"The effect of lanthanides and their nanoparticles on pathogenic bacteria"
ABSTRACT:

The main aim of this study is to obtain data based on an experimental procedure and check the effectiveness of some antibacterial agents against several pathogenic bacteria. In the present experiment, the cultivation of four different bacterial strains was carried out: *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli* and Methicillin Resistant *Staphylococcus aureus*. Two different nanoparticles (Cadmium Telluride Quantum dots with Lanthanides: Gadolinium and Terbium) were used to check their antibacterial properties. The Cadmium Telluride Quantum dots without the Lanthanides, Gadolinium nitrate and Terbium nitrate were also tested on those bacteria. In the present experiment, four methods were employed: disc-diffusion test, determination of the minimal inhibitory concentration, the determination of the growth properties of the bacteria and the analysis of live and dead cells using fluorescence microscopy. From the results, it has been found that the tested Cadmium Telluride Quantum dots with Lanthanides (Gadolinium and Terbium) have good antimicrobial effects. The Gram-negative bacterial strains were found to be more susceptible to the Cadmium Telluride Quantum dots with Lanthanides (mostly Gadolinium) compared to Gram-positive bacteria.

KEYWORDS:

Bacteria, pathogenic, Lanthanides, quantum dots, nanoparticles.
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MAIN OBJECTIVE:

The bacteria selected for the experiment have a great importance at a clinical level, especially methicillin-resistant \textit{S. aureus} (MRSA), as it developed resistance to the antibiotics that are used more commonly. Thus, the main target of this project is to find out if the Cadmium Telluride Quantum dots (CdTe QD) work against these bacteria and if their effectiveness increases if they are modified with Lanthanides.

INTRODUCTION:

\textit{Staphylococcus aureus} is a gram-positive, round-shaped bacterium that is a member of the Firmicutes, and is frequently found in the nose, respiratory tract, and on the skin. It is often positive for catalase and nitrate reduction and is a facultative anaerobe that can grow without the need for oxygen\cite{1}. \textit{S. aureus} is not always pathogenic, but pathogenic strains often promote infections by producing virulence factors such as potent protein toxins, and the expression of a cell-surface protein that binds and inactivates antibodies.

\textit{Staphylococcus} was first identified in 1880 in Aberdeen, Scotland, by surgeon Sir Alexander Ogston in pus from a surgical abscess in a knee joint\cite{2}. Friedrich Julius Rosenbach, who was credited by the official system of nomenclature at the time, amended this name to \textit{Staphylococcus aureus}. An estimated 20\% to 30\% of the human population are long-term carriers of \textit{S. aureus}\cite{3} which can be found as part of the normal skin flora, in the nostrils\cite{4}, and as a normal inhabitant of the lower reproductive tract of women\cite{5}. \textit{S. aureus} can cause a range of illnesses, from minor skin infections, such as pimples, impetigo, boils, cellulitis, folliculitis, carbuncles, scalded skin syndrome, and abscesses, to life-threatening diseases such as pneumonia, meningitis, osteomyelitis, endocarditis, toxic shock syndrome, bacteremia, and sepsis. It is still one of the five
most common causes of hospital-acquired infections and is often the cause of wound infections following surgery.

*Escherichia coli* is a gram-negative, facultative anaerobic, rod-shaped, coliform bacterium of the genus *Escherichia* that is commonly found in the lower intestine of warm-blooded organisms (endotherms)[6, 7]. Most *E. coli* strains are harmless, but some serotypes can cause serious food poisoning in their hosts, and are occasionally responsible for product recalls due to food contamination[8]. The harmless strains are part of the normal flora of the gut, and can benefit their hosts by producing vitamin K2, and preventing colonization of the intestine with pathogenic bacteria, having a symbiotic relationship[9, 10]. *E. coli* is expelled into the environment within faecal matter.

