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Research Article

Antibacterial Activity of Zinc Oxide Nano Particles against Bacteria Isolated from Infants with Urinary Tract Infection

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Abstract

This study included the collection of 70 sample of urine from infant aged between (2 days-2 years) divided into 35 sample of healthy children and 35 sample of children suffering from urinary tract infection, Through which get 40 isolated of different types of bacteria which included: 19 isolated of the *E.coli*, 9 isolated of the *Proteus mirabilis*, 5 isolated of the *Klebsiella Aerogenes*, 4 isolated of the *Pseudomonas aeruginosa* and 3 isolated of the *Enterococcus Faecalis* And at different rates ranged between (27, 12.8, 7.1, 5.7, 4.2) % As percentages of the total isolation and (47.5, 22.5, 12.5, 10, 4.2) % As percentages of isolation ratios qualitative for *E.coli*, *Proteus mirabilis*, *Klebsiella Aerogenes*, *Pseudomonus aeruginosa*and *Enterococcus Faecalis* respectively ,While the number of bacteria in sick children was (15, 8, 5, 3, 2) isolated for *E.coli*, *Proteus mirabilis*, *Klebsiella Aerogenes*, *Pseudomonus aeruginosa*and *Enterococcus Faecalis* respectively with isolation rates (42.85, 22.85, 14.28, 8.57, 5.7) % Compared with healthy children who were (4) isolated for *E.coli* and with isolated ratios have (11.42%)and one isolated for each of *Proteus mirabilis*, *Pseudomonus aeruginosa* and *EnterococcusFaecalis* with isolated ratios (2.85 %) for this types respectively, also the preparation of zinc oxide nano particles done in Nanotechnology and Advanced Materials Research Center (NAMRC) in university of technology and Prepared of Zinc Oxide NanoParticles were investigated via using Scanning Electron Microscopy (SEM, the VEGA Easy Probe), X ray powder diffraction (XRD) and antibacterial activity and the results of Zinc Oxide Nano Particles against bacteria explain ability of its to inhibition growth of bacteria in different rate of bacteriostatic when used in different concentration.

Keywords: infant, bacteria, urinary tract infection, zinc oxide nano particles, Antibacterial activity.

الخلاصة

تم في هذه الدراسة جمع (70) عينة ادرار من اطفال رضع تتراوح اعمارهم ما بين (2 يوم -2 سنة) مقسمين الى مجموعتين (35 عينة) اخذت من اطفال اصحاء و (35 عينة) من اطفال يعانون من التهاب المجاري البولية, تم خلالها الحصول على (40) عزلة لانواع مختلفة من البكتريا تضمنت : (19) عزلة لبكتريا (*E.coli*) و (9)عزلات لبكتريا (*Proteus mirabilis*) و (5) عزلات لبكتريا (*Klebsiella Aerogenes*) و (4) عزلات لبكتريا (*Pseudomonas aeruginosa*) و (3) عزلات لبكتريا (*Enterococcus Faecalis*) و بنسب عزل مختلفة تراوحت بين (27, 12.8, 7.1, 5.7, 4.2 %) كنسب عزل كلية و (47.5, 22.5, 12.5, 10, 4.2 %) كنسب عزل نوعية للانواع (*E.coli, Proteus mirabilis, Klebsiella Aerogenes, Pseudomonus aeruginosa*and *Enterococcus Faecalis*) على التوالي, بينما تراوحت اعداد العزلات للعينات الماخوذة من الاطفال المرضى (15, 8, 5, 3, 2) عزلة للانواع (*E.coli, Proteus mirabilis, Klebsiella Aerogenes, Pseudomonus aeruginosa*and *Enterococcus Faecalis*) على التوالي و بنسبة عزل (42.85, 22.85, 14.28, 8.57, 5.7)(%) مقارنة مع الاطفال الاصحاء الذين كانت اعداد البكتريا فيها (4) عزلات لبكتريا (*E.coli*) و نسبة العزل فيها (11.42%) وعزلة واحدة لكل من (*Proteus mirabilis, Pseudomonus aeruginosa* and *EnterococcusFaecalis*) و بنسبة عزل (2.85 %), و تم تحضير اوكسيد الزنك النانوي في مركز بحوث النانوتكنولوجي و المواد المتقدمة في الجامعة التكنولوجية و و تم دراسة الخواص التركيبية لأوكسيد الزنك النانوي باستخدام المجهر الالكتروني الماسح (SEM) وجهاز حيود الاشعة السينية (XRD) و الفعالية المضادة للبكتريا باستخدام طريقة العد (طريقة التخفيف) و التي اوضحت نتائج تأثير اوكسيد الزنك النانوي ضد هذه الانواع و قدرة هذه المادة على تثبيط الانواع البكتيرية المختلفة عند استخدامها بنسب مختلفة .

