

Synergistic evaluation of AgO₂ nanoparticles with ceftriaxone against CTXM and blaSHV genes positive ESBL producing clinical strains of Uro-pathogenic *E. coli*

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Abstract: The silver oxide nanoparticles (AgO₂-NPs) were synthesised using silver foil as a new precursor in wet chemical method. X-ray diffraction analysis shows crystallographic structures of AgO₂-NPs with crystallite size of 35.54 nm well-matched with standard cubic structure. Scanning electron microscopy analysis clearly shows the random distribution of spherical-shaped nanoparticles. Energy dispersive X-ray analysis confirmed the purity of the samples as it shows no impurity element. Fourier transforms infra-red analysis confirmed the formation of AgO₂-NPs with the presence of Ag-O-Ag stretching bond. All the techniques also confirmed the loading of ceftriaxone drug on the surface of AgO₂-NPs. This study also described the effect of AgO₂-NPs having synergistic activity with β lactam antibiotic i.e. ceftriaxone against ESBL generating *Escherichia coli* (*E. coli*). Among isolated strains of *E. coli*, 60.0% were found to be ESBL producer. The synergistic activities of AgO₂-NPs with ceftriaxone suggest that these combinations are effective against MDR-ESBL *E. coli* strains as evident by increase in zone sizes. The present study observed rise in MDR-ESBL *E. coli* with polymorphism of blaCTXM and blaSHV causing UTI infections in Pakistani population. The antibiotic and AgO₂-NPs synergistic effect can be used as an efficient approach to combat uro-pathogenic infections.

1 Introduction

In the treatment of bacterial infections, use of cephalosporins has led to the development of a novel class of broad-spectrum enzymes called extended spectrum β -lactamases (ESBLs) [1]. *Escherichia coli* (*E. coli*) strains producing ESBL enzymes are considered as the well-known reasons of infections in urinary tract of children [2]. *K. pneumoniae* producing ESBL was first described in 1983 [3] and consequently, *E. coli* producing ESBL enzymes was reported in 1987 [4]. Since then, reports on the production of ESBL-producing microorganisms have been consistently expanding worldwide. ESBLs are encoded by mobile genes; the most frequently encountered ESBLs genes belong to the blaCTX-M, blaSHV, and blaTEM. TEM (Temoniera), SHV (sulphydryl variable), and CTX-M type are the most prominent variants [5]. The prevalence regarding antibiotic resistance among ESBL producing uro-pathogenic *E. coli* continues to be increased inside both healthcare and local community settings and become a challenge to the healthcare professionals in terms of disease prevention, therapeutic development, and epidemiology [6]. Alternate antimicrobial agents are immensely needed to fight ESBL-producing strains, nanoparticles in combination with existing antibiotics are an alternative technique to fight against infections caused by MDR bacteria [7]. Silver, due to its broad-spectrum antimicrobial property, could represent a favourable option especially in its nanoform [8]. Silver oxide has been used to treat bacterial infections in the start of nineteenth century but when the era of antibiotics began, along with the discovery of penicillin [9]. Among the wide range of nanoparticle choices available, silver oxide nanoparticles due to its wide range of antibacterial properties constitute a very encouraging loom for the advancement of new antimicrobial agents [10]. Ag₂O nanoparticles exhibit antibacterial activity against both gram-positive as well as gram-negative bacteria but its efficiency is higher in case of gram-negative

bacteria. [10] It has been observed by Harshita Negi *et al.* during their research that by increasing the concentration of silver oxide nanoparticles, their inhibition zone increases. They exhibit higher efficiency due to the larger surface to volume ratio. Similarly, Xin Wang *et al.* [11] showed the efficiency of different shapes of Ag₂O nanoparticles. They found that antibacterial activity of Ag₂O nanoparticles against *E. coli* gram-negative bacteria depends on the shape of nanoparticles. Silver oxide NPs have received rigorous attention in medicine, as they are able to act as antimicrobials, and elicit anti-angiogenesis, anti-inflammatory, and anti-platelet activities [10, 12]. There are detailed reports regarding anti-biofilm adequacy of silver nanoparticles against biofilm produced by expanded range β -lactamase isolates of *E. coli* [13]. Several studies about the synergistic action of nano-materials in conjunction with antibiotics have been reported [14], nano-silvers combined to polymixin B also confirmed synergistic consequences with regard to gram-negative microorganisms [15].