*E. coli* and other facultative anaerobes constitute about 0.1% of gut flora[11], and faecal–oral transmission is the major route through which pathogenic strains of the bacterium cause disease. Cells are able to survive outside the body for a limited amount of time, which makes them potential indicator organisms to test environmental samples for faecal contamination. A growing body of research, though, has examined environmentally persistent *E. coli*, which can survive for extended periods outside of a host[12].

The bacterium can be grown and cultured easily and inexpensively in a laboratory setting, and has been intensively investigated for over 60 years. *E. coli* is a chemo-heterotroph whose chemically defined medium must include a source of carbon and energy. *E. coli* is the most widely studied prokaryotic model organism, and an important species in the fields of biotechnology and microbiology, where it has served as the host organism for the majority of work with recombinant DNA[13].
*Pseudomonas aeruginosa* is a common Gram-negative, rod-shaped bacterium that can cause disease in plants and animals, including humans. A species of considerable medical importance, *P. aeruginosa* is a multidrug resistant pathogen recognised for its ubiquity, it is intrinsically advanced antibiotic resistance mechanisms, and its association with serious illnesses – especially hospital-acquired infections such as ventilator-associated pneumonia and various sepsis syndromes.

The organism is considered opportunistic insofar as serious infection often occurs during existing diseases or conditions – most notably cystic fibrosis and traumatic burns. It is also found generally in the immunocompromised but can infect the immunocompetent as in hot tub folliculitis. Treatment of *P. aeruginosa* infections can be difficult due to its natural resistance to antibiotics. When more advanced antibiotic drug regimens are needed, adverse effects may result.

It is citrate, catalase, and oxidase positive. It is found in soil, water, skin flora, and most man-made environments throughout the world. It thrives not only in normal atmospheres, but also in low-oxygen atmospheres, thus has colonized many natural and artificial environments, as it is capable of extensive colonization. It uses a wide range of organic material for food; in animals, its versatility enables the organism to infect damaged tissues or those with reduced immunity. The symptoms of such infections are generalized inflammation and sepsis. If such colonizations occur in critical body organs, such as the lungs, the urinary tract, and kidneys, the results can be fatal\[14\]. Because it thrives on moist surfaces, this bacterium is also found on and in medical equipment, including catheters, causing cross-infections in hospitals and clinics. It is also able to decompose hydrocarbons and has been used to break down tarballs and oil from oil spills\[15\].
Methicillin-resistant *Staphylococcus aureus* (MRSA) is a gram-positive bacterium responsible for several difficult-to-treat infections in humans. MRSA is any strain of *Staphylococcus aureus* that has developed, through horizontal gene transfer and natural selection, multiple drug resistance to beta-lactam antibiotics. β-lactam antibiotics are a broad-spectrum group which includes some penams – penicillin derivatives such as methicillin and oxacillin, and cephems such as the cephalosporins. MRSA evolved from horizontal gene transfer of the mecA gene to at least five distinct *S. aureus* lineages[16]. Strains unable to resist these antibiotics are classified as methicillin-susceptible *Staphylococcus aureus* (MSSA). The evolution of such resistance does not cause the organism to be more intrinsically virulent than strains of *S. aureus* that have no antibiotic resistance, but resistance does make MRSA infection more difficult to treat with standard types of antibiotics and thus more dangerous.

MRSA is prevalent in hospitals, prisons, and nursing homes, where people with open wounds, invasive devices such as catheters, and weakened immune systems are at greater risk of nosocomial infection (hospital-acquired infection). MRSA began as a hospital-acquired infection, but has developed limited endemic status and is now community-acquired as well as livestock-acquired. The terms HA-MRSA (healthcare-associated MRSA), CA-MRSA (community-associated MRSA) and LA-MRSA (livestock-associated) reflect this distinction.

The lanthanides comprise the fifteen metallic chemical elements with atomic numbers 57 through 71, from lanthanum through lutetium. These fifteen lanthanide elements are often collectively known as the rare earth elements. Lanthanide elements have a practically unlimited abundance[17].

