyl groups (-OH) from PVA. Boiling the PVA solution at 100°C for 60 min in the presence of AgNO3, results to formation of silver nanoparticles stabilized in PVA, which protects the silver nanoparticles from agglomeration and ensure the homogenous distribution of silver nanoparticles. The formation of silver nanoparticles was proven by UV-Vis spectroscopy and transmission electron microscopy (TEM) [45]. The formation of silver nanoparticles is evidenced from UV-Vis spectroscopy by the appearance of strong absorption bands at 420 nm which indicates the formation of AgNPs.

5.2. Microbiological tests.

The sinthezized solution of hybrid material was diluted with sterile injection water to concentration 30 mg/l. This concentration of silver was chosen on the basis of performed in advance tests onto more than 100 clinical antimicrobial resistant isolates which showed strong bactericidal and fungicidal activity using this silver content. It was taken into account the threshold value for cytotoxicity for the silver nanoparticles cited in the literature [46].

For testing the antimicrobial properties of the synthesized hybrid PVA/AgNps material following methods are used [47, 48, 49, 50, 51]:

- DDM (Disk Diffusion Method);
- MIC (Minimal Inhibitory Concentration) by the agar dilution method;
- Method with the macro dilutions;
- Chess method for testing the presence of synergism of PVA/AgNps hybrid material and Pi or Ce;
- Modified method for testing the presence of synergism of the material to antimycotics;
- *In vivo* tests: dermal test; test for bio toxicity; application as an aseptic agent in the treatment of skin and wound infections in animals and humans.

5.2.1. Disk Diffusion Method (DDM).

The influence of the stabilizer (disk impregnated with PVA) compared to the impact of the entire hybrid material, was an evaluated in the absence of the zone of the inhibition on the control bacterial and yeast strains.

Bactericidal properties of the hybrid PVA/Ag Nps materials was initially established through DDM.

Figure 2. Testing the bactericidal activity of PVA/AgNps hybrid material to control bacterial strains by DDM.

Calculations of the silver concentration in the synthesized hybrid sample were in this case against the inputted starting concentration of the silver nitrate. A sample of hybrid PVA/Ag Nps material with a concentration of silver precursor of 3.9 mg/ mL (3900 μg/mL) was used.

5.2.2. The Agar Dilution Method.

The Agar Dilution Method is very convenient for the simultaneous determination of the Minimum Inhibitory Concentration (MIC) of a large number of strains.

The same PVA/Ag Nps sample (concentration of silver precursor 3.9 mg/ mL) against 21 clinical isolates from Staphylococcus sp., 24 clinical strains E. coli and 26 P. aeruginosa was tested [51].

The tested clinical strains of S. aureus and S. saprophyticus have demonstrated resistance to six antimicrobial substances. MIC (calculated from the outgoing concentration of silver precursor) for all staphylococci was ≥24.4 μg/mL with the exception of four strains, where in it is lower. In two of the tested strains S. aureus MIC was 12.2 μg/mL, while in to the other two the MIC was 6.1 μg/mL.