The first objective of the present study is to synthesise AgO₂-NPs using a novel method. There are many reports on silver oxide synthesis [16, 17] but our methodology is unique in utilisation of Ag foil as precursor. The second objective is the isolation of uro-pathogenic strains of *E. coli* producing ESBL enzymes and to analyse existence of blaCTX-M gene, blaSHV gene, and blaTEM gene among *E. coli* strains showing phenotypic ESBL characteristic on antibiotic sensitivity plates. Furthermore to combat these ESBL-producing uro-pathogenic *E. coli* strains, AgO₂-NPs synergistic interaction with β -lactam antibiotic (ceftriaxone) was evaluated against ESBL-producing *E. coli* strains. The prevalence of diseases caused by broad spectrum β -lactamases (ESBLs) is a worldwide problem. Urinary tract infection caused by *E. coli* strains producing ESBL enzymes is known as a noteworthy hazard of childhood kidney diseases. Furthermore to combat these ESBL-producing uro-pathogenic *E.*

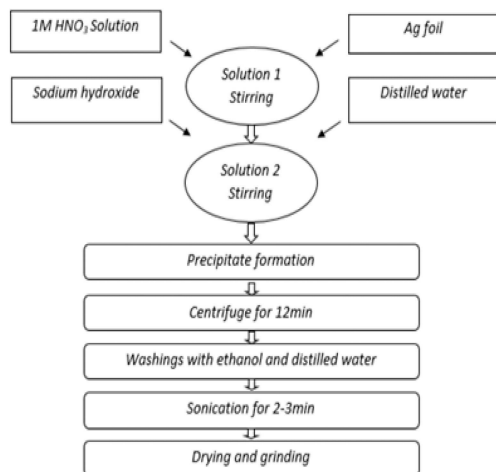


Fig. 1 Schematic diagram of silver oxide nanoparticles (NPs)

coli strains silver nanoparticles synergistic interaction with β -lactam antibiotic ceftriaxone was evaluated against ESBL-producing *E. coli* strains.

2 Experimental procedures

2.1 Materials and methods

Silver foil, sodium hydroxide (NaOH), nitric Acid (HNO₃), antibiotics discs: Augmentin (20 μ g amoxicillin + 10 μ g clavulanic acid), Cefazidime (30 μ g), Ceftriaxone (30 μ g), Aztreonam (ATM-30 μ g), and Cefotaxime (CTX-30 μ g) (Oxoid, UK). Culture media CLED agar, MacConkey agar, TSI agar, and Muller Hinton agar (MH) were purchased from (Oxoid, UK).

2.2 Synthesis of silver oxide nanoparticles

Silver foil was used as a precursor for synthesis of silver oxide nanoparticles by adopting wet chemical method. First of all, one molar aqueous solution of nitric acid (HNO₃) was prepared and silver foil was dissolved into it with constant stirring (solution 1). Then, 40 mL of 0.5 M solution of sodium hydroxide (solution 2) was added into it drop-wise until brownish precipitates are formed and allowed to stir for an hour. The solution was centrifuged for 12 min and washed with ethanol and distilled water. Then, it was sonicated for 5 to 10 min and dried. The schematic representation of the synthesis of silver oxide nanoparticles is shown in Fig. 1.

2.3 Sample collection and bacterial strains identification

The urine samples of paediatric patients suffering from urinary tract infection from nephrology department of tertiary care hospital of Pakistan were brought into microbiology lab of International Islamic University and the collected urine samples were processed for the isolation of *E. coli*. The urine samples were cultured on the standard media including cytosine lactose electrolyte deficient (CLED) and MacConkey agar plates. After culturing, the plates were incubated at 37°C for 24 h. The colony showed fermentation of lactose on MacConkey agar and CLED media were refined and recognised by their morphology as round, rose-pink to red states on MacConkey agar medium and yellow colonies on CLED agar. The bacterial isolates were identified by standard microbiological techniques as described in the Bergey's manual of systematic bacteriology, which involves colony characterisation, cell morphology, and biochemical tests i.e. biochemical reactions e.g. oxidase test, catalase, Indole and methyl red test, vogesproskaur reaction, urease, and citrate and H₂S production in triple sugar iron medium was examined [18].