They share many similar characteristics, which include the following:

- Similarity in physical properties throughout the series
- Adoption mainly of the +3 oxidation state. Usually found in crystalline compounds
- They can also have an oxidation state of +2 or +4, though some lanthanides are most stable in the +3 oxidation state.
- Adoption of coordination numbers greater than 6 (usually 8-9) in compounds
- Tendency to decreasing coordination number across the series
- A preference for more electronegative elements (such as O or F) binding
- Very small crystal-field effects
- Little dependence on ligands
- Ionic complexes undergo rapid ligand-exchange

Lanthanides have similarities in their electron configuration, which explains most of the physical similarities. These elements are different from the main group elements in the fact that they have electrons in the f orbital. After Lanthanum, the energy of the 4f sub-shell falls below that of the 5d sub-shell. This means that the electron starts to fill the 4f sub-shell before the 5d sub-shell.

Lanthanides have also magnetic characteristics. The major magnetic properties of any chemical species are a result of the fact that each moving electron is a micromagnet. The species are either diamagnetic, meaning they have no unpaired electrons, or paramagnetic, meaning that
they do have some unpaired electrons. The diamagnetic ions are: La\textsuperscript{3+}, Lu\textsuperscript{3+}, Yb\textsuperscript{2+} and Ce\textsuperscript{4+}. The rest of the elements are paramagnetic\textsuperscript{[18, 19]}.

**MATERIALS & METHODS:**

**Chemical compounds**

All the reagents for quantum dots synthesis, standards, and other chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA) in ACS purity, unless noted otherwise. Media for cultivation of microorganisms were purchased from OXOID CZ (ThermoFisher Scientific - CZ)

**PREPARATION OF HOSPITAL SAMPLES AND THEIR CULTIVATION**

**Collection of wound swabs from the patients with bacterial infections**

The smears, collected from infected wounds with the agreement of patients from Trauma Hospital in Brno, were sampled by rolling motion at the wound using a sterile swab sampler. All patients were divided into two subgroups, on the grounds of infection severity: deep and superficial wound. A detailed description of comorbidities and duration of treatment was obtained. Patients were classified according to the Classification of surgical wounds – SSI (surgical site infections). Infected wounds were sampled by using disposable tampon swabs maximizing collection of representative microflora. Tampons were subsequently stored in transport medium (inorganic salts, sodium thioglycolate, 1% agar, activated charcoal). The important part of our work-flow process comprised sampling in duplicates with further transport in both aerobic and anaerobic conditions to preserve bacterial viability\textsuperscript{[20]}. 
Cultivation of clinical specimens

Four types of selective nutrient media (blood agar enriched by 10% NaCl, Endo agar, blood agar without any other component, and blood agar with amikacin) we employed for further microbiological selection. Petri dishes, containing the above mentioned media were subsequently incubated according to conventional protocols, as described elsewhere, to maintain suitable conditions for growth of all types of bacteria. These Petri dishes were incubated for 24–48 h at 37°C supplemented by TGY medium (1 g L−1glucose, 5 g L−1tryptone, 2.5 g L−1yeast extract). Subsequently, individual colonies were collected from each Petri dish and stored in 1 µL of enriched media. These samples were processed and utilized for both — MALDI-TOF MS identification and PCR with subsequent sequencing. The glycerol stocks were prepared from bacterial cultures and 80% glycerol for long-term storage and further use [20].

Cultivation of bacterial strains

Four identified pathogenic bacterial strains *Staphylococcus aureus; Escherichia coli; Pseudomonas aeruginosa* and methicillin-resistant *Staphylococcus aureus* from the infected wounds of the patients were cultivated. The composition of cultivation medium was as follows: Mueller Hinton broth 21 g and 1000 mL distilled water with 18 MΩ. Mueller Hinton agar 38 g into the 1 000 mL of distilled water. The pH of the cultivation medium was adjusted to pH 7.4. Prior experiments, the cultures were diluted by Phosphate-buffered saline (PBS) to OD 600 nm = 0.5 McF standard [20]. PBS was prepared with NaCl 8 g, KCl 0.2 g, Na₂HPO₄ 1.44 g, KH₂PO₄ 0.2 g and 1000 mL of miliQ water [20].