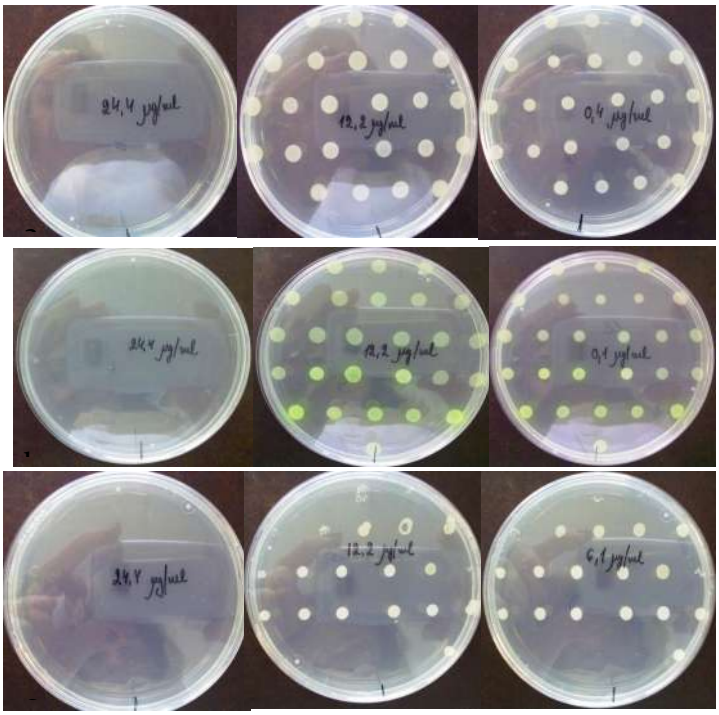

Figure 3. Agar dilution method for testing the MIC of the PVA/AgNps to: a) *E.coli*, b) *P.aeruginosa*, c) *S.aureus* and *S. saprophyticus*.

The tested clinical strains of E. coli have established resistance to 11 antibiotics, and the explored clinical P.aeruginosa strains,they were resistant to eight antibiotics. MIC (calculated from the outgoing concentration of silver precursor) for all tested Gram negative bacteria was ≥ 24.4 μg/mL.

5.2.3. Determination of Minimal Bactericidal Concentration (MBC) of the tested bacterial strains.

For the determination of the MBC according to the method of macro dilution PVA/Ag Nps sample with determined by ICP silver concentration 156, 902 mg/L was used.

Four multi resistant clinical isolates were selected - two strains P.aeruginosa, one strain E.coli, isolated from humans and one strain A. Baumanii isolated from an ear infection in a dog.

The MBC for all of them was established as ≥ 0,12 mg / L. These multidrug-resistant clinical strains were P.aeruginosa 1773, resistant to Pi, Cz, Ct, Ce, Azt, I, G,Cp; P.aeruginosa 1570, resistant to Pi, Ct, Cz, ,Ce, Azt,G,Cp; E.coli № 5, resistant to A, A/S, AmC, Cx, Cz, Ct, Cm, Cft,Ce, Azt, G,Cp and A.baumanii, resistant to Pi, A/S, Cz, Ct, Cft, Ce, I, G, Tb, Am, T, D, Cp, S/T (Piperacillin (Pi) , Ampicillin/sulbactam (A/S), Ceftazidime (Cz), Cefuroxime (Cx), Cefotaksime (Ct), Ceftriaxone (Cft), Cefepime (Ce), Imipenem (I), Gentamicine (G), Tobramicine (Tb), Amikacine (Am), Cephamandole (Cm), Tetracycline (T), Doxycycline (D), Ciprofloxacin (Cp), Sulfamethoxazole/ Trimethoprim (S/T), Aztreonam (Azt), Amoxicilin/Clavulanic acid (AmC)). Other 30 Pseudomonas sp., Klebsiella sp. and Salmonella sp. Iliev, M., 2013; Iliev, M., 2013) clinical strains, tested with synthesized sample PVA/AgNps (ICP 175.9 mg/L) were with established MBC ≥ 0.5 mg/L.

5.2.4. Determination of Minimal Fungicidal Concentration (MFC) of the tested yeasts.

The hybrid materials (PVA/AgNps) were investigated using with a serial macro-dilutions method. To determine the Minimal Fungicidal Concentration (MFC) of the control and the first five clinical strains, standarts of CLSI M26-A and CLSI M27-A2 were applied in modified variant.

It was established that the MFC (calculated from the outgoing concentration of silver precursor) for the control strains Candida albicans and Candida krusei was lower than 14.5 mg/L, for Candida tropicalis MFC was 28.99 mg/L, for Candida glabrata – 115.98 mg/L and for Aspergillus brasiliensis MFC was 927.81 mg/L [51].

