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Original Article

Effect of ZnO nanoparticles on ESBL producing *Escherichia coli* & *Klebsiella spp.*

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Abstract. Zinc oxide (ZnO) nanaoparticles have been observed to have significant antibacterial properties against different gram positive and gram negative organisms. This study was done to determine the effect ZnO nanoparticles against ESBL producing organisms. Which are acting as menance in treatment of severe infective conditions. ZnO nanoparticles were tested against ESBL producing *E.coli* and *Klebsiella.* Inhibition of growth was observed by spectrophotometry. There was significant reduction in growth of ESBL positive isolates of *E.coli* and *Klebsiella.* Thus nanoparticles have a potential to be used as antibacterial agents in case of infections with ESBL positive organisms, which can be cause of high mortality and morbidity.

Key words: Nanoparticle, extended spectrum β- lactamase (ESBL), Klebsiella

1. Introduction

Increasing emergence and injudicious use of antimicrobial agents among the members of enterobacteriacea, especially *E.coli* and *Klebsiella* poses serious threat in management of such infections. Resistances in these organisms are mainly mediated by enzymes called β-lactamases which open β-lactam ring of penicillins and cephalosporins thereby destroying their antibacterial properties. Plasmid encoded β-lactamases which are capable of hydrolyzing broader spectrum of β-lactam antibiotics than simple parent β-lactamases are known as extended spectrum β-lactamases, they can inactivate β-lactam antibiotics containing oxyimino group, such as oxyimino-cephalosporins like cefotaxime, ceftazidime and oxyimino-monobactams like aztreonam (1,2).

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Nanoparticle metal oxides represent a new class of important materials that are increasingly being developed for use in research and health related applications. High ionic metal oxides are interesting not only for their wide variety of physical and chemical properties but also for their antibacterial activity (3,4). Although the in-vitro antibacterial activity and efficacy of regular ZnO have been investigated, little is known about the antibacterial activity of ZnO nanoparticles (5,6). Some study suggested the antibacterial properties of ZnO nanoparticles on gram positive and gram negative bacteria. The mechanism of the antibacterial activity of ZnO particles is not well understood, Sawai et al (7) proposed that the generation of hydrogen peroxide is a main factor, while Stemenov et al (8) indicated that the binding of the particles on the bacterial surface due to electrostatic force could be mechanism.

2. Material and methods

The study was conducted in Department of Microbiology, J.N. Medical College, A.M.U, Aligarh (U.P) India. *E. coli* and *Klebsiella* isolated on blood agar, MacConkey's agar from different clinical specimens like pus, urine, cervical swab, blood, bronchial secretion and CSF were identified by battery of standard

biochemical reactions, Antimicrobial drug susceptibility pattern was determined by using Kirby-Bauer disc diffusion technique using antibiotics of different classes, like aminoglycosides, quinolones, cephalsporins etc. Inhibition zones were noted matched with standard zone for that particular agent.

Phenotypic detection of ESBL was done using double disc synergy test using co-amoxiclav and piperacillin/tazobactam discs as a source of inhibitors. The test inoculum (equivalent in turbidity to that of a 0.5 McFarland standard) was streaked on Muller Hinton agar, discs of co-amoxiclav (20/10 µg) or piperacillin/tazobactam were placed 20mm and 30 mm centre to centre, from disc containing cefotaxime (30 µg), ceftazidime (30 µg) and plates were incubated at 37° C overnight (9). Enhancement of zones of inhibition of cephalosporins towards pipercillin/tazobactam or co-amoxiclav was considered as positive for ESBL. Genotypic characterizations of all isolates were done using PCR designed to detect and identify CTX-M genotype using primers CTXM-U-f and CTX- M-U-r and sequences TGTGCAGYACCAAGTAAR GT and TGTGCAGYACCAAGTAARGT,

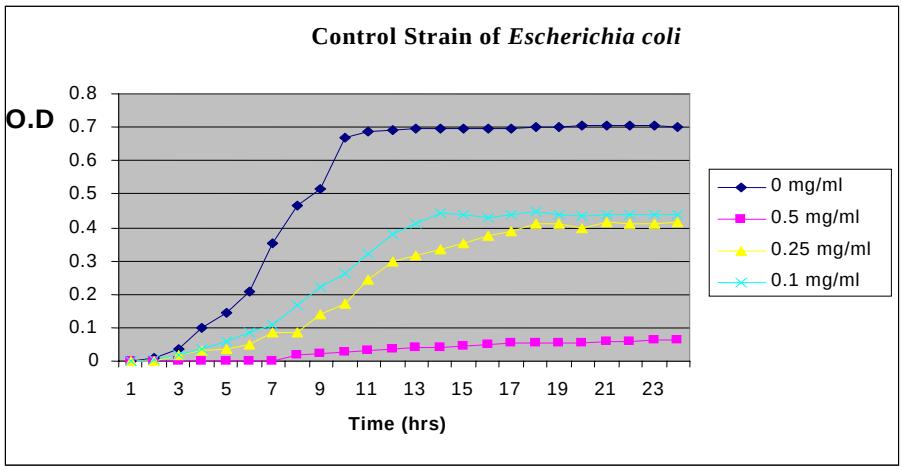


Fig.1. Growth curves of Control Strain of *Escherichia coli* in LB medium in presence of different concentrations of ZnO.

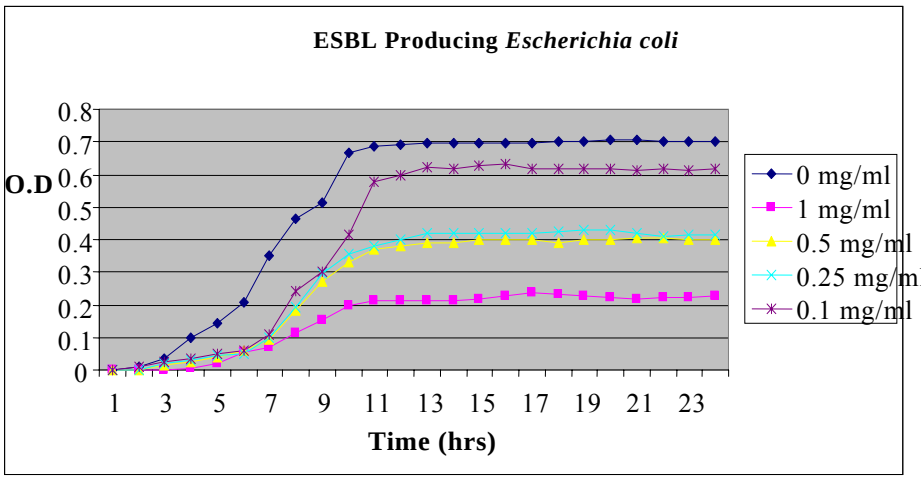


Fig.2. Growth curves of ESBL producing *Klebsiella spp.* strain in LB medium in presence of different concentrations of ZnO.

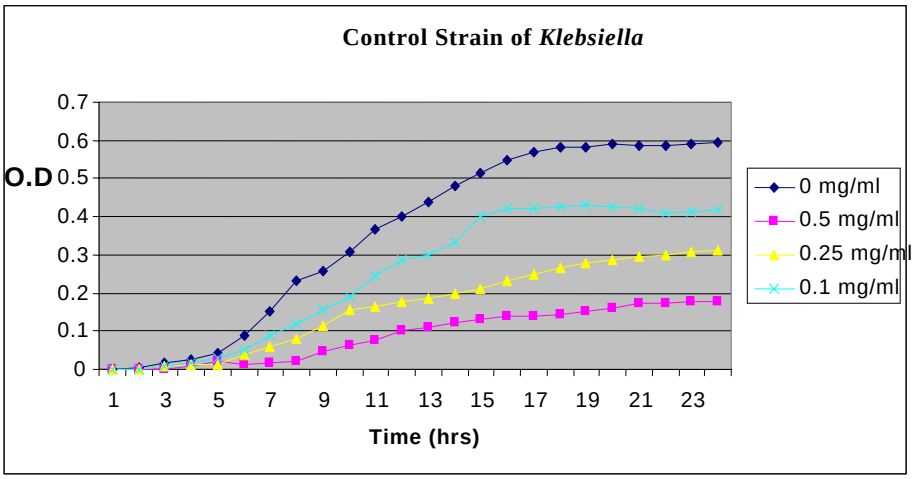


Fig.3. Growth curves of control Strain of *Klebsiella* in LB medium in presence of different concentrations of ZnO.

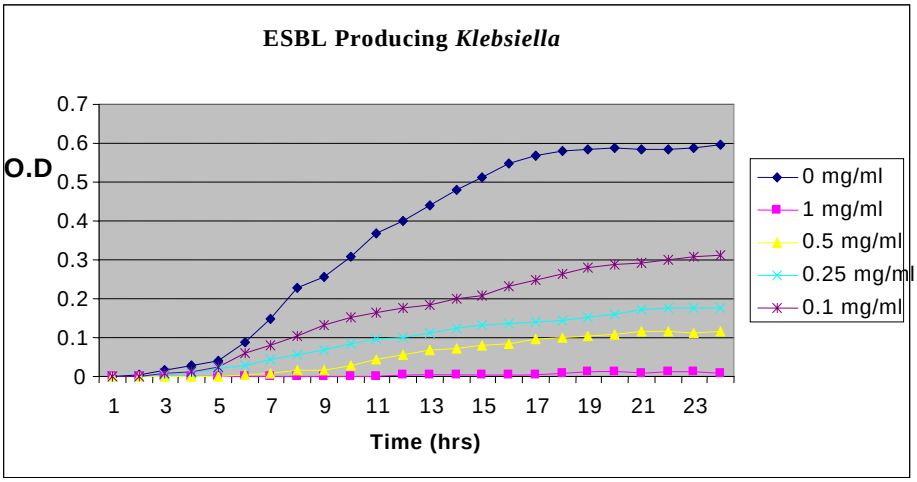


Fig. 4. Growth curves of ESBL Producing *Klebsiella* strain in LB medium in presence of different concentrations of ZnO.

cycling conditions used for PCR, initial denaturation 94˚C for 7 min; 35 cycles of 94˚C for 50 sec, 50˚C for 40sec, 72˚C for 60 sec, final extension at 72˚C for 5 min. All ESBL positive strains of *Klebsiella* and *E.coli* were CTX-M positive (10,11). (Fig.6 showing CTX-M detection by PCR).

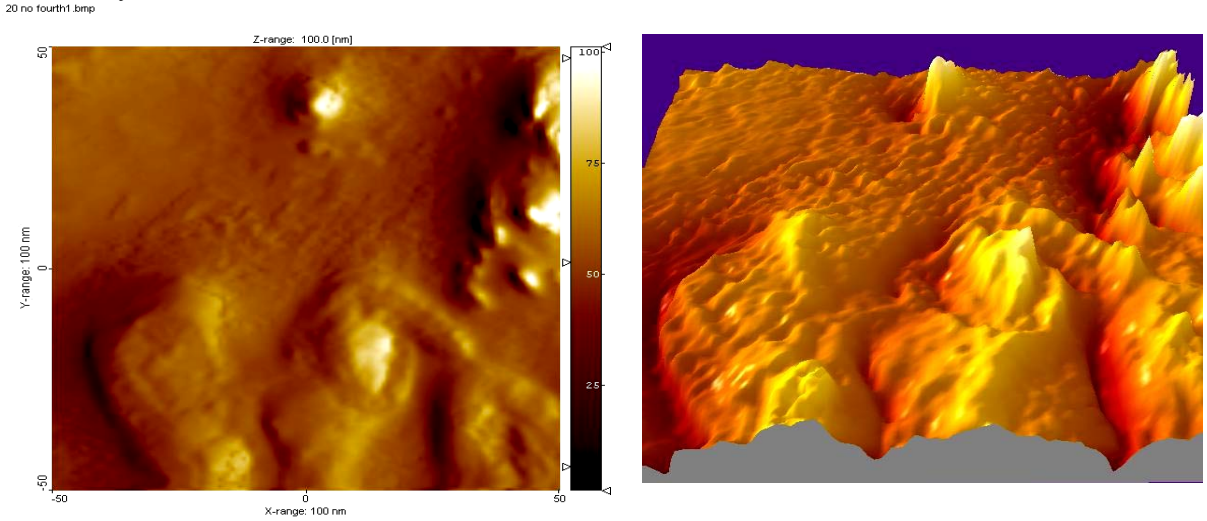
2. 1. Preparation of Nanoparticle mixture

ZnO nanoparticle of 70nm procured from Sigma Aldrich, India was used to prepare solution, using a preset amount of dry nanoparticles which were mixed with distilled water in a glass beaker with aid of magnetic stirrer, and then the beaker was placed in an ultrasonicator for breaking agglomerates of nanoparticles. After 30 mm of sonication master nanofluid prepared, diluted with distilled water to different concentration for antibacterial test. Four different concentrations of ZnO were 1 mg/ml, 0.5 mg/ml, 0.25 mg/ml, and 0.1 mg/ml.

2.2.Test for antibacterial activity of nanoparticles

1. Turbidity measurement of bacterial growth is done by using Lobalite Spectrophotometer. The density of bacterial cells in liquid cultures were estimated by optical density (OD) measurements at 600nm wavelength.