2.4 Disc diffusion test for ESBL detection

Identification of ESBL-producing *E. coli* was done using the Kirby–Bauer disc diffusion test on Muller–Hinton agar plates inoculated with the *E. coli* strains as recommended for standard

Table 1 Sequence of the primers used for the genotyping of blaSHV, blaCTX-M and blaTEM

Gene	Primers	Sequence	Product size, bp
bla TEM	forward primer	ATG AGT ATT CAA CAT TTC	858
	reverse primer	CG CCA ATG CTT AAT CAG TGA GG	—
bla SHV	forward primer	ATG CGT TAT ATT CGC	859
	reverse primer	CTG TG AGC GTT GCC AGT GCT CGA TC	—
bla CTX-M	forward primer	SCS ATG TGC AGY ACC	581
	reverse primer	AGT AA ACC AGA AYV AGC GGB GC	—

disc diffusion susceptibility test CLSI (2012) [19]. Augmentin disc (20 μ g amoxicillin + 10 μ g clavulanic acid) were positioned at the middle of the plate, and discs containing ceftazidime (30 μ g), ceftriaxone (30 μ g), Aztreonam (ATM-30 μ g), and cefotaxime (CTX-30 μ g) were placed at a distance of 25 mm from Augmentin disc. After 24 h incubation at 37°C, a zone of inhibition around one or more cephalosporin discs was extended on the side nearest to the Augmentin disc. This synergy was designated as ESBL positive.

2.5 Antibiotics susceptibility profiling

The antibiotics susceptibility patterns of the ESBL-producing *E. coli* were determined by disc diffusion method on MH plates as per CLSI rules 2012 [20]. Total 17 antibiotics discs of known concentration, i.e. Augmentin (30 μ g), Cefipime (30 μ g), nitrofurantoin (300 μ g), Cefixime (5 μ g), ceftriaxone (30 μ g), amikacin (30 μ g), ciprofloxacin (5 μ g), sulphanethoxazole SXT (25 μ g), gentamicin CN (10 μ g), piperacillin/tazobactam (110 μ g), sulbactam (105 μ g), norfloxacin NOR (10 μ g), impipenum (10 μ g), ampicillin AMP (10 μ g), and nalidixic acid NA (30 μ g) were placed on the surface of these plates pre-inoculated by ESBL-producing *E. coli*. The plates were incubated overnight at 37°C for 24 h. Diameters of the zone of inhibition were measured in mm with the help of a ruler, and interpreted as per CLSI guidelines 2013 [19].

2.6 Genomic DNA extraction and genotyping

The genomic DNA of bacteria was extracted by phenol and chloroform routine extraction method [21]. The genomic DNA extraction was performed in strict understanding to the following steps: (i) quantitative determination of DNA by UV-spectrophotometry using Nanodrop 2000c (Thermo Scientific, USA); (ii) PCR analysis of TEM, SHV, and CTX-M genes in all isolated for the detection of ESBL resistance blaTEM, blaSHV, and blaCTX-M genes, using primers provided in Table 1. The process of PCR was done in 96-well thermal cyclers (Thermo Electron Corporation, Mill passage, USA) utilising amplification protocol comprising of 30 cycles with an underlying initial denaturation at 94°C for 5 min and final extension at 72°C for 4 min. Each cycle comprised of denaturation at 94°C for 1 min, annealing for 1 min at 57°C for blaSHV, 55°C for blaCTX-M, and at 50°C for blaTEM and final extension at 72°C for 1 min. The amplified PCR products were detected by electrophoresis using 2.0% of agarose gel to obtain better resolution of the amplified product which size was checked with standard molecular weight marker.