Introduction

Urinary tract infection (UTI) is a disease cause by bacterial common occurring between children [1] more than 30% of infant exposure to infection between period of six month to one year after began of UTI[2] the infection of UTI was diagnosed between boys and girls in different percentage about 1% in male and 3-8% in female .In first period of infant life UTI occurred in boys more than in girls, it is about 2.7% in boys compared with 0.7% in girls [3] in the first three month from boys life occurred most infection with UTI[4] but this situation different in school age, its increased in girls and decreased in boys [3] .

Other studies shown about 10-12 fold increased infection with UTI in uncircumcised male [5] .The occurring rate with UTI infection is a bout 12-30% in infants their ageing more than six months and severe from vesico-ureteric reflux and abnormal nuclear renal scans in the beginning of disease [6] .The symptoms of UTI different between young and older children [7], the infants bossed high prevalence compared with older children, with a male predominance [8].

The common pathogen that caused UTI between children is *E. coli*, at the principal occurrence of UTI, it's infecting more than 80% of male and 90% of female [9].*E.coli* has the capacity to stick to endothelium of urinary tract and this is one of the factors important for the dominance of these bacteria. There are different types of bacterial spectrum in male UTI especially in uncircumcised boys because The lower region colonizes various types of Gram-negative bacteria other than colorectal bacteria .In repetitive infections, more than of non-*E. coli* is *Klebsiella spp*., *Enterococci, Enterobacteriaceae,* and *Proteus spp.* And when urine is culture a lot of microbes were discovered and which are associated with a congenital anomalies of the kidney and urinary tract (CAKUT). [10].

the infections are a worldwide risk to human, especially in kids because of pathogenic strains, and Widespread of her, the resistance of bacteria to an antibiotic, development new mutations of bacteria, the absence of reasonable vaccine in immature nations, hospital-binded diseases,. For instance, diseases by *Shigella flexneri* cause 1.5 million deaths yearly, because of polluted nourishment and beverages by these microscopic organisms [11].

