http://doi.org/10.36582/j.Alkuno.2023.06.10
Al-Kunooze University College
Journal homepage: http://journals.kunoozu.edu.iq/1/archive &
http:// www.iasj.net

Use of green synthesized ZnO NPs to increase antibiotic activity against *E. coli* isolated from children infected with rotavirus.

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ABSTRACT

The current study aimed to find the main bacterial co-infection with rotavirus, antibiotic sensitivity of dominant isolates and attempting to increase the efficiency of antibiotics by using green synthesized zinc oxide nanoparticles. For this purpose 30 fecal samples collected from patients with diarrhea and bacterial isolation and identification were conducted. The effect of green ZnO NPs on *Escherichia coli* isolates were also studied, in addition to MIC and MBC of antibiotic with and without ZnO Nanoparticles.

Seven types of bacteria were isolated and identified, and they were as follows: *E. coli*, *Salmonella spp. Shigella spp*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and coagulase negative staphylococcus spp. The highest isolation rate was for *E. coli* in rate of 53.35 % and the lowest were *Salmonella spp and Proteus mirabilis* in rate 6.6%. MIC of ZnO NPs, Ceftriaxone, Vancomycin, Amoxicillin, Rifamycin and Ciprofloxacin against *E. coli* were 100, 200,100,200, 50 and 50 µg/ml respectively, while the MBC were 200,400,200,400, 100 and 100 µg/ml respectively. The mixing of antibiotic with ZnO NPs lead to decrease in the MIC and MBC for all antibiotics used in the current study.

KEYWORDS: ZNO, green nanoparticles, diarrhea, Rota virus

INTRODUCTION

Diarrhea is the most common health complaints. It range from mild, to a potentially threatening of life, and about 2 billion diarrheal cases occur each year, from which

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about 1.9 million children under the age of 5 years were die from diarrhea every year. [1]. Diarrhea can be defined as the loose, watery stools three or more times a day. It may be acute, persistent, or chronic [2]. The main complications of diarrhea are dehydration (loss of fluid and electrolytes) and malabsorption (no absorption of enough nutrients) [3]. Main diarrheal signs are abdominal cramps or pain, bloating, nausea, vomiting, fever, blood in the stool, mucus in the stool, urgent need to have a bowel [4]. According to pathology feature, diarrhea can be classified in to inflammatory and infectious diarrhea, osmotic diarrhea and secretory diarrhea [5].

Multidrug resistance bacteria are a serious problem for the world, community well-being and attracted worldwide attention. The Antimicrobial resistance by bacterial pathogens increases due to mutations, insufficient dosage, long-term use, intense and unsuitable application of antibiotics in veterinary and clinical medicine and agricultural environments [6].

Nano particular were suggested for solvent of antibiotic resistance and as alternative antibacterial substances, their action due to wide surface area in compare with volume ratio, this character make it able to binding with bacterial and surface receptors, NPs have potential antimicrobials actions by penetrating bacteria and interacting with a number of internal components [7].

The current study aimed to find the effect of mixing ZnO Nps with antibiotic against bacteria isolated from diarrhea.

MATERIAL AND METHODS

- The current study conducted on AL-Anbar province in period from January to May 2022
- Patients : thirty children under 5 years from both gender suffer from diarrhea who have been confirmed to have had rotavirus infection (data from previous study).
- Bacterial isolation and identification: The stool samples were kept on trypton soya broth (Himedia- India) and incubated at 37°c for 24h. The isolated bacteria were sub-cultured on MacConkey agar, Mannitol salt agar, Nutrient agar, EMB agar, Chocolate agar and Blood agar and incubated at 48°c for 24h. The single colony purified in same culture medium and incubation condition. Gram stain and group of biochemical tests were applied according to [8].

Biosynthesis of ZnO NPs

a- Preparation of cinnamon bark extract: 2.5 grams of cinnamon bark powder add to 100ml of distilled water then heated until boiling for 30 minutes, then cooled. The mixture was filtered by filter paper and the filtered liquid stored in the refrigerator at 4°C [9].

b- Five gram zinc nitrate added to cinnamon bark extract and heated to 80° C by magnetic stirrer heater, (the solution turns into a deep yellow color paste). The paste put on ceramic crucible and heated at 400° C for 2 h. A light yellow color powder of ZnO NPs was obtained, then leaved to cold and kept at refrigerator for chemical analysis [9].