Minimal inhibitory concentration (MIC) Determination
The susceptibility of bacterial cultures was determined by the standard broth microdilution method (EUCAST) and detected by the unaided eye. The MIC was defined as the lowest concentration of antimicrobial agent that inhibited the bacterial growth. The MIC of antimicrobial agents was determined by preparation of solutions with increasing concentrations. The different concentrations of solutions were added to the microplate wells and were mixed with bacterial cultures ($\text{OD}_{600\text{nm}} = 0.5$ McF and next dilution 1:100 with MH medium). The microplate with antimicrobial agents and bacterial cultures was incubated at 37°C for 24 hours. The following day the results were evaluated. The MIC was the concentration of the higher dilution well in which the absence of bacterial growth occurred. As positive control was used MH medium with inoculation [21].

**Analysis of inhibition zones using broth microdilution method**

To determine the antimicrobial effect of compounds on bacterial cultures of *Staphylococcus aureus; Escherichia coli; Pseudomonas aeruginosa* and methicillin-resistant *Staphylococcus aureus*, the measurement of the inhibition zones was performed. Agar surface in Petri dish was covered with a mixture of 0.5 McF standard bacterial cultures (100 µL of 24 h bacterial cultures in the exponential phase of growth, and 3 mL of MH broth). Discs (Ø 0.6 cm) were filled with 10 µL of 2 mM antimicrobial agents. Soaked discs were then laid on a Petri dish. Petri dishes were insulated against possible external contamination and placed in a thermostat (Tuttnauer 2450EL, Israel) at 37°C for 24 h. After 24 hours of incubation, the inhibition zones were measured and photographed in each Petri dish.[22]
**Determination of growth properties**

The second procedure for the evaluation of an antimicrobial effect of antimicrobial agents employed apparatus Multiskan EX (Thermo Fisher Scientific, Germany) for analysis of bacterial growth curves. The diluted cultures (OD\textsubscript{600nm} = 0.5 McF and next dilution 1:100 with MH medium) were pipetted into a microplate (total volume of 200 μL) alone as a control variant, or with various concentrations of antimicrobial agents. The concentrations of compounds were 0; 15.6; 31.25; 62.5; 125; 250; 500; 1000 μM. Measurements were carried out at time 0, then each 30 min for 24 hours at 37°C, at a wavelength of 600 nm[22].

**Live/dead cell analysis**

Microscopic assay for evaluation of live/dead bacterial cells was performed using an inverted Olympus IX 71S8F-3 fluorescence microscope (Olympus, Tokyo, Japan) equipped with Olympus UIS2 series objective LUCPlanFLN 40× (N.A. 0.6, WD 2.7 – 4 mm, F.N. 22) and a mercury arc lamp X-cite 12 (120 W, Lumen Dynamics, Mississauga, Canada) for illumination. Two fluorescent dyes were used for the assay: propidium iodide (PI) for staining of cells with damaged membranes and SYTO9 (Invitrogen AG, Basel, Switzerland) permeating both intact and damaged membranes of the cells [23]. The fluorescence mirror used had excitation wavelength of 460-495 nm, emission wavelength of 510-550 nm and dichroic mirror 505 nm for SYTO9 fluorescence and excitation wavelength of 545-580 nm, emission wavelength of 610 nm and dichroic mirror 600 nm for PI fluorescence, respectively. Images were acquired with a Camera Olympus DP73 (Olympus, Tokyo, Japan) and processed by Stream Basic 1.7 software (Olympus Soft Imaging Solutions GmbH, Münster, Germany) with the software resolution of 1,600 × 1,200 pixels. 5 μL of 10× diluted
bacterial suspension with different substances after 24 h shaking on a shaker at 600 rpm and 37°C was stained with 0.5 μL of fluorescent dye.[24]

**Statistical analysis**

Software STATISTICA (data analysis software system), version 10.0 (Tulsa, Oklahoma, USA) was used for data processing. The general regression model was used to analyse differences between the measured values. To reveal differences between the cell lines, Tukey’s post hoc test within homogenous groups was employed. Unless noted otherwise, $p < 0.05$ was considered significant [23, 24].