2.7 Evaluation of synergism of AgO₂-NPs with ceftriaxone

The tests were performed by using the disk diffusion (DD) strategy on Müller-Hinton agar plates. In this examination, three kinds of disks were utilised (i) paper disc (10 mm) immersed with 10 μ L of AgO₂-NPs; (ii) ceftriaxone discs of two different concentrations (10 μ g/mL and 20 μ g/mL and (iii) ceftriaxone discs saturated with

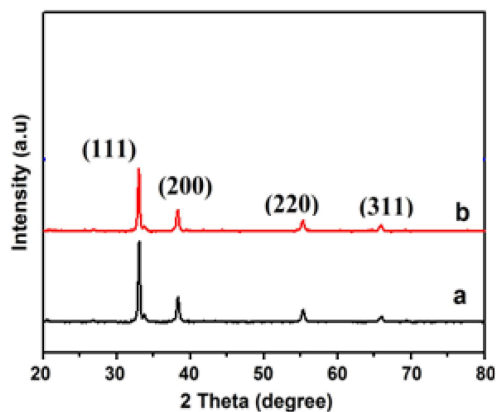


Fig. 2 X-ray diffraction analysis of various samples
(a) Ag₂O-NPs and, (b) drug loaded Ag₂O-NPs

10 and 20 μL of Ag₂O-NPs). Fresh cultures of ESBL *E. coli* with <12 h incubation were prepared at a concentration of 0.5 McFarland Scale29 (1.5×10^8 CFU/mL) and used as inoculums for swabbing on plates. After swabbing the prepared discs containing synergistic formulation were placed on inoculated plates and were incubated at 37°C for 24 h, the zones of inhibition were measured next day. The synergism was assessed by the equation $\{(C - B)/B\} \times 100$, where, B = the zone of inhibition of the ceftriaxone alone and C = Ceftriaxone + Ag₂O-NPs. This equation used to assess the expansion of the zone of inhibition around the bacteria caused by the antibiotic in combination with Ag₂O-NPs.

2.8 Observation of phenotype changes of silver oxide nanoparticles and antibiotic-treated cells

Culture of ESBL *E. coli* (A_{600} , 0.05) at its exponential stage was grown in Luria broth and was treated with silver oxide nanoparticles and antibiotic formulation (10 $\mu\text{g}/\text{mL}$ + 10 $\mu\text{g}/\text{mL}$) and incubated for 15 min at 37°C and slides were made in 1.0% agarose and observed under phase contrast microscope, to find their morphology, specific care was taken to reduce the sample exposure to UV light, image grabbing were performed basically as depicted by Edwards and Errington [22].

3 Results and discussion

Inappropriate and arbitrary utilisation of β -lactam drugs are the prominent reasons of emergence of resistant strains and their episodes. As per the current investigation, the predominance of β -lactamase genes in Pakistan is high. Therefore, with a specific end goal to control resistant strains, suitable strong measures, new antimicrobial formulations, and additional research to determine antibiotic resistance patterns in different regions are crucial. In addition, there is a pressing need to build up alternate antibacterial agents. The past decade has observed as a considerable rise in the global use of nano-medicines as inventive tools to fight antimicrobial resistance dilemma. Silver oxide nanoparticles (Ag₂O-NPs) are one of the notable antibacterial substances and showed up as reasonable possibility for use in combinations with traditional antibiotics [23]. Antibiotic resistance has evolved as a serious area under discussion against life threatening infections and remains a crucial rationale of morbidity and mortality. Infections in the urinary tract of the children are the well-known microbial disease with an increasing incidence rate in girls [24]. *E. coli* distant as the main cause of UTI with prevalence ranging from 80 to 90% and are progressively revealed as causative agents of UTI diseases [4] and production ESBLs by uro-pathogenic *E. coli* causes inactivation of large number of antibiotics and present major therapeutic dilemma [1]. Studies to recognise the microorganisms causing UTIs and determining the resistance prototype to antimicrobial agent's exhibit specifically geographical area prompts the choice of reasonable treatment possibilities. The current study investigated the antimicrobial susceptibility patterns of ESBL enzymes producing *E. coli* isolates separated from urine

sample of paediatric patients experiencing infections in the urinary tract in tertiary-care hospitals of Pakistan. We have prepared the Ag₂O-NPs using Ag foil as cheaper precursor. These Ag₂O-NPs are utilised in combination with antibiotic and their synergistic effect is studied. The current study includes 120 uro-pathogenic *E. coli* strains isolated from paediatric patients having UTI infections, the isolated strains of *E. coli* were screened for the production of ESBL enzymes by double-disc synergy method and the existence of bla CTX-M, bla SHV, and bla TEM genes were investigated by using PCR. High rate of resistance was observed against ceftriaxone (100%), followed by ciprofloxacin (94.0%) cotrimoxazole (79.0%), whereas 71.0% of the isolates exhibited blaCTX-M genes, 29.0% isolates were positive for blaSHV gene, 29.0% isolates harboured both blaCTX-M and blaSHV genes, while blaTEM gene was not detected in any of the isolated strain. Rather than treatment with antimicrobials alone, we additionally analysed antibiotic/Ag₂O-NPs combinations, our results revealed a positive synergism marked by significant increase in sizes zone of inhibition against MDR-ESBL *E. coli* strains.

3.1 Characteristics of silver oxide nanoparticles

3.1.1 X-ray diffraction spectroscopy: Silver oxide nanoparticles (Ag₂O-NPs) have been synthesised successfully using a cheaper and easily available precursor i.e. silver foil. X-ray diffraction (XRD) analyses of the samples were carried out using X-ray diffractometer in which Cu K_{α} of wavelength 1.54 Å radiations were used. Intensity of the diffracted beam was recorded as a function of position (2θ). Fig. 2a presents the X-ray diffraction pattern of the prepared sample. It gives the confirmation of Ag₂O-NPs by comparing them with the standard crystal structures, crystallinity, and value of lattice parameters by using JCPDS card. It is evidently showed that all of the peaks of all the three samples well-matched with the Bragg reflections of the standard cubic structure (Space group: Pn-3m, $a = 4.7360$, $b = 4.7360$, $c = 4.7360$, JCPDS: 012-0793). The five sharp peaks at 32.88, 37.98, 55.28, 65.34, and 68.59 can be allotted to their characteristic (111), (200), (220), (311), and (222) indices, respectively. All the reflected peaks were matched with the Ag₂O phase having simple cube structure. In addition, it can be analysed that the product has a high purity as no characteristic peaks of other impurities are observed. The crystallite sizes of NPs were also calculated from Debye-Scherrer's formula was 35.54 nm. Fig. 2b depicts the X-Ray diffraction pattern of ceftriaxone drug loaded Ag₂O material. The loaded sample shows all characteristic peaks of Ag₂O with change of intensity. There is no appearance of any peak related to drug loading due to less contents. Although there is change in intensity of (111) peak value which confirms the incorporation of dye molecules on the surface of Ag₂O.

3.1.2 Scanning electron microscopy: The morphology of prepared sample is shown in for pure (Figs. 3A and B and ceftriaxone drug loaded samples Figs. 3C and D). It is clear from the image that the particles were highly agglomerated in nature. The SEM pictures showed clearly, random distribution of nanoparticles of spherical shape which were grown in a very high density. The image revealed the average particle size of 100 nm. The sample after drug loading shows agglomeration of Ag nanoparticles with appearance of large-sized crystal of drug material of ceftriaxone.

3.1.3 Energy dispersive X-ray analysis: The energy dispersive X-ray analysis of the prepared samples indicated the presence of oxygen and silver elemental composition. Fig. 3E shows the EDS analysis of sample. It can be clearly seen that the sample just comprises on silver (wt.% 83.31) and oxygen (wt.% 16.69). It shows the purity of the sample as no other peak related with any impurity has been indicated by XRD analysis. Elemental analysis of sample Ag₂O-NPs is given in Table 2.