- Antibiotic and ZnO NPs sensitivity test: MIC and MBC were determine by the lower twofold dilution method and the lower concentration of antibiotic caused inhibition (clear broth media) and kill (septic broth media) [10] as the following:
- Ceftriaxone: dissolved by distilled water in concentration of 25 µg/ml, 50 µg/ml,100 µg/ml, 200 µg/ml and 400 µg/ml
- Vancomycin: dissolved by distilled water in concentration of25 µg/ml, 50 µg/ml,100 µg/ml, 200 µg/ml and 400 µg/ml
- Amoxicillin dissolved by Buffer phosphate in concentration of 25 µg/ml, 50 µg/ml,100 µg/ml, 200 µg/ml and 400 µg/ml
- Rifamycin: dissolved by DMSO in concentration of25 µg/ml,50 µg/ml, 100 µg/ml, 200 µg/ml and 400 µg/ml
- Ciprofloxacin: dissolved by Acidic water (pH5) in concentration of 25 µg/ml, 50 µg/ml,100 µg/ml, 200 µg/ml and 400 µg/ml
- ZnO NPs: dissolved by DMSO in concentration of 25 µg/ml, 50 µg/ml, 100 µg/ml, 200 µg/ml and 400 µg/ml.

Preparation of ZnO NPs –antibiotic mixture

For determine the effect of ZnO NPs on antibiotic activity, the mixture prepared in same concentration in rate 1:1for example 25 µg antibiotic+25 µg Zn Nano per ml, 50 µg antibiotic+50 µg ZnO NPs per ml.

Antibiotic loading on ZnO NPs: antibiotic loading on ZnO NPs according to (Borges *et al*.,2005) (11)

RESULTS AND DISCUSSION

Bacterial isolation: according to colony morphology, gram stain and biochemical test 7 bacterial type were isolated which were *E. coli*, Salmonella spp. Shigella spp, *Proteus mirabilis , Pseudomonas aeruginosa, Staphylococcus aureus* and coagulase negative staphylococcus spp (Table 1).

Table 1. Bacterial species isolated from diarrhea cases

Bacterial type	Number of isolates	Isolation rate
E. coli	16	53.3%
Shigella spp	4	13.3%
Pseudomonas aeruginosa	3	10.0%
Salmonella spp	2	6.6%

Proteus mirabilis	2	6.6%
Staphylococcus aureus	7	23.3%
coagulase negative staphylococcus	5	16.6%

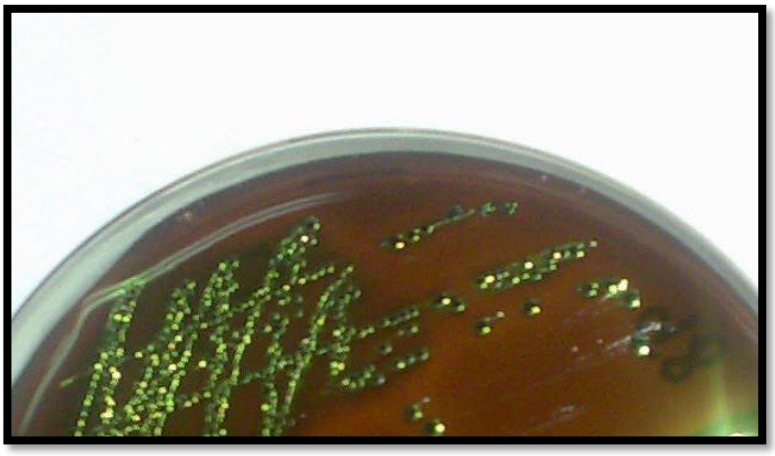

Figure (1): *E. coli* grow on EMB. Shows metallic sheen colony

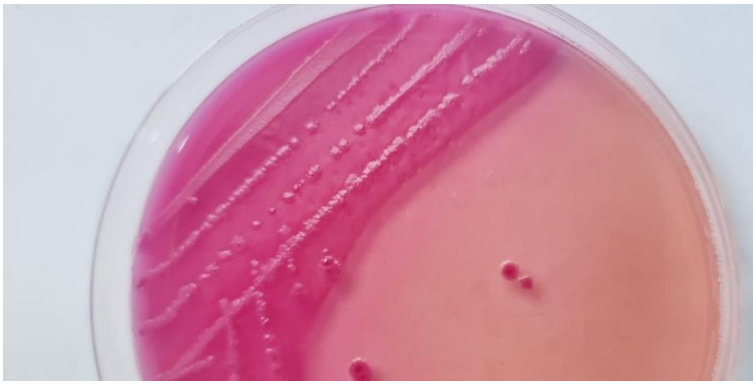

Figure (2): *E. coli* grow on MacConkey agar

The results showed that *Escherichia coli* was the main cause of diarrhea cases in children, as more than 50% of diarrhea cases were caused by this bacterium. Microbial causes of diarrhea include variety of bacterial, viral, and parasites. Among bacterial causes of diarrhea, E. coli play a major role in causing diarrhea in children under 5years (11, 12, 13).

Rota virus causes enteritis or intestinal damage, this lead lose normal flora barrier and this

lead activation of pathogenic and opportunistic bacteria to replication and caused diarrhea,
also the damage on intestinal mucosa may be lead to activation of immunity and produce
pro-inflammatory cytokines and change in physiology of intestine , all these cases may be
lead to diarrhea(11).

Analysis of green synthesized ZnO NPs by ultraviolet-visible spectroscopy and Atomic force microscope AFM: In this device, the absorption peak at 450.1 Nm three-dimensional cross-sections showed the surface topography of zinc particles of spherical shape, and their average sizes were 21.32 nm. Figures 7, 8, and 9.

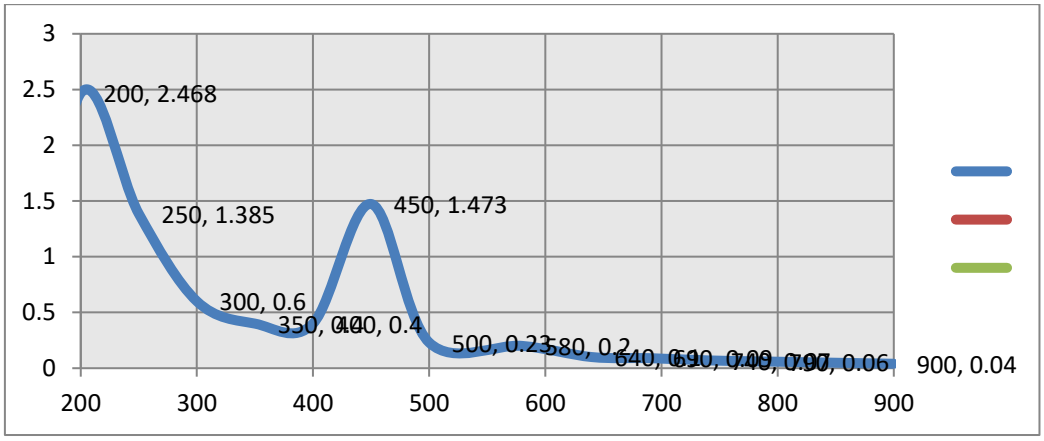

Figure 7. Nanoparticles ultraviolet-visible spectroscopy analysis.

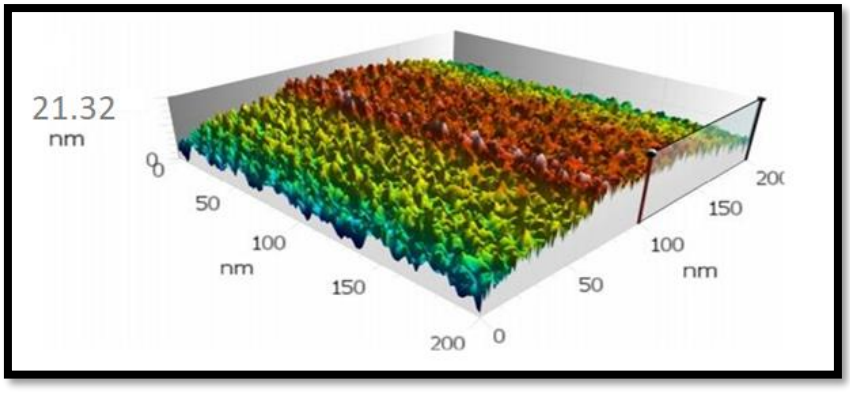

Figure 8. Three-dimensional cross-section of the surface topography of ZnO NPs

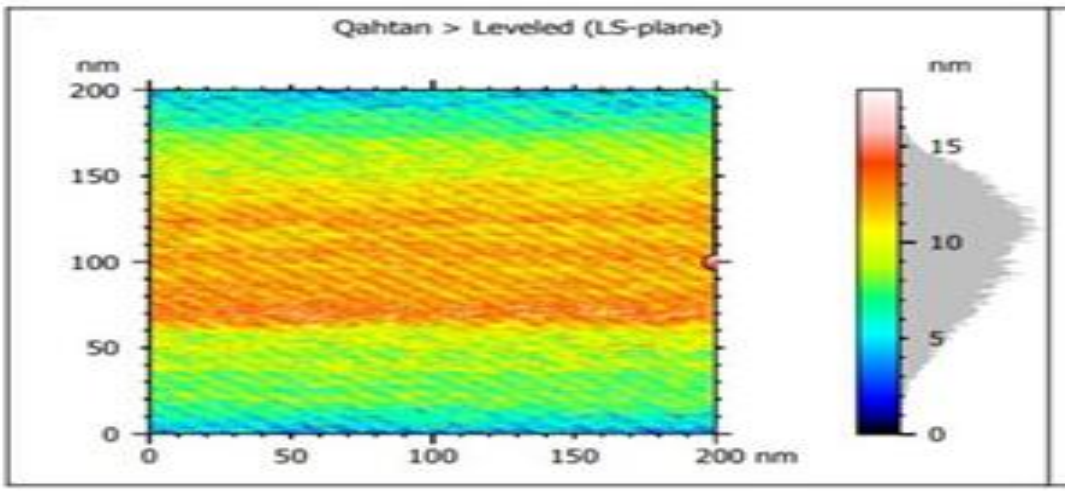

Figure 9. Two-dimensional image of ZnO NPs

From results showed in figures (7, 8, and 9) we can conclude the ability of cinnamon bark extract to produce Zn nanoparticle from ZnO NPs

MIC and MBC of ZnO NPs and antibiotic against *E. coli*: according to result of table (2) it is clear that the MIC for Zn Nano, Ceftriaxone, Vancomycin, Amoxicillin, Rifamycin and Ciprofloxacin were 100, 200, 100, 200, 50 and 50 µg/ml respectively, while the MBC were 200, 400, 200, 400, 100 and 100 respectively.

Table 2: MIC and MBC of ZnO NPs and antibiotic against *E. coli*

Zn Nano / Antibiotic type	concentration				
	25 µg/ml	50 µg/ml	100 µg/ml	200 µg/ml	400 µg/ml
ZnO NPs	growth	growth	Clear	Septic	Septic
Ceftriaxone	growth	growth	Growth	clear	Septic
Vancomycin	growth	growth	Clear	clear	Septic
Amoxicillin	growth	growth	Growth	clear	Septic
Rifamycin	growth	clear	Septic	Septic	Septic
Ciprofloxacin	growth	clear	Septic	Septic	Septic

The antibacterial effect of ZnO NPs against *E. coli* recorded by[14,15], the difference in MIC and MBC recorded in current study in compare with other study may be due to difference in sources of ZnO NPs , size, form, surface area and chemical structure. ZnO NPs is inorganic nanoparticles have multifunctional group, chemical and physical stability, unique optical, piezoelectric, and magnetic properties. There are three proposed mechanisms for the action of ZnO NPs as an antibacterial. The first mechanism is the throw reactive oxygen species (ROS) such as (H_2O_2), hydroxyl radicals OH^- and peroxide O_2^{-2} these groups internalized into the bacteria and

caused destruction of cellular components. Second mechanisms by release of ions in medium, these ions have toxic effect. Finally ZnO NPs cause change in bacterial membrane permeability as progressive release of lipopolysaccharides for Gram negative bacteria and cell lysis [16, 17].

Table 3. MIC and MBC of ZnO NPs with antibiotic against *E. coli*

ZnO NPs Antibiotic type	concentration				
	12.5+12.5 µg/ml	25+25 µg/ml	50+50 µg/ml	100+100 µg/ml	200+200 µg/ml
Ceftriaxone	Grow	Grow	clear	Clear	Septic
Vancomycin	Grow	Clear	Clear	Septic	Septic
Amoxicillin	Grow	Grow	Clear	Septic	Septic
Rifamycin	Clear	Septic	Septic	Septic	Septic
Ciprofloxacin	Clear	Clear	Septic	Septic	Septic

Table 3 showed that ZnO NPs increases the activity of antibiotic by decreasing the MIC and MBC, for example the MBC and MIC of vancomycin alone were 400 and 200 mg/ml respectively, while the MBC and MIC of the same antibiotic become 100 and 50 mg/ml when mixed with ZnO NPs.

The synergistic effect of ZnO with these antibiotic may be due to the effect of ZnO NPs by increasing the uptake of antibiotic by bacteria or due to their effect on cell wall and permeability change [20;21;22].

CONCLUSION: *E. coli* is main bacterial isolates from diarrhea accompanying with Rota infection Green syntheses ZnO NPs have antibacterial activity and synergistic effect when mixed with antibiotics.

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