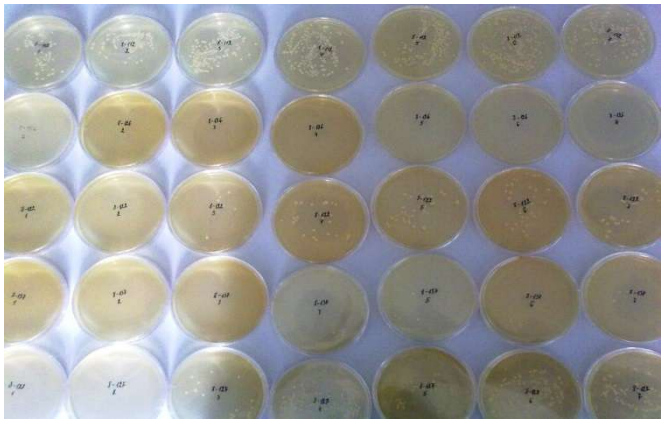

Figure 4. Determination of MFC of PVA / AgNps for clinical Candida spp.isolates.

In order to establish the fungicidal activity of PVA/Ag N ps against clinical strains with proven resistance to one or more anti mycotics, five clinical yeast strains were used and their MFCs were determined. The established values for MFC differ from the results obtained for the control strains. The MFC (calculated from the outgoing concentration of silver precursor) for *Candida albicans* 8-127 and *Candida glabrata* 8-122 was 463.91 mg/L, for *Candida albicans* 8-137 - 231.95 mg/L, for *Candida krusei* 8-126 MFC was lower than 14.5 mg/L. Summary all results demonstrated a pronounced fungicidal activity against all tested control and clinical strains with MFC (calculated from the outgoing concentration of silver precursor) in the range of 980 to 30 mg/L. An exception was *C. krusei* 8-112, where no fungicidal activity of the tested PVA/AgNps was observed [52].

The results indicate that the most sensitive control strains were *C. tropicalis* and *C. krusei* with MFC (calculated from the outgoing concentration of silver precursor) <30 mg/L akin to PVA/AgNps. Further, C. krusei 8-112 strain was found to be resistant to silver in PVA/AgNps respectively at as high as 1960 mg/L Ag concentration. This concentration (1960 mg/L) was more than 5 times greater than those established for the other types of clinical strains, which is indicative for the presence of silver resistance strain.

Table 1. Determined with macro-dilution method MBC and MFC of the tested with PVA/AgNps clinical microorganisms.

№	Clinical strains of microorganism/s	MBC/MFC (mg/L)
1.	*2 strains P.aeruginosa*	≥ 0,12
2.	*E.coli*	≥ 0,12
3.	E.coli O104	0.3 ± 0,1
4.	*A.baumanii*	≥ 0,12
5.	*Bacterium NLAE*-zl-H515	6,25
6.	*Serratia marcescens*	3.125
7.	9 strains *Klebsiella pneumonia*	≥0,5
8.	1 strain *Klebsiella pneumonia*	≥1,1
9.	4 strains *Salmonella cholerasuis*	≥0,5
10	*6 serovars Salmonella enterica*	≥1,1
11	*10 strains Pseudomonas aeruginosa*	≥1,1
12	*C. krusei* 8-48	<0,27
13	*C. parapsilosis* 0-115	<0,27
14	*C. glabrata* 0-73	<0,27
15	*C. nivariensis* 383	<0,27

An another four clinical *Candida strains* (*C. krusei* 8-48, *C. parapsilosis* 0-115, *C. glabrata* 0-73, C. nivariensis 383) were tested with the second sample (silver concentration determined by ICP: 140 mg/L) using another validated method, by adding such a quantity of the suspension to each tube of the reaction system, which guarantees the submission of 105-106 CFU /mL [53]. MFC of the PVA/Ag Nps sample for all of them was defined as less than 0.27 mg/L.

Summarized data from the established MBC and MFC (determined on the base of ICP or AAA silver concentration) for all of the tested with this hybrid material bacterial and yeast clinical strains are given in Table 1.

5.3. Silver resistance.

The wide use of Nano silver in consumer goods, medical devices and other products may also increased the susceptibility of bacteria to acquire resistance to silver. There are a sufficient number of reports of established silver resistance in bacteria. In

1975, McHugh *et al*. describe the first case of strain Salmonella typhimurium, resistant to silver isolated in a hospital ward for burns. In other studies is identified silver resistance in members of the family *Enterobacteriaceae* and *P. aeruginosa*, isolated too from patients with burns [54].

