ANTI BACTERIAL ACTIVITY OF COMMERCIAL ANTIBIOTICS AND ZINC OXIDE NANOPARTICLES AGAINST SELECTED UTI PATHOGENS - A COMPARATIVE STUDY

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Abstract: The present study is concerned about the synthesis, characterization of zinc oxide nanoparticles and their use as antibacterial agent. Zinc oxide nanoparticles were synthesized by Co-precipitation method using zinc acetate and thiourea. The synthesized zinc oxide nanoparticles were characterized with X-ray diffraction analysis. The antimicrobial activity of zinc oxide nanoparticle was tested against UTI pathogens like, *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Proteus vulgaris* and *Staphylococcus aureus* using Well diffusion method. Similarly the antibacterial activity of standard antibiotics was tested against UTI pathogens using the disc diffusion method. The efficiency of zinc oxide nanoparticles was compared with that of standard antibiotics. The results showed that zinc oxide nanoparticles have strong antimicrobial activity against all tested pathogens except *Proteus vulgaris*.

Introduction:

The infectious diseases remain one of the greatest challenges to global health. Urinary tract infection (UTI) is the second most common clinical disease and possesses a significant healthcare burden. This infectious disease can alter the urinary system either structurally or functionally (Foxman, 2010). Worldwide about 150 million people are diagnosed each year with UTI's costing in excess of 6 billion dollars (Gupta *et al.,2001)* . UTI's are predominantly caused by bacteria. The most common bacteria implicated as causative agents of UTI generally originate in the intestine and include but not limited to *E.coli, Pseudomonas spp, Streptococcus spp, Proteus spp., Klebsiella spp., Staphylococcus spp*(El-Sweih *et al*., 2008). About 80 to 90 percent of UTIs are caused by a single type of bacteria *Escherichia coli* (Barnett and Stephens.,1997)

There is an urgent need to produce the new antibacterial agents from different sources. The terrestrial plant such as *Phylanthusamarus* and *Parquetinanigrescens* showed potential antibacterial activity against UTI pathogens(Oluwafemi F and Debiri F.,2008). Moreover, the marine resources such as mangroves, seaweeds, sponges, and sea grasses already showed antibacterial, antifungal(Ravikumar *et al*.,2010) and antiplasmoidal activities(Ravikumar *et al*.,2011).

Nanotechnology is emerging as a rapidly growing field with its application in science and technology for the purpose of manufacturing new materials at the nano scale level(Albrecht *et al*.,2006). The word "nano" is used to indicate one billionth of a meter or 10^{-9}. The term nanotechnology was coined by **Norio Taniguchi,** researcher at the Universityof Tokyo, Japan in a 1974. Nanotechnology is the application of science to control matter at the molecular level. It is the most promising field for generating new applications in medicine (Mukherjee *et al*., 2008).

Reports are available on the considerable antibacterial activity of inorganic metal oxides like TiO_2, SiO_2, MgO, CaO, CeO_2 and ZnO exhibiting bacteriostatic, antimicrobial or biocidal action (Hamouda *et al., 2000; Fu etal.,2005)*. Recent studies have shown that these nanoparticles have selective toxicity with bacteria but exhibit minimal effect on human cells (Brayner *et al*., 2006; Thill *et al*.,2006;Reddy *et al*.,2007;Zhang *et al*.,2007). ZnO nanoparticles have been shown to have a wide range of antibacterial activities against both Gram-positive and Gram-negative bacteria.Zinc oxide (ZnO) is listed as "generally recognized as safe" (GRAS) by the U.S Food and Drug Administration (21CFR182.8991).

Materials and Methods

Collection of UTI samples

A total of 25 urine samples from 12 male and 13 female patients admitted in the hospitals as UTI problems were collected from different hospitals and laboratory, in a separate sterile wide mouth bottle.

Isolation of pathogens

The collected samples were streaked on nutrient agar plates and MacConkey agar plates. The plates were incubated at 37ºC for 24 hours. After incubation, individual colonies were selected and identified on the basis of morphological characteristics, Gram's staining and Biochemical characters.