The studies of zinc oxide nano particles as antibacterial factor appeared possibility of connect different specialties like biologists, medicine, chemists, and physicists; consequently, there is a broad of usage. The important of the basic usage is in the sustenance manufacture as an anti-microbial factor in sustenance packaging and against sustenance borne microbe. Nano materials have a high reactivity, improved bioavailability, and bioactivity and have creative surface possessions therefore posses great important in sustenance technology [12]. Nanotechnology explains that nanoparticles that are atomic or molecular groups can characterized by their size that less than 100nm. This is the result of the transformation of the basic elements derived by changing the atomic and molecular properties of these elements [13]. nanotechnology is new science in 21st century .because of Important specifications of Nano materials it's used in Different processes.one of the important metals nanoparticles is zinc oxide nanoparticles its used in biomedical, gas sensors, drug-delivery systems, biosensors, cosmetics and agriculture etc. Zinc oxide (ZnO) Nano powders are accessible as scatterings and powders. These nanoparticles, show antifungal, anti-corrosive, antibacterial and against destructive properties. [14].A portion of the principle advantages of utilizing Nano Particles in nourishment Nano technology are the expansion of Nano Particles onto sustenance surfaces to repress bacterial development, additionally utilizing of Nano Particles as important packaging substances and for Nano sensin [15]. P. Kaur 2011 and P. Narayanan, *et al.*, 2012 Studied that ZnO-NPs can effected on bacteria from killing or inhibition a lot of food borne bacteria the bactericidal affectivity of ZnO Nano Particles (8–10 nm size) against *S. aureus* and *E. coli DH5a* Its effectiveness was tested and found to be the most effective at 80 and100 gm/ L. this concentration of ZnO Nano particles can effect on bacteria from the Smashing of the cell membrane and lose of cytoplasm. a lot of studies made about zinc oxide nanoparticles as an antibacterial factor against *E. coli*, *E. faecal ,P. aeruginosa, and S. aureus.* explained the ability of these nanomaterials against many human pathogens [16-17]. The release of the NPs, which work as bacteriostatic or bactericidal factors onto the nourishment substances surface where microorganisms live, stops the development and these keeps the nourishment substances from damage [18]. This kind of active bundling is additionally called antimicrobial bundling, where coordinate interaction communication happens between the product and the Nano Particles prompting the bacteriostatic and bactericidal development on nourishment substances surfaces [19]. The Insert of antibacterial factors helps either inhibition or killing bacteria materials to step by step diffuse into the nourishment substances origin. Subsequently, lessening the likelihood of microbe infection and therefore a protected product with an expanded timeframe of realistic usability was acquired towards *P. aeruginosa* and *Escherichia coli* that separated from frozen ice cream and mint leaf extricate. Both microorganisms indicated diminished development rate at the most noteworthy concentration100 lL and they clarified ZnO Nano Particles penetration cell membrane of microorganism and damage it that lead to growth inhibition as a result of cell death. ZnO-Nano Particles synthesized by the wet chemical technique are potential antimicrobial factors is because of its inalienable capacity to absorb UV illumination and optical straightforwardness, ZnO Nano particles used in the makeup industry, typically in facial creams and sunscreens [20]. In this study, we wanted to clarify the most important causes of urinary tract infection in children under the age of two years and how to form nanomaterial against these causes, especially after the resistance of many of them to antibiotics.

Materials and Methodologies

Collecting and culturing of samples

It was collected 70 sample of urine from infant Ages ranging from 2 days to 2 years. Samples divided to 35 sample from healthy infants and 35 sample from infants suffered from urinary tract infection and cultured on MacConkey Agar and blood agar (Hi media) for 24 h and under 37 C and later it was examined general urine examination .

Examination of isolated

Isolated was examined by using Cultural and Microbial Examination by gram stain and then using catalase, oxidase and biochemical tests when culture on peptone water (Hi-media – India), urea agar base, Kligler Iron Agar, Simmon Citrate Agar and manitol salt agar (oxoid), and by used of Api E20, later this isolated was kept in nutrient broth with 15% glycerol and kept in refrigerator (-20 C) For while in use.

Preparation of Zinc Oxide Nano Particles (ZnO NPs)

Preparation of Zinc Oxide Nano Particles was in Nano Technology and Advanced Materials Research Center (NAMRC) in University of Technology in Iraq via sol gel technique, we dissolved 8 g of Sodium Hydroxide in 10 ml of (DW) distilled water and 2 g of Zinc Acetate Dihydrate were dissolved in 10 ml of distilled water after weighted this materials utilizing a weighting balance and measured distilled water via a measuring cylinder. The solution was blended with a continuous stirring for around five minutes. After the solution mixed well, sodium hydroxide solution was mixed with the solution containing zinc acetate by addition its drop after drop with a continuous stirring via a magnetic stirrer for five minutes. White precipitate formed after the reaction completed, then filtered the solution and dry the matter in the oven at 70C for 24h and later grind to become white powdered. [21].

Making Laboratory Tests

X ray powder diffraction, Scanning Electron Microscopy (SEM, the VEGA Easy Probe), and antibacterial activity was tested for identification of zinc oxide and clarification of important in inhibition of bacterial growth when used in different concentration (0.8, 1.6, 2.4 mg/ml).