Resistance to silver can also be induced in the laboratory and it is easiest to develop into bacteria with an already established mechanisms of resistance to antibiotics, such as methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin resistant enterococci (VRE), enterobacteria with production of a broad spectrum beta lactamases (ESBL), multiresistant *Pseudomonas aeruginosa*" [55].

Recognized are multiple mechanisms of resistance to heavy metals [56]:

1. Bacteria generate cell surface proteins that bind heavy metals by forming a barrier which prevents penetration of metal into the cell.
2. Other metal-detoxifying proteins are produced in the cell cytoplasm of bacteria, yeasts and other fungi, even in the multicellular invertebrates and vertebrates. These small (30 to 50 kd) cytoplasmic proteins are called by various names, such as metal lothionein, metal binding proteins rich in cysteine membrane associated proteins, protein sequencing, and etc.. They all bind copper, silver and many other heavy metals which may interact with amino acids (cysteine) and can't initiate toxic effect. Metal lothionein are simple products of single genes amplified easily and thus develop a metal resistance.
3. Efflux pump is active biochemical transport system which is associated with the silver or copper, and transports it to the cell surface where discarded it. This mechanism of resistance may be a reason for the emergence and cross-resistance to silver and various antibiotics.
4. Often enzymes or other proteins which are subject to the toxic effects are modified, resulting in reduced their sensitivity to copper and silver, and thus avoiding the other detoxification mechanisms.
5. From bacteria were isolated also plasmids encoding resistance to silver, arsenic, cadmium, chromium, copper, mercury, nickel, lead, antimony, thallium and zinc. Bacterial resistance to silver can be transferred between bacteria by plasmid DNA (pMGH100), which includes 9 genes in three transcriptional units. According to the publication [24] plasmid encoding pMGH100 silver resistance was found in Salmonella. Centrally located six genes are found and are functioning in the chromosome of Escherichia coli K-12, as is found in the genome of E. coli O157: H7.

Currently is not fully understood how irreversible development has the resistance to silver products in clinical conditions.

5.3.1. Combination of silver nanoparticles with different antimicrobial.

In different publications are presented data for established synergistic effect by combination of silver nanoparticles alone or as component in hybrid materials with: amoxicillin [57], rifampicin [58], ampicillin, kanamycin, erythromycin, chloramphenicol, polymyxin B [59]. This kind of materials are broad-spectrum but not selective and their combination with antimicrobials could can solve the problem with this disadvantages.

The diameter of the inhibition zone is evidence for bactericidal activity and is also result of the released in the agar media silver ions. But the absence of any inhibition zone of the silver ion does not be interpreted as lack of activity. It could be also result of impossibility of the functional ion to diffuse in the nutrient media. That`s why by analyzing the results of Disk diffusion testing for synergism they must be interpreted very carefully. In the literature are reported data for investigation of the effect by combination of silver nanoparticles and different antibiotics (penicillin G, amoxicillin, carbenicillin, cephalexin, cefixime, erythromycin, gentamicin, amikacin, tetracycline, Co-trimoxasole, clindamycin, nitrofurantoin, nalidix acid and vancomycin) toward S. aureus and E. coli using DDM with disks, impregnated with solution of both of the tested substances. The increased activity was established by their combination with penicillin G, amoxicillin, erythromycin, clindamycin and vancomycin.

5.3.2. A checkerboard testing method of the synergism of clinical isolates resistant to antibiotics, in combining antibiotics with PVA / Ag Nps.

Resistant to Pi an Ce strains when tested by DDM on CLSI [51] were included in experiment with checkerboard method.