**PREPARATION OF CdTe QDS MODIFIED BY A GADOLINIUM-SCHIFF BASE COMPLEX (GDQDS)**

The Schiff base, \[(2-\{(E)\}-2\text{-pyridylmethyleneamino\}}-\text{N}-\{2-\{(E)\}-2\text{-pyridylmethyleneamino\}}\text{ethyl\}}\text{ethanamine}\]} was prepared according to Kopel et al.[25] with few modifications. Briefly, 2-pyridinecarboxaldehyde and diethylenetriamine were mixed and heated. After cooling, MeOH was added to prepare the desired Schiff base solution. In a separate beaker, methanol was mixed with an aqueous solution of gadolinium nitrate, which was subsequently added to the Schiff base solution. The solutions were mixed and the volume was brought to 100 mL with deionized water (Sigma-Aldrich). The prepared Gd-SB solution was stored at 25°C until use.

Microwave preparation of the CdTe QDs was carried out according to our previous study[26] with necessary modifications. Briefly, 53.2 mg of cadmium acetate was mixed with 86 mL of ACS-grade water on a magnetic stirrer, followed by the addition of 60 mg of MSA. Next, 1.8 mL of an ammonia solution (1 M) was then added to attain neutral pH (tested by a pH meter,
inoLab pH 720, WTW, Weilheim, Germany). Then, 1.5 mL of a sodium tellurite solution (221 mg/mL) was added, and the solution was mixed well. Subsequently, 50 mg of sodium borohydride was added to the solution, which was stirred for approximately 2 h until bubble formation ceased, and the volume of the solution was brought to 100 mL with deionized water. Two millilitres of this solution were removed; placed in a small glass vessel and heated at 300 W under microwave irradiation (Multiwave 3000, Anton-Paar GmbH, Graz, Austria) to prepare the CdTe QDs. Next, the Gd-SB solution was added to the prepared CdTe QD solution, followed by heating using 300 W under microwave irradiation to prepare the GdQDs. The sample and control particles were filtered through 0.22 µm membranes and subsequently dialysed against deionized water several times to remove the unreacted initiators. Then, the particles were dispersed in deionized water for further characterization and use.

**PREPARATION OF CdTe QDs MODIFIED BY A TERBIUM-SCHIFF BASE COMPLEX (TBQDS)**

The Schiff base, \((2-\{(E)-2\text{-pyridylmethyleneamino}\}-N-\{2-\{(E)-2\text{-pyridylmethyleneamino}\}\text{ethyl}\text{ethanamine}\})\) was prepared according to Kopel et al.[25] with few modifications. Briefly, 2-pyridinecarboxaldehyde and diethylenetriamine were mixed and heated. After cooling, MeOH was added to prepare the desired Schiff base solution. In a separate beaker, methanol was mixed with an aqueous solution of Terbium nitrate, which was subsequently added to the Schiff base solution. The solutions were mixed and the volume was brought to 100 mL with deionized water (Sigma-Aldrich). The prepared Tb-SB solution was stored at 25°C until use.

Microwave preparation of the CdTe QDs was carried out according to our previous study[26] with necessary modifications. Briefly, 53.2 mg of cadmium acetate was mixed with 86 mL of ACS-grade water on a magnetic stirrer, followed by the addition of 60 mg of MSA. Next, 1.8 mL of an ammonia solution (1 M) was then added to attain neutral pH (tested by a pH meter,
inoLab pH 720, WTW, Weilheim, Germany). Then, 1.5 mL of a sodium tellurite solution (221 mg/mL) was added, and the solution was mixed well. Subsequently, 50 mg of sodium borohydride was added to the solution, which was stirred for approximately 2 h until bubble formation ceased, and the volume of the solution was brought to 100 mL with deionized water. Two millilitres of this solution were removed; placed in a small glass vessel and heated at 300 W under microwave irradiation (Multiwave 3000, Anton-Paar GmbH, Graz, Austria) to prepare the CdTe QDs. Next, the Tb-SB solution was added to the prepared CdTe QD solution, followed by heating using 300 W under microwave irradiation to prepare the TbQDs. The sample and control particles were filtered through 0.22 µm membranes and subsequently dialysed against deionized water several times to remove the unreacted initiators. Then, the particles were dispersed in deionized water for further characterization and use.