Identification of the pathogens

A. Microscopic observation

a) Gram's staining

Gram's staining is usually performed to differentiate the bacteria into Gram positive and Gram negative (Dr.Christian Gram., 1884).

B. Biochemical Identification

The isolated organisms were confimed by IMVIC, Catalase uease and oxidase test

Antimicrobial Activity of selected commercial Antibiotics against the Isolated Pathogens

Assay of Antibiotic activity was performed by Kirby – Bauer disc diffusion method (Bauer *et al., 1966)* following the definition of the Clinical and Laboratory Standards Institute (CLSI, 2006). The Muller Hinton agar plates were prepared and the isolated organism was swabbed over it using a sterile cotton swab. The antibiotic discs(Amikacin(30mg),Amoxacillin(30mg),AmoxacillinClavulanicacid(30mg),Azithromycin(30mg),Aztreonam(30mg),Cefdinar(30mg),Cefixime(30mg),Cefotaxime(30m)Ceftriaxone(30mg),Ceftazidime(30mg),Cefuroxime(30mg),Ciprofloxacin(30mg),Chloramphenicol(30mg)Cephalexin(30mg), CoTrimoxazole(30mg),Erythromycin(30mg), Pencillin(30mg),Piperacillin(30mg),Gentamycin(30mg),Nalidixicacid(30mg),Nitrofurantoin(30mg),Norfloxacin(30mg),Ofloxacin(30mg),Tetracycline(30mg) were placed on the surface of the agar plates and then, the plates were incubated at 37^0C for 24 hours. After incubation, the zone of Inhibition was measured.

ZnO Nanoparticle preparation

The zinc oxide Nanoparticles were prepared by co-precipitation method. The analytical region grade Zinc acetate and thiourea along with ethylene glycol were used for the preparation of ZnOnanocrystals. Zinc acetate &thiourea are taken in 1:3 molecular ratio were mixed and dissolved in 100ml ethylene glycol and kept in a domestic microwave oven.

The microwave irradiation was carried out with the solvent get evaporated. The collected precipitate obtained was cooled to room temperature and washed with distilled water and then acetone used by purification ZnO NP characterization (or) XRD.

Characterization of Zinc oxide Nanoparticle

X-ray powder diffraction (XRD) was used to characterize the zinc oxide powders. Particle size of the samples was determined using x-ray diffraction technique. The patterns of prepared ZnO were recorded by diffractometer (AXS Bruker D8 Advance) using a Cu Kα radiation (λ = 1.5406A^0).

Particle size is calculated using Schrrer formula, $$D = \frac{0.94\lambda}{\beta \cos\theta} \text{ nm}$$

where β is the measured FWHM (full-width at half maximum), θ is the Bragg peak angle of the peak, λ is the X-ray diffraction wavelength .

Antibacterial activity of ZnO Nanoparticles against isolated UTI pathogens

The antibacterial activity of theZnO Nanoparticles was performed by modified Kirby-Bauer disk diffusion method(Bauer *et al.*,1966). The Muller Hinton agar plates were prepared and the

isolated organisms were swabbed over it using a sterile cotton swab. The solid medium was gently punctured with the help of cork borer to make a wells. About 30mg of ZnO NPs were mixed with 1 ml of DMSO solution.After getting colloidal stock solution, from the stock load the different concentrations ofZnOnanoparticles with 20µl,40µl upto 100µl to the wells. After 24 hours incubation each plate was examined and measured for the diameters of zones of complete inhibition.

Results and Discussion:

Isolation of pathogens

The collected samples were streaked on Nutrient agar and MacConkey agar plates and incubated at 37^0 for 24 hours. After 24 hours *E.coli, Klebsiella pneumoniae, Proteus vulgaris, Pseudomonas aeruginosa and Staphylococcus aureus.* The colonies on each media were represented in plates 1,2,3,4and 5.

Identification of the pathogens

The isolated organisms were subjected to microscopic observation and biochemical identification. The biochemical characteristics were tabulated in Table 1 and represented in plates 6, 7, 8,9,10,11and12.