2. The Atomic Force Microscope (AFM) is one of the foremost tools for imaging, measuring and manipulating matter at nanoscale, it's a scanning probe is used (both control and ESBL positive) in our study. Different strains of bacteria treated with ZnO nanoparticles were visualized using AFM in both two and three dimensional planes. The sizes of different types of nanoparticles used were assessed using X-ray diffraction technique.



(a) Two dimensional **(b)** Three dimensional

Fig. 5. Atomic Force Microscope image showing embedment of ZnO nanoparticles on cell surface in *Escherichia coli*

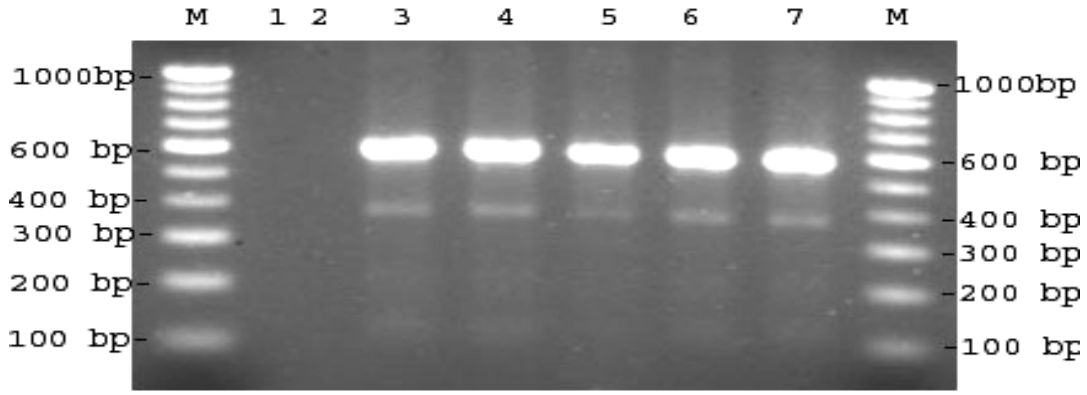


Fig .6. CTX-M gene detected by PCR

3. Results

ZnO nanoparticles were used in different concentrations of 0.5 mg/ml, 0.25 mg/ml, & 0.1 mg/ml. Growth curves of control strain of *Escherichia coli* in LB medium inoculated with 10^7 Colony Forming Unit (CFU) of bacteria in the presence of different concentrations of ZnO nanoparticles At all these concentrations, the nanoparticles caused a growth delay of *Escherichia coli*. Increasing the concentration of nanoparticles increased this growth delay hence all these concentrations showed antibacterial activity and this antibacterial activity increased with increase in concentration of nanoparticles. Nanoparticles with highest concentration showed almost no growth for up to 24 hrs representing a bactericidal effect at this concentration (fig 1,2).

ESBL producing *Klebsiella* strain in LB medium inoculated with 10^7 CFU of bacteria in

the presence of different concentrations of ZnO nanoparticles showed antibacterial activity. Antibacterial activity increased with increase in concentration of nanoparticles. There was an inhibitory action at all the concentrations of the ZnO nanoparticles (1, 0.5, 0.25 and 0.1 mg/ml). Nanoparticles with highest concentration (1 mg/ml) showed almost no growth for up to 24 hrs representing a bactericidal effect at this concentration (fig 3,4) AFM imaging showed embedment of nanoparticles on surface of bacteria (fig 5).

4. Discussions

Resistance to antimicrobial agents is a major public health problem, present world wide with high rate of resistance to third generation cephalosporins. CTX-M gene, a major factor for ESBLs is prevalent worldwide. It is previously being reported in various studies in India. So we tested ZnO nanoparticles antibacterial activity against ESBL producing isolates of *E.coli* and *Klebsiella* (Fig.1-4). Significant antibacterial activity of ZnO at different concentration was found in control as well as ESBL positive strains. Similar antibacterial behavior of ZnO was observed by Zhang et al (12) on control strains no data is available till date to our knowledge on antibacterial effect of ZnO nanoparticles on ESBL producing isolates of *E.coli* and *Klebsiella spp*.

5. Conclusion

ZnO nanoparticles can be used alone or in combination to different antimicrobial agents to combat serious infection caused by ESBL producing strains of *Klebsiella* and *E.coli* but in vivo studies are yet to be conducted for analysis of toxic side effects, these are safe agents for topical application**.**

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