## **RESEARCH PAPER**

# Cytotoxic and Antibacterial Activity of Yttrium Oxide Nanoparticle Y<sub>2</sub>O<sub>3</sub> Against Serratia Fonticuli and Citrobacter Kasseri Isolated fFrom Cholangitis Patients

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## **ABSTRACT**

The rise of antibiotic-resistant bacteria necessitates the exploration of novel antimicrobial agents. Yttrium oxide nanoparticles (Y2O3) have shown potential due to their unique physicochemical properties and antibacterial activities against various pathogens. This study investigates the cytotoxic and antibacterial effects of Y2O3 nanoparticles against Serratia fonticuli and Citrobacter koseri, bacteria isolated from cholangitis patients. Bacterial strains were isolated from bile specimens and confirmed using standard microbiological techniques. The methods of X-ray diffraction (XRD), (SEM), and Frequency transforminfrared spectroscopic (FT-IR) were used to characterize YO<sub>3</sub> particles. Using a microdilution technique, the minimum concentrations of inhibition (MICS) were calculated for a variety of nanoparticles concentrations. MTT and LDH tests were used to evaluate mortality on cell lines derived from humans. Substantial antimicrobial activity was demonstrated by Y2O3 small particles, which efficiently broke down microbial cellular membranes and produced reactive oxygen molecules (ROS). At different doses, the MIC values showed strong suppression of both species of bacteria. Different reactions from cells of various types were shown by viability experiments, indicating that Y<sub>2</sub>O<sub>3</sub> nanomaterials might be less harmful at particular dosages. Yttrium nanoparticles made of oxide show great promise as disinfectants versus cholangitis-causing microorganisms that are sensitive to antibiotics. In order to promote their use in hospitals, our work emphasizes the necessity of more research into the protective characteristics and mechanism for action of Y2O3 particles.

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#### **INTRODUCTION**

One area in healthcare that is being revolutionized by nanotechnology is the creation of novel drugs. owing to their unique properties,

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such as increased surface area, reactivity, and the potential to enhance drug delivery, oxides of metals, especially particularly their nanotechnology (NPs), have garnered a lot of attention throughout different nanomaterials. The development of new medications is one aspect of medical treatment that nanostructures is revolutionizing. Metallic oxides, and in specifically their nanoparticles (NPs), have attracted a lot of interest among other types of nanostructures due to their distinct physical features, which include improved area of coverage, responsiveness, and the ability to improve drug administration.

The lower susceptibility of drugs to bacteria has made it necessary to research for new antimicrobial agents. Oxide particles have antibacterial properties against a variety of diseases. The experiments show that these nanoparticles can rend bacterial membranes and produce reactive oxygen species (ROS), all of which can lead to the death of cells [1].

Some tests can provide potential risks of NP use by estimating transmembrane health and the longevity of cells, such as MTT and LDH tests, which measure cell death rate and therefore evaluate the effect of these particles on human cells [2]. The main outcome of choleretics is the bacterial infection. This is an outstanding puff of the gastrointestinal tract. The *Cit koseri* and *scalloped fonticuli* bacteria are species associated with a condition called.

The microbe the ship Serratia fonticuli is a Gram-negative microbe that has been obtained from a range of healthcare settings. It is famous for existing in harsh environments and for its ability to cause damage to human life, particularly vulnerable individuals. Recent studies have found that it is powerful versus common therapies, making it a crucial area of research for novel antimicrobial techniques [3,4]. Citrus bacterium koseri are therefore a distinct type of Gramnegative bacteria associated with infections.

It is especially noteworthy for its capacity to spread throughout newborns' neurological systems. Different methods for treatment are desperately needed, as evidenced by the increasing prevalence of resistant to antibiotics amongst C. koseri genotypes in question [5].

In hospitals, serrate fonticuli and Citrobacter species koseri are acknowledged as aggressive and new infections. Despite being primarily ecological, living in soil and water, S. fonticuli continue to be linked more and more to illnesses related to healthcare, especially in immunocompromised people. Biofilm is a generation, biochemical virulence indicators (such as hemoglobin and

proteins), and inherent susceptibility to β-lactams because of Ampc a type of enzyme and extendedspectrum a type of enzyme (ESBLS) are the main causes of its pathogenic [6,7]. Infections of the urine tract (UTIS), pneumonia, which is a condition known as and few instances of newborn diarrhea are among the illnesses [8]. On the other hand, C. koseri is well-known for its preference for newborns, which results in fatalities and extensive encephalopathy with distinctive brain tumors. Its aggressiveness depends on attachment elements like type 1 fibrous structures and external membrane protein A (Ompa), as well as monocyte proliferation and the blood-brain barrier permeability [9,10]. It mostly caused pneumonia, device-associated bacteremia, and UTIS in people; it is frequently connected to the formation of fouling on catheters that are embedded [11]. In both diseases, different forms of drug resistance make therapies more difficult. As the number of diseases caused by these microorganisms rises, it is critical to enhance surveillance, especially in hospitals, and to comprehend their unique pathogenic mechanisms in order to inform preventative measures and tailored treatments.