Antibacterial activity of selected commercial Antibiotics against the Isolated Pathogens

The anti-bacterial activity of selected commercial antibiotics against isolated pathogens was done using Kirby-Bauer disc diffusion method. After 24hrs, the zone of inhibition was measured and tabulated in Table 2&3 and also represented in plates 13,14,15,16and 17.

XRD Analysis

Figure 4 shows the XRD pattern of ZnOnanocrystals. The diffraction peaks indicate the nanocrystalline nature (JCPDS Card no 0.3-0888). These peaks at scattering angles (2θ) of 31.3670, 34.6270, 35.8596, 47.1635, 56.2572, 62.5384, 67.6356 and 68.7978 correspond to the reflection from: 100,002,101,102,110,103,200 and 112 crystal planes respectively. The XRD Pattern is identical to the hexagonal phase with wurtzite structure with space group (c6v= P_{63} mc).The average grain size of the samples was estimated with the help of Schrrer equation.

$$D = \frac{0.94\lambda}{\beta \cos\theta} \text{ nm}$$

Where,

D = Grain size.

λ = wavelength of Cu K α x ray source ($1.5406\times 10^{-10}m$)

$\beta = Full\ width\ at\ half\ maxima\ (FWH\mu)$

d= Interplanar distance between the two lattice space.

The average grain size was obtained 38 nm.

Antibacterial activity of ZnO Nanoparticles against isolated UTI pathogens

The anti-bacterial activity of ZnO NPs against isolated pathogens was done using modified Kirby-Bauer disc diffusion method. After 24hrs, the zone of inhibition was measured and tabulated in Table 3 and also represented in plates 18,19,20,21 and 22.

Conclusion

In this study five bacterial isolates from UTI patients (*E.coli, Pseudomonas aeruginosa, Proteus vulgaris, S.aureus, Klebsiella pneumoniae)* were subjected to antibiotic sensitivity which consisted of fourteen different antibiotic groups. They are routinely used to treat UTI's. Among this *E.coli, P.vulgaris, Pseudomonas aeruginosa, Klebsiella pneumoniae* highly sensitive to Gentamycin, Amikacin, Ciprofloxacin,ofloxacin whereas *E.coli, Pseudomonas aeruginosa* ,exhibited sensitive to Nitrofurantoin and Nalidixic acid.

The high rate of resistance to Cefuroxime, Aztreonam, Cefotaxime, Ceftriaxone, Cefixime, Cefdinir and Ceftazidime observed. *Staphylococcus aureus* highly sensitive to Piperacillin, Tetracycline and Erythromycin and not sensitive to most of the antibiotics. In this study may reflect the fact that these are the most commonly prescribed antibiotics in the hospital and also the most available in the community without prescription and because they are also very cheap in terms of cost and so subject to abuse and misuse (Ako-Nai *et al*., 2005; Nwanze *et al*., 2007; Kolowale *et al*., 2009; OKeSolandoni , 2009).

In another experiment we analysed the antibacterial activity of ZnO NP against isolated UTI pathogens. In this studies different concentration of ZnO NPs ie., 20µl ,40µl upto 100µl for each of the five isolates. *Staphylococcus aureus* and *Klebsiella pneumonia* showed maximum sensitivity at all the concentration.

Pseudomonas aeruginosa exhibit sensitivity at 16mm diameter in 60µl,16mm diameter in80µl and 17mm diameter in100µl, whereas *E.coli* showed sensitivity at 13mmdiameter in60µl, 14mmdiameter in80µl and 16mmdiameter in100µl. *Proteus vulgaris* is not susceptible at all the concentrations.

Thus, in this report, ZnO nano particles have shown the best antibacterial behavior compared to commercial antibiotics. Therefore, in the future, ZnO nanoparticle- containing formulations may be utilized for external uses as antibacterial agents in oinments, lotions, mouth washes and surface coatings on various substrates to prevent micro-organism from attaching, colonizing, spreading and forming biofilms in undwelling medical devices. The antibacterial activity increased with increasing concentration of zinc oxide Nanoparticles. The results of the study confirmed that the ZnO Nanoparticles may serve as promising antibacterial agents.

Table: 1

Biochemical identification for isolated organisms.

Characteristics	***P.aeruginosa***	***E.coli***	***K.pneumoniae***	***P.vulgaris***	***S.aureus***
Grams staining	-	-	-	-	+
TSI Slant	K	A	A	K	A
Butt	K	A	A	A	A
Gas	-	G	G	G	-
H_2S	-	-	-	+	-
Motility	Motile	Motile	Non-Motile	swarming	Motile
Indole test	-	-	-	+	-
Methyl red test	+	+	-	+	-
Voges-Proskauer test	-	-	+	-	-
Citrate test	-	-	+	-	-
Urease test	+	-	+	+	-
Oxidase test	+	-	-	-	-
Catalase test	+	-	-	+	+

+:positive ; - : negative ; k:alkaline; a:**acid:G:Gas**

Table: 2

Comparison of commercial antibiotics against isolated pathogens

S.no	**Antibiotics**	***E.coli***	***K.pneumoniae***	***P.vulgaris***	***P.aeruginosa***
1	CR	-	-	-	-
2	GM	16mm	15mm	14mm	14mm
3	AK	16mm	15mm	15mm	14mm
4	CL	15mm	15mm	15mm	14mm
5	NF	15mm	-	-	14mm
6	AT	-	-	-	-
7	CX	-	14mm	-	-
8	FR	-	-	-	-
9	NA	13mm	-	-	17mm

10	FU	13mm	-	-	-
11	FX	-	-	-	-
12	CN	-	-	-	-
13	OF	18mm	15mm	16mm	19mm
14	CZ	-	-	-	-

Table: 3

Antibacterial activity of commercial antibiotics against *S.aureus*

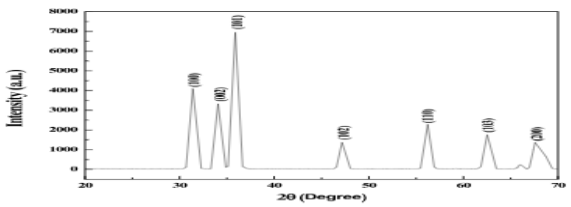
S.No	Antibiotics	Zone of inhibition
1	CT	-
2	CP	-
3	CF	14mm
4	CR	-
5	ER	16mm
6	CK	14mm
7	CL	14mm
8	PG	-
9	AX	11mm
10	OF	-
11	PC	17mm
12	AZ	11mm
13	TE	20mm

Table :4

Comparison of ZnO NPs against isolated pathogens

Isolated organisms	Zone of inhibition				
	20µl	40µl	60µl	80µl	100µl
Escherichia coli	-	-	13mms	14mm	16mm
Klebsiella pneumoniae	13mm	16mm	17mm	19mm	21mm
Proteus vulgaris	-	-	-	-	-
Pseudomonas aeruginosa	-	-	16mm	16mm	17mm
Staphylococcus aureus	15mm	15mm	17mm	18mm	19mm

XRD pattern of Zinc oxide nanoparticle



ISOLATION OF ORGANISMS FROM UTI PATIENTS

Plate: 1

***Escherichia coli* on MacConkey Agar** ***Escherichia coli* on Nutrient Agar**

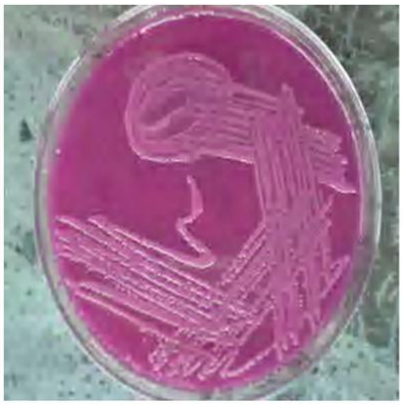


Plate : 2

***Klebsiella pneumonia* on MacConkey Agar** ***Klebsiella pneumonia* on Nutrient Agar**

Plate:3

***Proteus vulgaris* on MacConkey Agar** ***Proteus vulgaris* on Nutrient Agar**

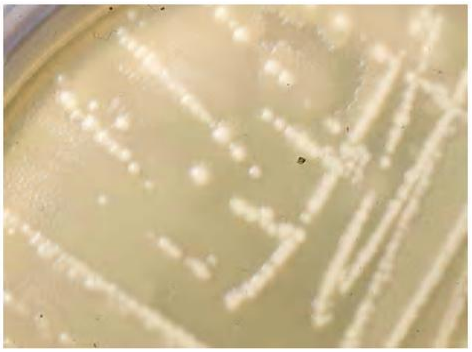


Plate:4

***Pseudomonas aeruginosa* on Mac Conkey Agar** ***Pseudomonas aeruginosa* on Nutrient**

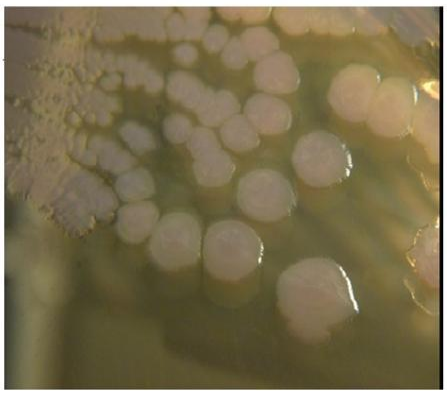