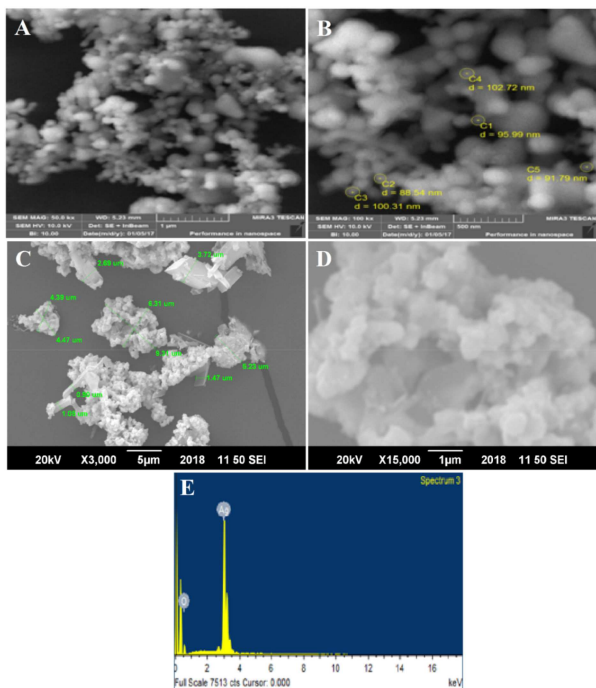


Fig. 3 SEM images of AgO_2 NPs and drug loaded AgO_2 NPs at different magnifications and Energy dispersive X-ray analysis of AgO_2 -NPs (A, B) SEM images of AgO_2 NPs, (C, D) SEM images of drug loaded AgO_2 NPs, (E) Energy dispersive X-ray analysis of AgO_2 -NPs

Table 2 Elemental analysis of sample AgO_2 -NPs

Elements	Weight, %	Atomic, %
O K	16.69	57.47
Ag L	83.31	42.53
Total	100.00	100.00

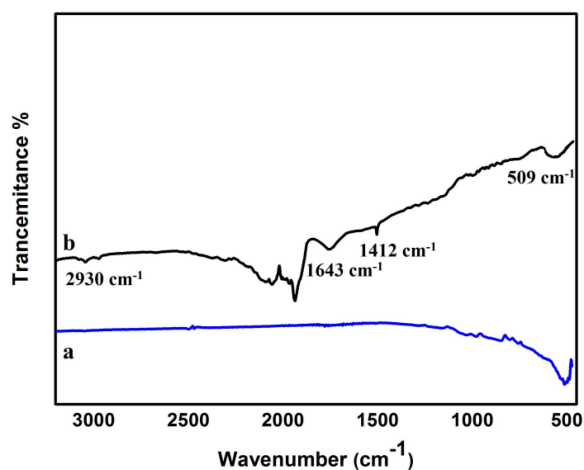


Fig. 4 FTIR spectra of AgO_2 -NPs and ceftriaxone-loaded AgO_2 -NPs (a) AgO_2 -NPs, (b) ceftriaxone-loaded AgO_2 -NPs

3.1.4 Fourier transforms infra-red: Fig. 4 shows FTIR spectra of pure AgO_2 -NPs and ceftriaxone loaded AgO_2 -NPs. It can be seen clearly from Fig. 4a that the sample of pure AgO_2 -NPs shows a vibration band at 509 cm^{-1} which represents Ag-O-Ag stretching vibration in correspondence with literature. The ceftriaxone-loaded AgO_2 sample showed the characteristic peaks of ceftriaxone in addition to Ag-O-Ag stretching vibration. These bands are recorded at 2930 cm^{-1} for C-H stretching, 1412 cm^{-1} of stretching $C=N$ and 1640 cm^{-1} peak represents amide group $C=O$ axial deformation.

3.2 Morphological and biochemical characterisation of *E. coli* strains

On the basis of colonial morphology and gram staining, *E. coli* strains were recognised. On MacConkey agar, *E. coli* colonies were lactose fermenter. Gram staining showed that all of the strains of *E. coli* were gram negative and appeared as rod-shaped. All of the isolated strains of *E. coli* were oxidase negative. In indole test, a red ring was appeared after adding Kavoc's reagent that was considered positive. Acidic reactions and gas formation were observed in TSI tubes inoculated.