RESULTS AND DISCUSSION:

The analysis and discussion of the results are going to be separated depending of the method that was used to get them.

DISC-DIFFUSION TEST

The graphics and pictures below show the results of inhibition zones after the application of circular discs with antibacterial compounds against the different bacterial strains. All bacterial pathogens (OD = 0.5 McF) were exposed to a 2mM antibacterial agent and the incubation was carried out at 37 °C for 24 hours.

- *Staphylococcus aureus*
It can be noticed with the graphic and picture above, that the two compounds with lanthanides have the best results, with a significant difference between the QD CdTe and them. Although GdQD is working better than TbQD against the strain of *S. aureus*.

- *Escherichia coli*

With the strain of *E. coli*, it can be found mostly the same size of the inhibition zones as against *S. aureus*, being GdQD the compound with better results.

- Methicillin-resistant *Staphylococcus aureus* (MRSA)
In these measurement of the inhibition zones can be noticed that all the compounds are less effective against methicillin-resistant *S. aureus*, than with other strains (*S. aureus; E.coli; P. aeruginosa*), but GdQD still being working better.

- *Pseudomonas aeruginosa*
The measurement of the inhibition zones size for *P. aeruginosa* are similar also as with *S. aureus*.

*Figure 5.*: Disc-diffusion method of all compounds against different bacterial strains (Measurement of inhibition zones)

Here we can compare antimicrobial effect by using disc-diffusion method of all the compounds with all the bacterial strains and definitely GdQD is more effective than the rest of them, especially against *E. coli* and *P. aeruginosa*.

**CALCULATION OF GROWTH CURVES**

- *P. aeruginosa*

  The graphics below show that GdQD is the compound with higher effectiveness against *P. aeruginosa* in both measurements (250 µM and 500 µM).
Figure 6.: Growth curves after application of different compounds on *P. aeruginosa* (concentration of the compound is 250 µM)

Figure 7.: Growth curves after application of different compounds on *P. aeruginosa* (concentration of compound is 500 µM)
Figure 7.: Measurement of absorbance of *P.aeruginosa* after 24 hours (concentration of compound is 250 µM)

Figure 8.: Measurement of absorbance of *P.aeruginosa* after 24 hours (concentration of compound is 500 µM)
• *E. coli*

In the graphics below it can be seen that GdQD is much more efficient against *E. coli* with a significant difference between the other compounds. Also, with higher concentration of the compounds (500 µM), higher antibacterial activity is shown.

*Figure 9.*: Growth curves after application of different compounds on *E. coli* (concentration of compound is 250 µM)
**Figure 10.** Growth curves after application of different compounds on *E.coli* (concentration of compound is 500 µM)

**Figure 11.** Measurement of absorbance of *E.coli* after 24 hours (concentration of compound is 250 µM)
Figure 12.: Measurement of absorbance of E.coli after 24 hours (concentration of compound is 500 µM)

- *S. aureus*

In the graphics below can be seen that all the compounds with quantum dots are really efficient against *S. aureus*. Additionally with a higher concentration of the compounds (500 µM), a higher antibacterial activity is shown by the QDs with lanthanides.
Figure 13.: Growth curves after application of different compounds on *S. aureus* (concentration of compound is 250 µM)

Figure 14.: Growth curves after application of different compounds on *S. aureus* (concentration of compound is 500 µM)
Figure 15.: Measurement of absorbance after 24 hours S. aureus (concentration of compound is 250 µM)

Figure 16.: Measurement of absorbance after 24 hours S. aureus (concentration of compound is 500 µM)
- MRSA

In the graphics below it can be seen that all the compounds are efficient against MRSA. They are not lethal to the bacteria but they can decrease it. Nevertheless none of them are enough effective to die out the bacteria.