Antibacterial activity

Antibacterial affectivity of Zinc Oxide Nano Particles was tested by used different concentration of this Nano Particles against all types of bacteria that isolated. Antibacterial effectiveness of Zinc Oxide Nano Particles is performed via plate count method against all types of bacteria that isolated from infants this procedure made in (Biotechnology lab in Center of Nanotechnology and Advanced Material Research Center(NAMRC), University of Technology, Iraq).After diagnostic the isolation bacteria every type was culture on Nutrient Agar (N. Agar) and incubated at 37°C for 24 h, bacterial suspension prepared by used normal saline (0.9%) to obtain bacterial samples with concentration ~10^7-10^8 CFU/ml by 0.5 McFarland standards.

Then one milliliter of each bacterial solution was add to the samples (0.8, 1.6, and 2.4) mg/ml of Zinc Oxide Nano Particles and incubated at 37 ^{0}C in incubator shaker at 165 rpm for 24h. Then, the mixtures was serially diluted in normal saline and cultured on Molar Hinton Agar (using 100µl spread out on M.H. agar), incubated at 37°C for 24 h. Then colonies are counting after the incubation [22]. The formula to calculate the bacteriostatic rate is as following: Bacteriostatic rate 100%) = (1- colonies of test groups/ colonies of control group) ×100.

Results and Discussion

Diagnostic of bacteria

The samples were diagnosed by biochemical tests that appeared in Table 1 its appeared that *E.coli*, *Klebsiella Aerogenes*, *Enterococcus Faecalis*, *Proteus Mirabilis* and *Pseudomonus aeruginosa* were positive for catalase and negative for oxidase and indol except *Pseudomonus Aeruginosa* its positive for oxidase and *E.coli* for indol while *Proteus Mirabilis* it’s negative for indol test.In simmon citrate *E.coli* and *Proteus Mirabilis* its positive while the other its negative and also *E.coli* and *Enterococcus Faecalis* its negative for urea test while the other its positive (Figure 1, 2, and 3).

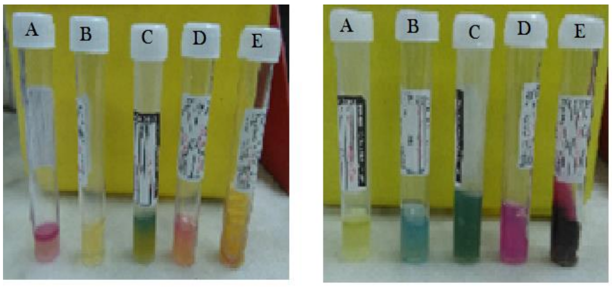

Figure 1: biochemical tests of bacteria (left picture is positive & right picture is negative) (A: positive and negative indole test, B: motile on semi solid manitol, C: Positive and negative simmon citrate, D: Positive and negative urea, E: Kliglar Iron test with H2S produced).

Figure 2: EPI20 used for diagnostic of *E.coli*

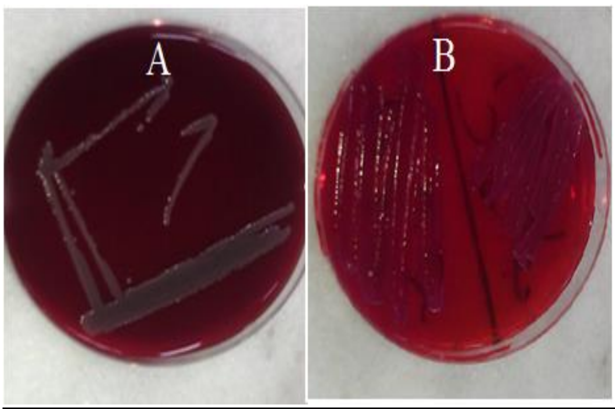

Figure 3: *E.coli* on(A)Blood agar,(B)MaccONcy agar.

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Table 1: biochemical tests of bacteria

Tests / Bacteria	KI H2S\G	Semi sold manitol	Urea	Citrate	Indol	Oxidase	Catalase
E.coli	A/A _\+	+	_	_	+	_	+
Klebsiella aerogenes	A/A __	_	+	+	_	_	+
Enterococcus faecalis	A/A _\+	_	_	+	_	_	+
Proteus mirabilis	A/A +\-	_	+	_	\+ _	_	+
P. aeruginosa	K/K __	_	+	+	_	+	+

Isolation of bacteria

This study included the collection of 70 sample of urine from infant aged between (2 days-2 years) divided into 35 sample of healthy children and 35 sample of children suffering from inflammation of the urinary tract, Through which get 40 isolated of different types of bacteria which included: 19 isolated of the bacterium *E.coli*, 9 isolated of the bacterium *Proteus mirabilis*, 5 isolated of the bacterium *Klebseilla aerogenes*, 4 isolated of the bacterium *Pseudomonas aeruginosa* and 3 isolated of the bacterium *Enterococcus faecalis* and at different rates ranged between (27, 12.8, 7.1, 5.7, and 4.2 %) As percentages of the total isolation of types *E.coli*, *Proteus mirabilis*, *Klebsiella aerogenes*, *P. aeruginosa* and *Enterococcus faecalis* respectively, While the insulation ratios qualitative for previous types were (47.5, 22.5, 12.5, 10, and 4.2 (%)) respectively, that is explained in Table 2. While the number of bacteria in sick children was (15, 8, 5, 3, and 2) isolated for *E.coli*, *Proteus mirabilis*, *Klebsiella aerogenes*, *Pseudomonus aeruginosa* and *Enterococcus faecalis* respectively with isolation rates (42.85, 22.85, 14.28, 8.57, and 5.7)% Compared with healthy children who were (4) isolated for *E.coli* bacteria and one isolated for each of *Proteus mirabilis*, *Pseudomonus aeruginosa* and *Enterococcus faecalis* with unarmed ratios have (11.42%) for *E.coli* and (2.85 %) for *Proteus mirabilis*, *Pseudomonus aeruginosa* and *Enterococcus faecalis* respectively, as seen in Figure 4.

Table 2: Total and insulation ratios qualitative of bact.

bacteria	Number of isolation for each type	Total and insulation ratios qualitative of bacteria (%)	
		qualitative	Total
E.coli	19	47.5	27
Proteus mirabilis	9	22.5	12.8
Klebsiella aerogenes	5	12.5	7.1
Pseudomonas aeruginosa	4	10	5.7
Enterococcus faecalis	3	7.5	4.2
Total	40		

Total represented the percentage (%) of (70) sample

Qualitative represented the percentage (%) of(40) isolated.

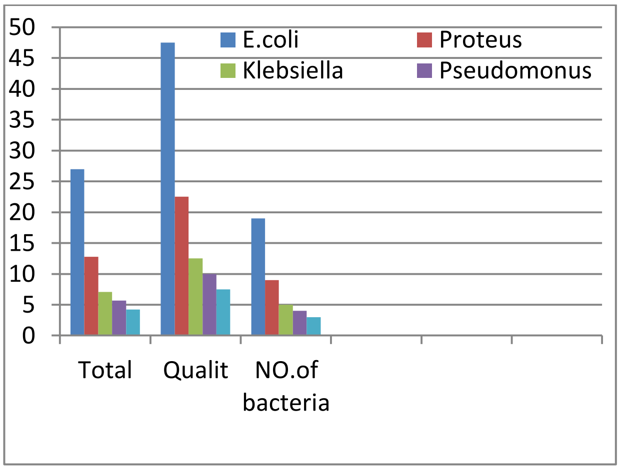

Figure 4: total, qualitative and NO. of bacteria.

The results explain that the most species of bacteria *E.coli* are isolated followed by *Proteus mirabilis, Klebsiella aerogenes*, *Pseudomonas aeruginosa and Enterococcus faecalis*and this results agree with (Riccabona 2003) that said the most types of isolation bacteria from urine samples of infants with complex UTI was Gram negative bacteria and *Escherichia coli* (*E. coli*) representing from 70 to 90% of disease [3], and (Arshad, 2015) noticed that in the first years of life most infections occurs because *Escherichia coli*, but later than in child life, *Klebsiella pneumoniae*, *Enterobacter spp.*, and *Pseudomonas spp.* were the more frequent, there is a great risk of uroseps is compared with young children [8].

Preparation of Zinc Oxide Nano Particles

XRD & Scanning Electron Microscopy

Figure 5 demonstrates XRD example of synthesis ZnO. The example demonstrates that all the diffraction tops filed to the hexagonal period of pure ZnO with a wurtzite structure, and detected peaks are at 2θ values of 31.8°, 34.5°, 36.3°, 47.6°, 56.7°, and 62.9° corresponding to the following lattice planes: (100), (002), (101), (102), (110), and (103) respectively.

Additionally, there is no diffraction peaks have a place with free Zn or some different debasements can be found in the range, this in result shows to the astounding nature of the synthesized products. Besides, the unmistakable and sharp pinnacles moreover revealed that the ZnO nanostructure has a high crystalline quality [23].

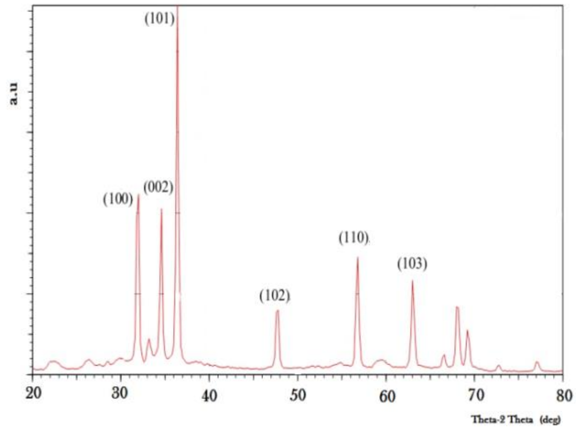

Figure 5: XRD patterns of ZnO nanostructures.

SEM images at different magnification, where SEM micrograph of the ZnO nanostructure, is shown in Figure 6. The ZnO structures have an average diameter of 100 nm and have found different in size, length and shape, in the nanorods. And in other regions, we have notice that pack of nanorods is ranged with each other in a various direction [23].

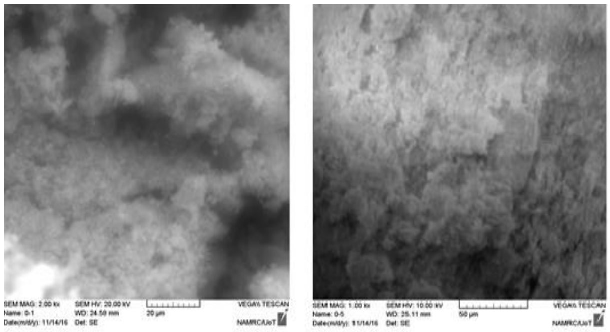

Figure 6: SEM images of ZnO nanostructures.

Antibacterial of Zinc oxide

The results of bacteriostatic rate of zinc oxide nanoparticles in (0.8 mg/ml) concentration was (99, 96, 90, 39, and 0 %) for (*E.coli*, *Enterococcus Faecalis*, *Klebsellia Aerogenes*, *Pseudomonus aeruginosa* and *Proteus Mirabilis*) while in (1.6 mg/ml) concentration was (100, 99 ,95 ,92, and 5 %) for the same types of bacteria and (100, 100, 100, 97, and 9%) when used (2.4 mg/ml) concentration of zinc oxide nano particles, this results show in Figure 7.