3.2.1 Phenotypic detection of ESBL production: A total of 120 *E. coli* isolates were analysed by the double-disc synergy method for the production of ESBL enzymes. Out of these 120 *E. coli* isolates, 72 isolates analysed evidenced ESBL-producing activity. So the overall incidence rate of ESBL-producing *E. coli* in our study was 60%. The double-disc synergism is shown in Fig. 5.

3.2.2 Antibiotic susceptibility profiling: Antibiotic susceptibility profiling of the ESBLs producing *E. coli* were determined by using disc-diffusion method. All of the ESBL-producing *E. coli* isolates ($n=72$) were resistant to ceftazidime (CAZ 100%), ceftriaxone (CRO 100%), cefixime (CFM 100%), ampicillin (AMP 100%), and nalidixic acid (NA 100%). All of the ESBLs isolates showed 100% susceptibility to imipenem, whereas 94% strains were susceptible to amikacin (AK), 81% to nitrofurantoin (F) 72% to cefoparazone, 62% piperacillin (TZP), 48% to Augmentin (AMC), 46% to Cefepime, 31% to Gentamicin (CN) 18% to Fosfomycin (FOS). Then, 13% to Co-trimoxazole (SXT) and 10% to Norfloxacin (NOR) MDR characteristic are evident in Fig. 5. The resistance pattern exhibited by isolates against the test antibiotics is summarised in Figs. 5 and 6.

3.2.3 PCR analysis of blaTEM, blaCTX-M and blaSHV genes: Among isolated strains, 21 isolates were positive for blaSHV genes and 51 showed prevalence of blaCTX-M gene, whereas blaTEM gene was not detected in any of studied isolate. This information is presented in Figs. 7 and 8. The percentage distribution of blaCTX-M, blaSHV, and blaTEM is summarised in Fig. 9.

The prevalence of MDR-ESBLs strains is growing resulting lack of usefulness of β -lactam class of antibiotics. In this study, 120 *E. coli* strains were screened from urine samples and among them 60% were ESBL-producing strains proposing a higher predominance of these enzymes in paediatric patients having infection in urinary tract in patients. The researches in different regions have also informed increase ESBL enzymes prevalence in uro-pathogens [25]. In this study, the prevalence of genes involved in MDR-ESBL *E. coli* strains was studied as β -lactamase genes are resulting from TEM1, thus blaCTX-M, blaSHV, and blaTEM genes were studied. Our data represent blaCTX-M as the most prevalent genotype which is 71% followed by blaSHV 29% and presence of both blaCTX-M-blaSHV genes was found in 29% of the strains. The frequency of blaCTX-M and blaSHV is in high agreement with the previously reported data [5, 26]. Masroor *et al.* reported 51% blaCTX-M in uro-pathogenic ESBL *E. coli* from tertiary care hospitals [27]. ESBLs belonging to the CTX-M types are also most prevalent in Europe [28]. The first occurrence of a CTX-M ESBL-producing *E. coli* outbreak intervened mother to neonate transmission in an Irish neonatal intensive care unit become mentioned by O'Connor *et al.* 2017 [29]. In light of the above discoveries, it is far clear that the frequency of a wide variety of ESBL enzymes is growing.

3.2.4 Antibacterial effect of AgO_2 -NPs in synergism with antibiotics: In our examination, we assessed the impact of AgO_2 -NPs alone and in combination with the antimicrobial agent ceftriaxone (CRO). $10\text{ }\mu\text{g/mL}$ concentration of ceftriaxone produced a zone of clearance of $10\pm 1\text{ mm}$; $10\text{ }\mu\text{g/mL}$ concentration of AgO_2 -NPs alone demonstrated a restraint zone of $14.5\pm 1\text{ mm}$, while combination of CRO + AgO_2 -NPs produced a