Figure 17.: Growth curves after application of different compounds on MRSA (concentration of compound is 250 µM)
Figure 18.: Growth curves after application of different compounds on MRSA (concentration of compound is 500 µM)

Figure 19.: Measurement of absorbance of MRSA after 24 hours (concentration of compound is 250 µM)
Figure 20: Measurement of absorbance of MRSA after 24 hours (concentration of compound is 500 µM)

MEASUREMENT OF MINIMAL INHIBITORY CONCENTRATION (MIC)

Table 1: Minimal inhibitory concentration of different compounds against pathogenic bacteria

<table>
<thead>
<tr>
<th>MIC (µM)</th>
<th>P. aeruginosa</th>
<th>E. coli</th>
<th>S. aureus</th>
<th>MRSA</th>
</tr>
</thead>
<tbody>
<tr>
<td>QD CdTe</td>
<td>≥ 1000</td>
<td>≥ 1000</td>
<td>270</td>
<td>≥ 1000</td>
</tr>
<tr>
<td>Gd QD</td>
<td>125</td>
<td>275</td>
<td>125</td>
<td>600</td>
</tr>
<tr>
<td>Tb QD</td>
<td>62.5</td>
<td>370</td>
<td>140</td>
<td>630</td>
</tr>
<tr>
<td>Gadolinium nitrate</td>
<td>≥ 1000</td>
<td>≥ 1000</td>
<td>≥ 1000</td>
<td>≥ 1000</td>
</tr>
<tr>
<td>Terbium nitrate</td>
<td>≥ 1000</td>
<td>≥ 1000</td>
<td>≥ 1000</td>
<td>≥ 1000</td>
</tr>
</tbody>
</table>
In the table above it can be seen on one hand, that the lanthanides without quantum dots need a high concentration to get the inhibitory activity for all of the strains. It is the same conditions for the QD without lanthanides, except with S. aureus that has a lower number. On the other hand, both Gd QD and Tb QD have lower concentration to get the inhibitory for the bacteria. Against P. aeruginosa Tb QD is succeeding in a better way, but with the rest of the strains Gd QD works better.

**LIVE AND DEAD CELLS ASSAY**

**P.aeruginosa**

- Control
- QD CdTe
- Gd QD
- Tb QD

**E.coli**

- Control
- QD CdTe
- Gd QD
- Tb QD
This assay was done by using a fluorescent microscopy. The concentration of the compounds used was 500 µM. In the pictures, the red spots are dead cells while the green ones correspond to live cells. We can see with these results that the QD CdTe with lanthanides are working better against the bacteria than QD CdTe themselves. Also, Gd QD is the compound that seems more effective.

CONCLUSION:

The aim of this bachelor thesis was to study the antimicrobial effects of Cadmium Telluride Quantum dots with Lanthanides (Gadolinium and Terbium) and the chemicals themselves without Quantum dots against four different pathogenic bacterial strains (Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli and methicillinresistant S. aureus). The antimicrobial
activity of prepared CdTe QD with Lanthanides was measured by a disc-diffusion method, determination of minimal inhibitory concentration, measurement of growth properties, Live/dead cell analysis.

In view of the results obtained with the experiments carried out, it can be established as a conclusion that the QDs with Lanthanides are working better against the bacteria that are gram-negative than the ones that are gram-positive as in general, they showed more effectiveness with the strains of *P. aeruginosa* and *E. coli*. Also it was found that the QDs with Lanthanides can decrease the bacterial growth of methicillin-resistant *S.aureus*.

Additionally, GdQDs showed stronger antibacterial effect than Tb QD and other tested compounds. The use of CdTe QD in combination with Lanthanides (mostly Gadolinium) appears to be a good way for the reduction of bacterial infection.
REFERENCES:


