# **Evaluation of antibacterial effect of gold nanoparticles and Antibiotic Resistant against Escherichia coli isolation from diarrheagenic stool of children with acute gastroenteritis**

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 **Abstract:** Enteropathogenic *Escherichia coli*  are a significant cause of acute and persistent diarrhea in humans. which are transmitted primarily through consumption of contaminated foods. This study was aimed study nano gold induces cell death by the production of cytotoxic reactive oxygen species (ROS). This principle of cell death can be utilized to kill bacteria. This integration of nanoparticles with biological molecules has lead to the development of diagnostic devices to *Escherichia coli* isolates from the diarrheagenic ofchildren. Isolates were tested for antibacterial resistance using the disk diffusion method found applications in nanobiotechnology in the development of antibiotic treatment of different bacterial infections.

 All specimens diarrheagenic ofchildren (n=55)were culturedon MacConkey,EMB agar plates, *E.coli* strains + isolate (n=35),Then *E. coli*  used antibiotic susceptibility testing by the disc diffusion method. Collacted bacteria *E.coli* by culture swape and identified by using standard microbiological tests.Then use of nano gold 100ppm,200ppm,300ppm to the media to khnow effecte both of *E.coli*

  *E. coli* were isolated in 35 from total sample 55 collected samples (63.6 %); The*E.coli* strains were sensitive to all the antibiotics were tested, except resistant to ampicillin 57.1%, Septrin71.4% ,Ofloxacin65.7%, Augmentin94.2 %,Ciprofloxacin71.4 % and chloramphenicol 91.4 %.

 Nano gold (nanopartecal ) in 300ppm,reducing *E. coli* faster then 100ppm,200ppm can be utilized to kill bacteria. Enteropathogenic *Escherichia coli* isolates which belonged to same serogroup were found to be highly diverse, as shown by their differing antibiotic susceptibility patterns. Gold nanoparticles is that they can induce toxicity at various degrees. It is suggested that higher concentrations of gold nanoparticles are toxic and can cause various health problems

**Key words**: Antibacterial activity, nano gold,antibacterial resistance, *Escherichia coli*.

**INTRODUCTION**

 Diarrhea is one of the important illnesses with high morbidity and mortality in children, resulting in about 1.6- 2.5 million deaths annually also it is still a health problem, especiallyin developing countries, where it is consideredone of the foremost causes of death in children,accounting for approximately 2 milliondeaths each year worldwide (1). Diarrhea is caused by a wide range of agents, including viruses, bacteria, and parasites (2). Among the bacterial pathogens, diarrheagenic *Escherichia coli* (DEC) is one of the important etiological agents of diarrhea (3).

 Diarrheagenic *E. coli* pathotypes represent a leadingbacterial cause of pediatric diarrhea in developingregions, with some responsible for traveler’sdiarrhea, and are also an emerging causeof diarrhea in industrialized countries (4). *E. coli* is usuallyfound in the commensal intestinal bacterial flora,but it could become a pathogen through acquisitionof genetic determinants, which mayenhanceadhesiveness or toxicity. These two factorsmake *E. coli* particularly aggressive in infants(5).

 Bacterial resistance to a particular antibiotic can be a natural property of the bacteria or a secondary acquired mechanism. It is a well-known fact that nano gold and gold -based compounds are highly toxic to microorganisms which include 16 major species of bacteria This aspect of gold makes it an excellent choice for multiple roles in the medical field. gold is generally used to induce antimicrobial effect, but when gold nanoparticles are used, there is a huge increase in the surface area available for the microbe to be exposed to. Though gold nanoparticles find use in many antibacterial applications, the action of this metal on microbes is not fully known. It has been hypothesized that gold nanoparticles can cause cell lysis or inhibit cell transduction. There are various mechanisms involved in cell lysis and growth inhibition (6).

**Materials and Methods**

**Bacterial strains:** A total of stool sample 55 and*E. coli* isolates 35 used in this study were obtained from children ≤5 years with diarrhea visited in Hilla teaching Hospitals. The samples were then transported by swab stick to the laboratorywhere they were processed within six (6) hours .

**Isolation of Escherichia coli from the stool samples:** The swab culture media and the antibiotic supplements which were used in the study were pro­cured from Hi-Media Laboratories, Each swab stick collected from each participant was dipped into 2ml sterile Normal Saline solution anda tenfold serial dilution was done. Using a standardized wire loop, a loopful of the second diluent was streakedon MacConkey agar plates and incubated for 24 hours at 37oC. Distinct colonies on the MacConkey agar plateswith *E.coli* morphology and appearance were aseptically streaked on Eosin Methylene Blue (EMB) agar plates.Colonies with green metallic sheen were tentatively identified as *Escherichia coli*. Further confirmatory tests were done by subjecting these colonies to gram staining and the following biochemical tests; catalase, citrate,indole and methyl red and Voges Proskaurhe confirmed colonies were then preserved on agar slants and refrigerated.(7)

**Antibiotic susceptibility testing:** Antibiotic susceptibility testing was performed by the disk diffusion method by use Muller Hinton Agar [8]. Different antibiotic disks like ampicillin, ceftazidime, ce­photaxime, ciprofloxacin, ceftriaxone, cefuroxime, chloramphenicol and gentamicin (Hi-Media Laboratories, Mumbai) were usedto test *E. coli*

**Effect nano gold Escherichia coli Antimicrobial**

Pure cultures were inoculated in100ml Nutrient Broth and incubated(370C, 24hrs) to allow complete growthin the liquid growth medium add nano gold 100 ppm,200 ppm, 300 ppm and one as control . Theovernight incubated broth culture wascentrifuged at 3000 rpm for 10 minutes. Supernatant Nutrient Brothwasdiscarded leaving bacterial pellet at thebottom of the centrifuge tube PhosphateBuffered Saline (PBS) was added to thetube and the bacterial pellet waswashedby shaking it gently. The washed pelletwas again centrifuged for 10 minutes at3000rpm . The pellet thus formed was then diluted withPBS. The initial population density of the sample wasmaintained at 1.5×108CFU/ml after comparing with 0.5McFarland standard solution i.e. Absorbance (450nm) =0.5(1.5×108CFU/ml) to know effecte nano Gold of bacteria *E.coli* isolation from diarrheagenic stool

Results

 Among the 55 stool samples which were screened, 35 samples 63.6%showed the growth of *E.coli -20*(36.3%) table1.

*E. coli* isolated and identified by using conventionalbiochemical method

The present study was performed to identify the incidence of *E. coli* as a potential aetiologic agentof diarrheal disease in a Hiella teaching Hospital. Previous reports have evaluatedthe prevalence of various non-Shiga toxin-producing *E. coli* as a cause of childhood diarrheain (19). Our study showed the importance of pathogenic*E. coli* gastroenteritis in clinical practice and inthe surveillance of incidence and complications.

A role of primary importance to reduce the diffusionof these pathogenic agents is played by improvementsin sanitary measures (i.e. hand-washingafter every nappy change and a clean watersupply) (20) .

**Table 1.**  **Number Bacterial(*E.coli***) **isolates from stool samples**

|  |  |
| --- | --- |
| **No. *E.coli* Isolates -%** | **No.*E.coli* Isolates +%** |
| (20:55) 36.3 % | (35:55) 63.6 % |

 *Escherichia coli*35 isolates were obtained from the stool samples screened. 18(51.4%)of the*Escherichia coli* isolates were from the male students while 17(48.6%) were from the female students(Table 2)

**Table 2: Distribution *E.coli* Isolates Obtained from Stool Samples from sex Pations.**

|  |  |  |  |
| --- | --- | --- | --- |
| **Total** | **No.*E.coli* Isolates -** | **No.*E.coli* Isolates +** | **Sex** |
| 31 | 13(65 ) | 18(51.4) | **Male** |
| 24 | 7( 35 ) | 17(48.5) | **Female** |
| 55 | 20 | 35 | **Total** |

 The diarrheagenic E. coli as a group were high frequently resistant to ampicillin 57.1%, Septrin71.4% ,Ofloxacin65.7%, Augmentin94.2 %,Ciprofloxacin71.4 % and chloramphenicol 91.4 % (Table3),Fig. 1 demonstrates the antibiotic sensitivity of of *E.coli* isolates Obtained from diarrheagenic .



**Fig 1: Antimicrobial susceptibility of of *E.coli* isolates Obtained from diarrheagenic Samples (n=35).**

 This study antimicrobial resistance in E. coli was associated both with a high frequency of antibiotic use for diarrhea (46%) and with a higher frequency of previous antibiotic exposure for non enteric infections. Recent antibiotic use, particularly one month before exposure, is a risk factor for developing infection or colonization with resistant bacterial pathogensE. coli is more resistant because they are exposed to antimicrobials more often, which may be because they cause persistent diarrhea and/or are often carried asymptomatically. Thus the long time within human hosts increases the chance that they will be exposed to antimicrobials and/or acquire resistant genes from the resident flora It is known that resistance to multiple antibiotics can be due to a variety of mobile genetic elements such as plasmids, transposons, and gene cassettes in integrons.(23)

The literature has reported varying rates of resistance against ciprofloxacin, which can be explained by the high prescription of this drug in some countries as a treatment for enteric infections caused by Gram-negative bacteria. .(24,25)

The high antimicrobial resistance observed In children with acute gastroenteritis in our study raises a broad discussion on the indiscriminate or improper use of antimicrobials which becoming an alarming situation in drug resistance. Monitoring drug resistance patterns of *E. coli* will give vital clues to clinicians regarding therapeutic regimens to be adopted against individual cases and will be an important tool to devise a comprehensive chemo-prophylaxis.The development of newer antibiotics may offer a short term solution to the problem of resistance among diarrheagenic bacteria especially *E. coli* but more effective measures, such as health education and further research on the prevention of infections through quality sanitation.(26)

**Table3:**  **Antibiotic susceptibility pattern of *E.coli* isolates S=sensitive; I=intermediate; R=resistant**

|  |  |  |  |
| --- | --- | --- | --- |
| **Resistant****NO. %** | **Intermediate****NO. %** | **Sensitive****NO. %**  | **Antibiotic** |
| 7 ( 20%) | 0 (0%) | 28 (80 %) | **Cefotaxime(CE)**  |
| 25(71.4 %) | 2 (5.8%) | 8 ( 22.8%) | **Ciprofloxacin(CPX)** |
| 1 (2.8%) | 1 (2.8%) | 33 (94.2%) | **Ceftriaxone** |
| 5 (14.2 %) | 0 (0%) | 30 ( 85.7 %) | **Gentamicin(CN)** |
| 3 (8.5 %) | 0 (0%) | 32 (91.4 %) | **Chloramphenicol(CH)** |
| 33 (94.2 %) | 0 (0%) | 2 (5.8%) | **Augmentin (AU)** |
| 23 (65.7 %) | 4 (11.4%) | 8 (22.8 %) | **Ofloxacin (OFX)** |
| 25 (71.4 %) | 3 (8.5%) | 7 (20 %) | **Septrin (SXT)** |
| 30 (57.1%) | 0 (0%) | 3 (8.5%) | **Ampicillin(AM)**  |

**N. isolates of *E.coli* =(35)**

**5. Conclusion**

 The *E.coli* isolate from stool children is one of the Enterobacteriaceae family have emerged as major pathogens of interest because of theirability to resist multiple antibiotics which confers on its a survival advantage with the propensity to acquire suchtraits as resistance determinant to various antimicrobials and other virulence factors, these organisms willcontinue to create new therapeutic problem and dilemmas.(27)The studysuggests that the use of antibiotics should be monitored to prevent the development of multiple antibioticresistant organisms especially in individuals living in crowded facilities.

**Nano gold Nanoparticles & Its Antibacterial Activities to the bacteria *E. coli*.**

Three dilutions of gold nanoparticles, this rate was possible after 60 minutes of disinfection. Thus 300ppm effect of gold nanoparticles on reducing *Pseudomonasaeruginosa***.**colony count is faster than the 100 ppm , 200 ppm in three tim 15 , 30, 45 minte (P value >0.05).



**Fig. 2 Effect of *E. coli* killing when add Gold Nano particles 100 ppm ,200 ppm, 300ppm compare of control from defferent time( 15,30,45) .**

Though there are many mechanisms attributed to the antimicrobial activity shown by gold nanoparticles, the actual and most reliable mechanism is not fully understood or cannot be generalized as the nanoparticles are found to act on different organisms in different ways. his has been increased due to resistance in some pathogenic bacteria strains to conventional antibiotics, which has initiated new studies to search for more effective treatments against resistant microorganisms  A longer irradiation time was required to killand add nano gold led to kill ***E. coli*** This has been attributed with the complex cell-wallstructure of gram-negative species compared to grampositiveones .(28)

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