The antimicrobial and cytotoxic properties of yttrium nanoparticles  $(Y_2O_3)$  against the *Cit koseri* and scalloped fonticuli bacteria, which have been isolated from patients with diarrhea, are the focus of this study. In determining how effective  $Y_2O_3$  NPs can be against these bacteria, we want to contribute to the development of new medicines for drug-sensitive diseases.

## **MATERIALS AND METHODS**

Microbial Diseases: Bile samples from patients with diarrhea were used to identify *Citrus koseri* and *Serbia fonticuli*. Following being confirmed using standard microbiological techniques, the bacteria were stored on micronutrients agar at 4°C prior to usage.

Antimicrobial Health Conditions: Bile samples from people who had dysentery were used to identify Grapefruit *koseri* and *Serbia fonticuli*. Before being used, the beneficial microbes were kept on multivitamin agar at 4°C after being examined using conventional bacterial methods.

#### Cultural diversity Communication

For the culture of bacterial cells, nourishment broth and agar (nutrition, blood, and bile culture) were utilized. Normal processes were followed in the preparation of every medium.

#### Methods

#### **Bacterial Culture**

- 1. The mutants of serrated fonticuli and a bacteria called Cit koseri were cultivated for a 24-hour period at 37°C in a broth containing nutrients.
- 2. Bacterial suspensions were prepared to achieve an optical density (OD) of 0.1 at 600 nm, corresponding to approximately  $1 \times 10^8$  CFU/mL.

The current study highlights the prevalence of Serratia fonticuli and Citrobacter koseri among 83 bacterial isolates, with S. fonticuli accounting for 6.0% (n=5) and C. koseri for 7.2% (n=6). The 6.0% prevalence of S. fonticuli reflects its role as an environmental organism capable of causing nosocomial infections, particularly in immunocompromised hosts or those with prolonged hospital stays.

Minimum Inhibitory Concentration (MIC)
Determination

Preparation of Y<sub>2</sub>O<sub>3</sub> Nanoparticle Solutions

Stock solutions of  $Y_2O_3$  nanoparticles were prepared at concentrations of 50, 75, 100, 150, 200, 250, 300, 350, and 400  $\mu$ g/mL.

## MIC Testing

- 1. A microdilution method was employed to determine the MIC against both bacterial strains.
- 2. In a 96-well microtiter plate, 100  $\mu L$  of nutrient broth was added to each well.
- 3. 100  $\mu L$  of each  $Y_2O_3$  nanoparticle solution was added to designated wells, followed by the addition of 100  $\mu L$  of bacterial suspension.
- 4. The final concentrations tested for S. fonticuli were 50, 75, 100,150, 200, 250, 300, 350, and  $400 \mu g/mL$ .
- 5. The final concentrations tested for C. koseri were 50, 75, 100, 150, 200, 250, 300, 350, and 400  $\mu g/mL$ .

6. The plates were incubated at 37°C for 24 hours.

#### Assessment of Bacterial Growth

After incubation, the wells were examined for turbidity. The lowest concentration exhibiting no visible growth was recorded as the MIC.

### Cytotoxicity Assays

Cytotoxicity of  $Y_2O_3$  nanoparticles was assessed using standard assays, including MTT and LDH assays, on human cell lines.

## Statistical Analysis

All experiments were performed in triplicate. Outcomes were presented as mean  $\pm$  deviations after data analysis using the proper tools for statistical analysis.

#### **RESULTS AND DISCUSSION**

X-ray diffractometer (XRD), electron microscopy by scanning (SEM), and Fourier transform-infrared spectroscopic (FT-IR) were used to determine the microscopic and compositional characteristics of  $y_2o_3$  NMs.

To find the functional families of Y<sub>2</sub>O<sub>3</sub> NMs, Fourier transform infrared spectroscopy (FTIR) spectroscopic study was employed.