When hybrid material (157 mg/L silver concentration determined by ICP-OES) was tested, the MBCs for both strains were determined as 0.12 mg/L. P. aeruginosa strain did not show sensitivity to piperacillin (Pi) even at 128 mg/L and the strain E. coli - is resistant even to 32 mg/L cefepime (Ce).

$$FBC(Pi)=\frac{MBC(Pi\text{ in the presence of PVA/AgNps})}{MBC(Pi\text{ alone})}$$

FBC (Pi)= 32/256= 0,125

$$FBC(PVA/AgNps)=\frac{MBC(PVA/AgNps\text{ in the presence of Pi})}{MBC(PVA/AgNps\text{ alone})}$$

FBC (PVA/AgNps)= 0,25/0,12= 2,1

ΣFBC= FBC (Pi)+FBC (PVA/AgNps)= 2,225

$$FBC(Ce)=\frac{MBC(Ce\text{ in the presence of PVA/AgNps})}{MBC(Ce\text{ alone})}$$

FBC (Ce)= 8/64=0,125

$$FBC(PVA/AgNps)=\frac{MBC(PVA/AgNps\text{ in the presence of Ce})}{MBC(PVA/AgNps\text{ alone})}$$

FBC (PVA/AgNps)= 0,25/0,12=2,1

ΣFBC= 2,225

Figure 5. Checkerboard method applied to PVA/AgNps combined with Piperaciline (Pi) or Cefepime (Ce) onto E. coli.

The results indicated that, self-administered, the hybrid material has a higher antibacterial activity than in combination with antibiotics. Thus, the hybrid material with a silver concentration 0.25 mg/L exhibits bactericidal effects on the test strain, while its combination in the same concentration with Pi: in concentration - 32mg/L and 16mg/L and with Ce: in concentration 16 mg /L does not exhibit such action.

If the definitions of the terms adopted by EUCAST were applied then this result could be defined as presence of antagonistic activity. At lower concentrations of piperacillin the bactericidal activity of the hybrid material was again exhibited. Calculation of Fractional bactericidal concentration (FBC) [60] with those obtained results are greater than 2, wherein the combined effect is evaluated as antagonism.

Testing for presence of synergism was performed by combining of PVA/AgNps with ceftazidime on Klebsiella 2494 [61]. The calculation of Fractional bactericidal concentration (FBC) [60] on the combined action of ceftazidime and the hybrid material was:

$$FBC(Cz) = \frac{MBC(Cz \text{ in the presence of } {}^{PVA}/_{AgNps})}{MBC\ (Cz\ alone)}$$

FBC(Cz)=0.25/2=0.125

FBC (PVA/AgNps)=0.234/0.937=0.25

ΣFBC= FBC (Cz)+FBC (PVA/AgNps)= 0.375

Figure 6. Checkerboard method applied to PVA/AgNps and Ceftazidime (Cz) onto Klebsiella 2494.

The result was lower than 2, and according to EUCAST, the combined effect was reported as sinergism.

Testing for presence of synergism was performed also by combining of PVA/Ag Nps with ceftazidime on Salmonella Paratyphi B 176 [62]. The calculation of Fractional bactericidal concentration (FBC) [60] on the combined action of ceftazidime and the hybrid material was:

$$FBC(Cz) = \frac{MBC(Cz \text{ in the presence of PVA/AgNps})}{MBC\ (Cz\ alone)}$$

FBC(Cz)=0,25/8=0,03

$$FBC(PVA/AgNps) = \frac{MBC(PVA/AgNps\ in\ the\ presence\ of\ Cz)}{MBC\ (PVA/AgNps\ alone)}$$

FBC(PVA/AgNps)=0,234/0,468=0,5

ΣFBC= FBC (Cz)+FBC (PVA/AgNps)= 0,53

Figure 7. Checkerboard method applied to PVA/AgNps and Ceftazidime (Cz) onto Salmonella Paratyphi B 176.

The result was lower than 2, and according to EUCAST, the combined effect was reported as sinergism.

Further, the presence of synergism was investigated combining PVA/AgNps (initial silver concentration 3900 mg/L) and antifungal agents, using a commercial product ATB™. A synergistic effect was observed when PVA/AgNps material with decreasing concentrations of antifungal agents was used. Similar effect was observed even for C.krusei 8-112 strain which was resistant to PVA/AgNps when used singularly. However, not all possible combinations of dilutions for both combined agents have been performed and will be a part of our future research reports [50].