Plate:5

***Staphylococcus aureus* on Nutrient Agar**

Plate :6

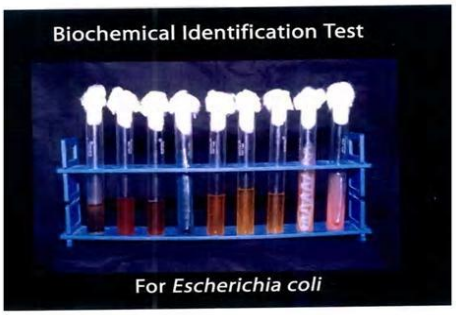


Plate : 9

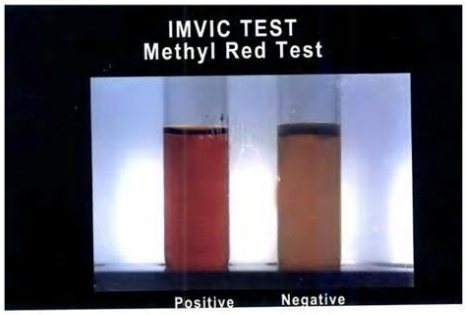


Plate :7

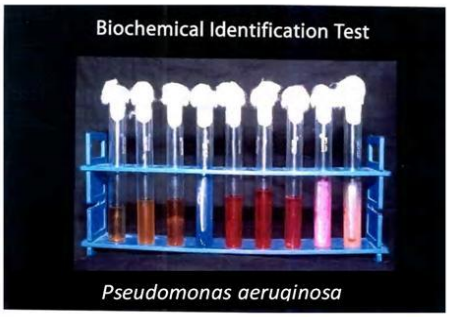


Plate :10

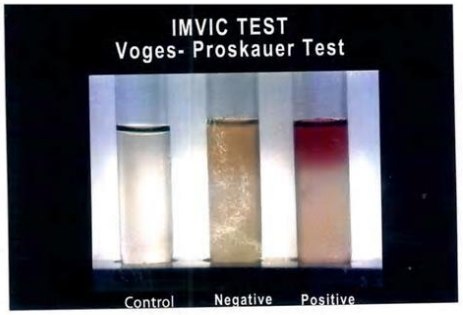


Plate :8

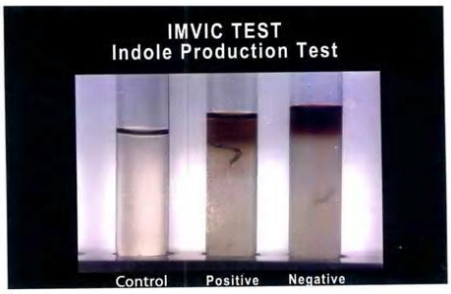
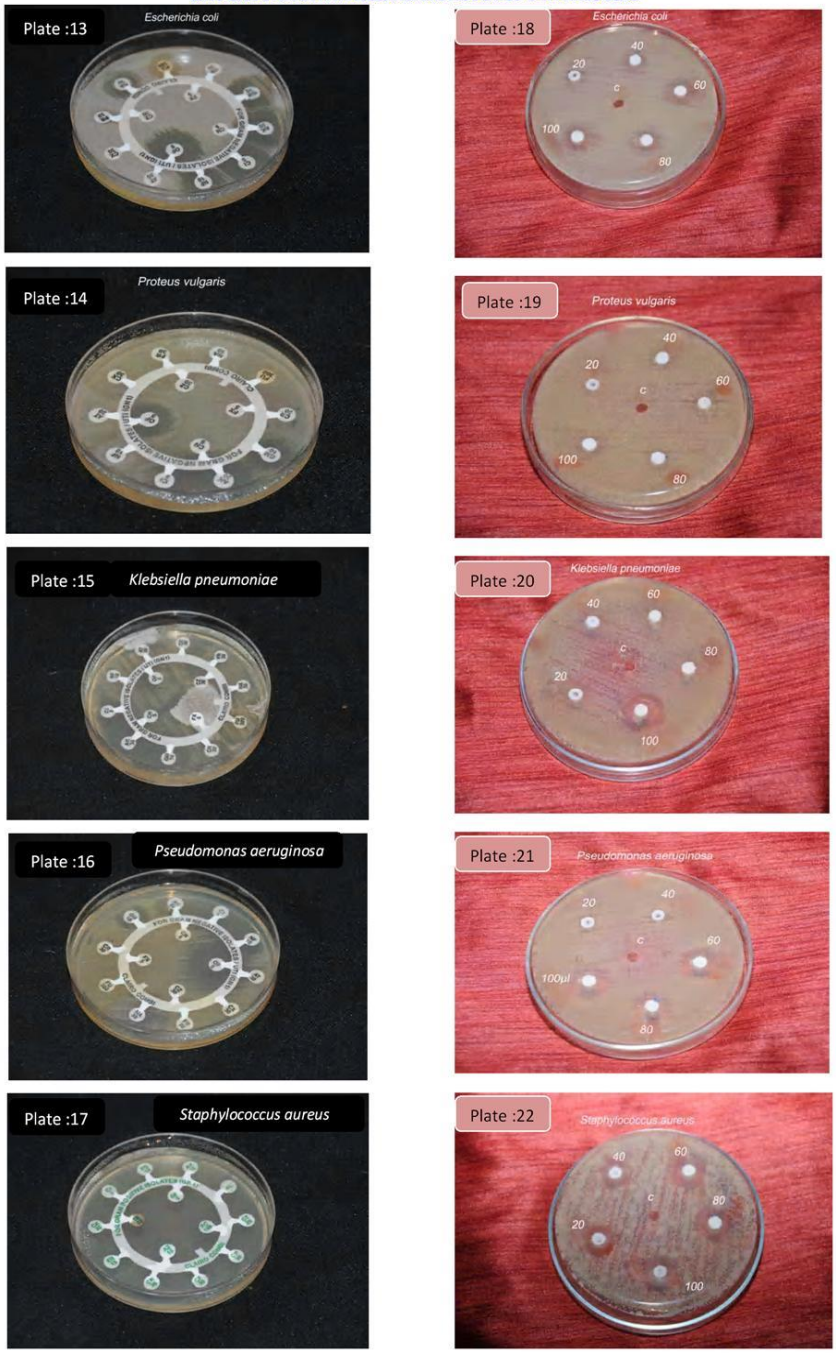


Plate :11

Plate :12

COMPARISON OF INHIBITORY EFFECT OF COMMERCIAL ANTIBIOTICS AND ZINC OXIDE NANA PARTICLES AGAINST ISOLATED UTI PATHOGENS



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