Fig. 1 shows the SEM image of prepared nanoparticles. The SEM images showed the uniform nanoparticles.

The existence of YO<sub>3</sub> nanomaterials in the 400–4000 cm<sup>-1</sup> region is confirmed through the acquired FT-IR spectroscopy. with additional peaks suggesting some adsorbed moisture or organic/carbonate impurities—common during synthesis or handling.as shown in figure at 3404 cm<sup>-1</sup> (Broad peak) This typically corresponds to O–H stretching vibrations, indicating adsorbed moisture or hydroxyl groups on the nanoparticle surface, at 1507 cm<sup>-1</sup> and 1397 cm<sup>-1</sup> These peaks can be

Table 1. Bacterial isolate.

Bacterial isolate	Number	Percentage
serretia fonticola	5	6.0
Citrobacter koseri isolated	6	7.2
Other bacterial isolates	72	86.8
Totally	83	100

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attributed to C=O and C=O stretching vibrations, possibly from carbonate species ( $CO_3^{2-}$ ) or trace organic contaminants (common in synthesis using organics or atmospheric exposure) and the characteristic Y=O stretching vibration at the fingerprint region (below 600 cm<sup>-1</sup>) Specifically at 558 cm<sup>-1</sup>confirming the formation of yttrium oxide ( $Y_2O_3$ ) while at 462 cm<sup>-1</sup> its consistent with metal-oxide bonding finally at 415 cm<sup>-1</sup> its further confirming the presence of  $Y_2O_3$  structure (Fig. 2).

Under specific circumstances, the framework

of the intersystem crystallographic function of coatings is examined using the shape of X-ray scattering observations.

Both the incident and reflective angles are scanned by the calculation that follows. The following are the requirements include the length of the beam is  $\lambda$  = 1.54056 Å, the value of the voltage is 40.0 KV, the current flowing is 30.0 ma, and the target is Cu K $\alpha$  irradiation. The 20 approach is used to scan the direct and reflection angles. Fig. 3 displays the XRD structure of Y<sub>2</sub>O<sub>3</sub>

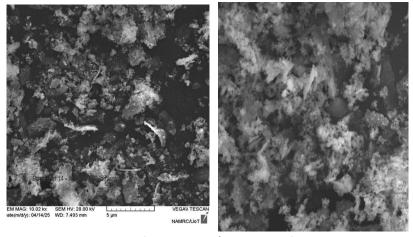


Fig. 1. The SEM images of  $\mathrm{Y_2O_3}$  nanoparticles.

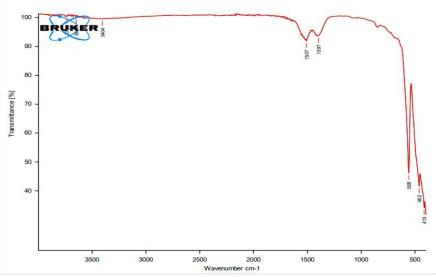


Fig. 2. FTIR spectra of Y<sub>2</sub>O<sub>3</sub> nanoparticles.

NMs, which confirms the extremely crystallized, homogeneous cubic phase in Y<sub>2</sub>O<sub>3</sub> by displaying crisp and powerful scattering lines. The major diffraction peaks are observed at 20.8º, 29.2º, 33.29, 29.29,33.29,36.09, 39.69,48.59,56.49,60.69 and 73.5°. The absence of extra peaks indicates no detectable impurities or secondary phases.

Results in Table 2 showed that the mean values for the HdFn cell line show a clear upward trend as the concentration decreases. Starting from 72.03 at 400 µg/ml, the mean increases consistently, peaking at 95.99 at 25 µg/ml. This implies that smaller amounts might increase HdFn cell turnover, suggesting a possible medical advantage at fewer dosages. The HepG2 cell line, on the other hand, has significantly smaller average numbers. The average value is just 30.21 at 400  $\mu$ g/ml, but it progressively increases to 80.48 at 25 μg/ml.

This suggests that Hepg2 cells are less responsive to the treatment than HdFn cells, which may reflect inherent differences in cell line behavior or metabolic responses.

The higher mean values observed in HdFn cells indicate their suitability for investigating cellular mechanisms at lower concentrations. However, Hepg2 may be more pertinent to research on particular routes of metabolism or safety evaluations.