5.3.3. Cyto toxicity.

Evaluation of cyto toxicity was an important part of the assessment of a potential antibacterial agent since the beneficial compound should be selective for bacteria-specific processes with little or no effects on the metabolisms of host-organism cells. After that we hold our experiments for determining the anti-bacterial activity of the PVA/AgNps hybrid material were preceded by determination of its cytotoxic effect *in vitro* of different cell lines. Cell viability was estimated by a modification of the MTT [3-4,5-dimethylthiazole-2-yl)-2,5dipheniltetrazolium bromide] assey [63]. At this way are determined, that mitochondria of the cells,which are metabolically active. The MTT reduction assay was one of the most frequently used methods for measuring cell proliferation and cytotoxicity. This test was based on the reduction of the soluble yellow MTT tetrazolium salt to a blue insoluble MTT formazan product by mitochondria succinic dehydrogenase [63]. Since its development, this assay has been modified by various investigators [64, 65, 66] and has been used primarily with tumour cells and, to a lesser extent, with fibroblast cell lines, to evaluate the cytotoxicities of chemotherapeutic agents [64, 67]. In our experiments we used three different monolayer cell lines of mammalian origin: two kidney lines - Madin-Darby canine kidney (MDCK) and monkey kidney (GMK) and one mouse fibroblasts cell line L20B. Cells were seeded in 96-well microplates (Nunclon) at a concentration of 5 x 104 cells/well for MDCK cell line and 6 x 104 cells/well for GMK and L20B cell lines. Confluent monolayer were washed, covered with media containing the tested compound in concentrations 150 µg/ml, 100 µg/ml, 50 µg/ml, 20 µg/ml, 5 µg/ml, and from 1 µg/ml to 0,00001 µg/ml and cultured at 37°C for 24h and 48h.

Cells grown in medium without compounds served as a control. After 24h or 48h incubation, the medium was replaced with MTT (Sigma) and dissolved at a final concentration of 5 mg/ml in serum-free medium, for further 3h incubation. Then, the MTT-formazan product was solubilised in ethanol : DMSO (1 : 1), and the optical density was measured at a test wave length of 540 nm [68].

Cytotoxic concentration 50% (CC50) was defined as the concentration of the test substance at which 50% of the cells die as a result of toxicity of the substance. Maximal non-toxic concentration (MNC) was defined as the highest concentration of the test substance which does not cause injury or death of the treated cells.

Using dose-response curves on the 24h and 48h we calculated maximal nontoxic concentration (MNC) and concentration required to inhibit cell viability by 50% (CD50). When microscopic observation of the morphology of the mono layers were carried out at 48h after the treatment with tested compound solution in concentration range from 150 µg/ml to 100 µg/ml we found some morphology changes of GMK and L20B cell lines in comparison with the cell control only in treated wells with highest concentration 150 µg/ml. The growth of all tested cell

lines was suppressed in a dose-dependent manner. When a treatment with lower concentrations was performed, no essential change was registered in the monolayer in comparison with cell control.

On the basis of the data from cyto toxicity experiments, we calculated the therapeutic efficacy (TE) - CC50 (Cytotoxic Concentration 50%) to MNC ratio. The ratio characterizes the tolerable concentration range in which the particular compound could be applied avoiding significant cell alterations.

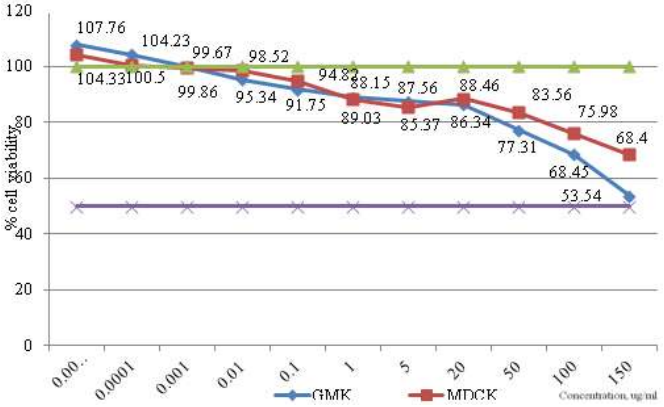

Figure 8. Cytotoxic activity of PVA/Nps on GMK and MDCK cell lines.