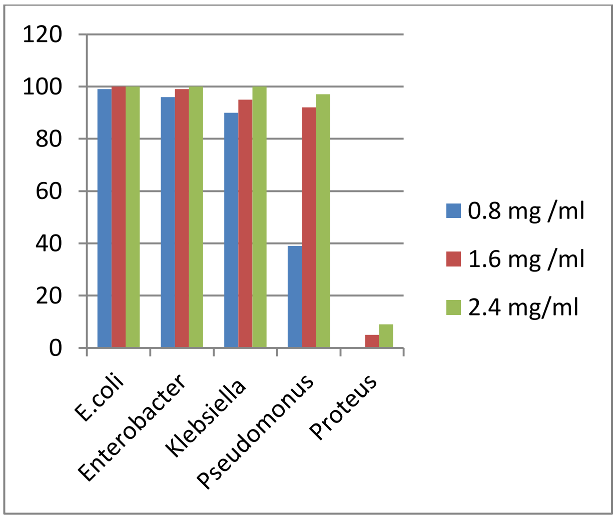

Figure 7: Antibacterial activity of zinc oxide nano particles in different concentration against different types of bacteria

The results explained ability of Zinc Oxide Nano Particles to inhibition of bacterial growth in different concentration and for all types of bacteria, this results accepted with Rizwan *et al*. (2010) In his opinion That large surface area of nanomaterials gives it the opportunity to interfere with bio-organics present on the living cell surface and also explain in his study the rate of inhibition growth of bacteria increased with increasing of ZnO nanoparticles concentration but according to different type of bacteria and concentration of ZnO nanoparticles [24].

Studies have shown that the ability of zinc oxide to affect the effect of large gram negative bacteria by affinity between the broad surfaces of nanoparticles and the surface of the bacterial cell, which leads to the penetration of the membrane of the bacterial cell and thus break the membrane, which leads to the exit of the contents of cytoplasm and cell death [25].

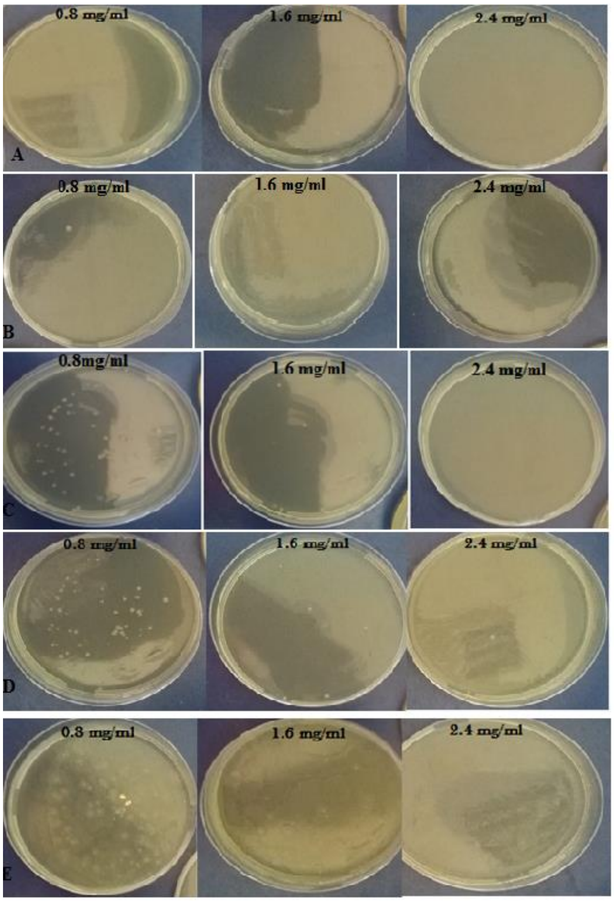

Figure 7 Antibacterial activity of zinc oxide nano particles against (A) *E.coli*, (B) *Enterococcus Faecalis* ,(C) *Klebsiella Aerogenes*,(D) *Proteus mirabilis* ,and (E) *Pseudomonus aeruginosa*.

Conclusion

The results of this study showed that most of the causes of urinary tract infections in infants are gram negative bacteria. The most common types of bacteria are E. coli, and it is the most isolating and also showed the ability of zinc oxide to affect all types of isolated bacteria when used in different percentages.

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