The data presented in Table 3 showed the inhibitory responses of HdFn and HepG2 cell lines to a specific inhibitor, The results indicate that the HdFn cell line exhibits a higher IC50 value (246.9 μM) compared to the HepG2 cell line (164.5 μM), suggesting that HepG2 cells are more sensitive to the inhibitor. There are variances among the two cell lines, such as the blocking reaction, as seen by

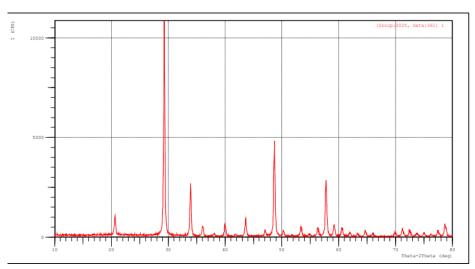


Fig. 3. XRD pattern of Y<sub>2</sub>O<sub>3</sub> nanoparticles.

Table 2. HdFn Cell Line and HepG2 Cell Line

Concentration —	HdFn Cell Line	HepG2 Cell Line
	Mean ± SD	Mean ± SD
400	72.03 ± 0.18	30.21 ± 2.10
200	85.19 ± 4.80	42.37 ± 2.00
100	93.70 ± 0.60	54.47 ± 2.49
50	94.57 ± 0.30	64.86 ± 2.82
25	95.99 ± 0.18	80.48 ± 2.41

the somewhat shallower slope of the Hill slopes for HepG2 and the pronounced reaction for HdFn.

The high  $R^2$  value (0.9603) demonstrates a good fit of the model to the data, indicating reliable results from the analysis.

Results reveals the dose-response relationship of two treatments, HdFn and HepG2. 246.9  $\mu g/ml$  was found to be the value of the IC50. The cells

from HepG2 significantly kinder to the compound than the HdFn cell conformity, as seen by their inhibitory concentration (IC50), which was 164.5  $\mu$ g/ml. This implies because at this dosage, the HdFn cells' lifetime is reduced by 50%.

Oxygen oxide is a highly uncommon chemical compound that has exceptional endurance and physiological friendliness. It can be customized

Table 3. HdFn and HepG2 Cell Lines' Detrimental Sensitivity.

REPORT 1	HdFn	HepG2
[Inhibitor] vs. response Variable slope (four parameters)		Interrupted
Best-fit values		
Bottom	66.23	-105.9
Тор	95.51	962.3
IC50	246.9	164.5
HillSlope	-2.901	-0.1306
logIC50	2.393	-3.784
Span	29.28	1068
95% CI (profile likelihood)		
Bottom	??? to 74.35	
Тор	93.41 to 100.4	
IC50	191.7 to ???	
HillSlope	??? to -0.8354	
logIC50	2.283 to ???	
Goodness of Fit		
Degrees of Freedom	11	
R squared	0.9603	
Sum of Squares	49.76	
Sy.x	2.127	
Constraints		
IC50	IC50 > 0	IC50 > 0
Number of points		
# of X values	15	15
#Y values analyzed	15	15

for specific applications. There are multiple techniques that can be employed to produce  $Y_2O_3$  nanomaterials, such as heating, sol-gel, and coprecipitate methods. Each method has a special advantage for controlling the particle size, shape, and condensation. All of which have effect on how they behave in biological influence [11].

If the Y<sub>2</sub>O<sub>3</sub> NPs interact with the microbial membranes, the framework might be damaged.  $Increased\ permeability, the loss\ of\ essential\ cellular$ components, and eventually cell lysis are the results of this disruption [12]. By producing (ROS), yttrium oxide nanomaterials can cause bacterial cells to become inflamed. DNA, cholesterol, and proteins can be damage by Hazardous chemicals, which can result in premature death of cells. Furthermore, it has been demonstrated that (Y2O3) NPs disrupt a number of biological functions, influencing the development and multiplication of microorganisms [13]. Despite promising antibacterial properties, their detrimental effects on human cells must be understood in order to assess their safety in medical [14].

#### CONCLUSION

Investigating hydrogen oxide particles as possible antiseptics is an interesting tactic to combat drug-sensitive bacteria. For their continued use in treatments in the years that followed, it was essential to comprehend their methods of use, harmful effects, and effectiveness against actual illnesses. The results of this study may lead to new approaches to treating illnesses related to pneumonia and related conditions. The findings will shed light on  $Y_2O_3$  NPs potential as antibiotics. The newly released study also emphasizes how crucial it is to comprehend how various cell types react to drugs in order to develop safer and more efficient treatments.

### **CONFLICT OF INTEREST**

The authors declare that there is no conflict of interests regarding the publication of this

manuscript.

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