The hybrid material with included silver nanoparticles was not cytotoxic on the three cell lines tested at concentrations ranging from 0.001 to 1 mg/L. Tested for cytotoxicity hybrid material PVA/Ag Np on MDCK, GMK and L20B cell lines showed very good therapeutic effect.

Specified minimum bactericidal concentration and some evidence of therapeutically efficiency (TE) can determine the nearest appropriate range of their application at given silver concentration.

5.3.4. *In vivo* experiments.

As a practical application of the examined hybrid material with the silver Nano particles it is applied as an experimental drug in pet-dogs. However, initially it was tested on laboratory animals - white mice and rabbits. The dermal cytotoxicity test and subcutaneous injections on white mouse BALB c showed PVA/AgNps as a non-toxic in the enclosed silver concentration [69]. The synthesized sample was used in a dermal test conducted on white mice at silver concentration determined by ICP as 156, 902 mg/L. After mechanical working on the test field there were not established skin wounds.

The used in the test hybrid material was with silver concentration 5.2 times higher than the announced in the literature on cytotoxicity when tested on human fibroblasts. However, there is not any redness in the experimental field. After 24 hours, the experiment was repeated and the result was the same.

Skin was calm and fur had grown by about 0,5 cm on the experimental field, which can be considered as aseptically processed and used as an operational field. Upon subsequent deep scarification is reported the absence of redness and dermal infection. Scarify areas healed without scarring.

This is the same sample hybrid material, but diluted to a concentration of the silver, 30 mg / l was used in two tests for bio-toxicity. In the first one subcutaneous injections of 2 ml of the hybrid material PVA / AgNps were injected on a white mouse “BALB c” with weight 20 g [50]. After the performed test for bio-toxicity the mouse used, stayed alive and in perfect health, traced for about a month after the intervention. In the second experiment the whole cycle of intravenous immunizations of the rabbit was finished successfully. An agglutinating antiserum E.coli O104 was produced with determined O-antigen titer 1600. The hybrid material PVA / AgNps has been used also for preservative of the obtained in consequence of the immunization hyper-immune E. coli O104 rabbit antiserum which stores its titer three years after the preservation [70].

In the intravenous immunization scheme, involving the killed via the hybrid material an antigen from control strain E. coli O104 Kopenhagen, at the end of the immunization cycle the hare stayed alive, clinically healthy and with normal vital signs. Previously proven highly bactericidal activity of the tested hybrid material PVA/AgNps, the occurrence of fungicidal activity at a concentration of silver nano particles 30 mg/L, and the positive test results for bio-toxicity are sufficient reason to start testing it as a vaccine preservative.

The hybrid material PVA/Ag N ps with a concentration of 30 mg/L was administered by a veterinarian for the local treatment of skin and wound infections in pets-dogs [51].

In the first case of purulent wounds in the leg, caused by awn, they are treated on the course of the wound using a sterile swab soaked in a hybrid material where upon its astringent effect is observed and subsequently confirmed the expected bactericidal action of that preparation, with the result that the wounds are healed uncomplicated. In the treatment of wounds in the ears with the hybrid material solution was reused, after heavy irrigation of a sterile swab with a long handle which allows local processing them even when they are located in the depth of the ear canal. There was again found an astringent and healing effect. There are good results of the clinical application of the hybrid material with silver concentration of approximately 200 mg/L in pets-dog as a therapeutic agent for cough [71] and by silver concentration 600 mg/L for application by recurrent otitis externa in Samoyed dog.

6. CONCLUSIONS

The hybrid nanomaterial PVA / Ag Nps has proven concentration dependent bactericidal and fungicidal properties, when they tested even at antibiotic resistant strains. Administered into appropriate dosages it is suitable for treatment of both superficial and subcutaneous purulent wounds, recurrent ear infections, and cough with mixed etymology. Data were obtained for synergistic clinical effect by combining of the PVA / AgNps with antibiotics. Positive tests exist in its combination with antibiotics and anti-allergic agents. These data confirm the possibilities for its use as a therapeutically agent with prospects for expanding the scope of its application.

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