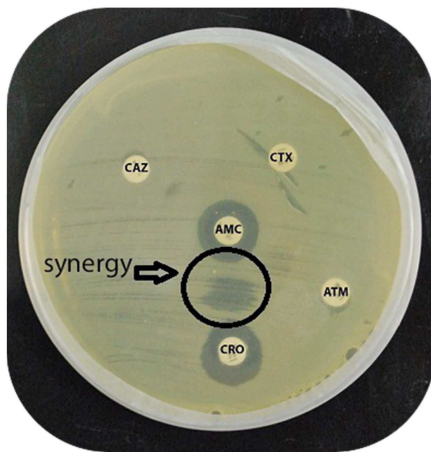


Fig. 5 Phenotypic detection of ESBLs between Ceftriaxone (CRO) and Augmentin (AMC) disc

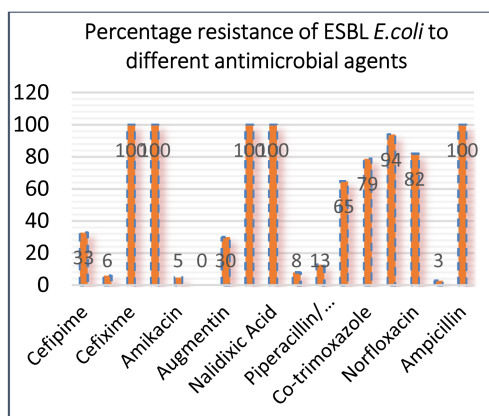


Fig. 6 Antibiotic resistance profile of uro-pathogenic ESBL *E. coli* strains

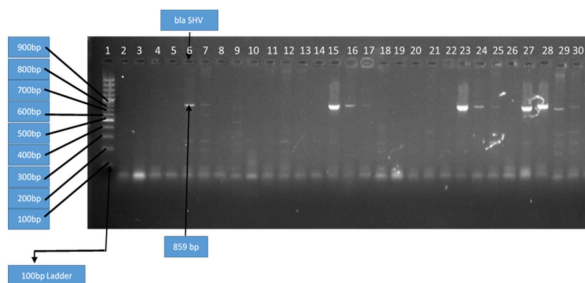


Fig. 7 Amplification of *bla*CTX-M gene by PCR with 100 bp DNA ladder. Key. Lane 1 was 100 bp DNA ladder. Lane 2, 3, 4, 5, ..., 31 was *bla*CTX-M positive

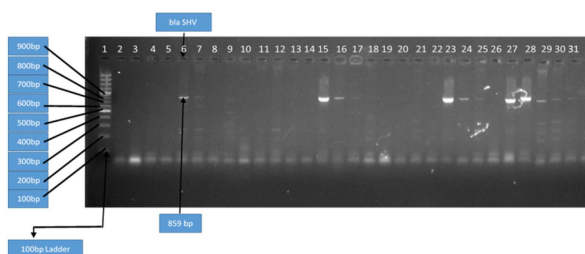


Fig. 8 Amplification of *bla*SHV gene by PCR with 100 bp DNA ladder. Key. Lane 1 was 100 bp DNA ladder. Lane 6, 7, 15, 16, 17, 23, 24, 25, 27, 28, 29 and 30 was *bla*SHV positive

zone of inhibition of 26.4 ± 2 mm (Fig. 5). The relationship of CRO+AgO₂-NPs created a 30% expansion in the zone of inhibition when compared and the antibiotic alone (Table 3). In Fig. 10, we can observe the activity of this affiliation; the inhibition zones on the disc containing CRO+AgO₂-NPs in both the tested

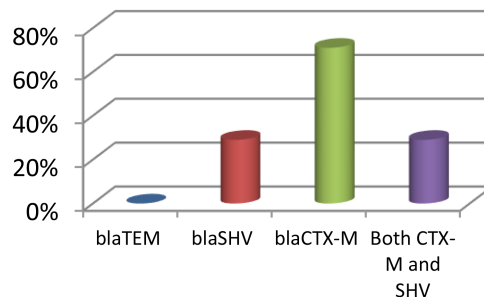


Fig. 9 Percentage distribution of *bla*CTX-M, *bla*SHV and *bla*TEM genes among ESBL *E. coli*

Table 3 Antibacterial activity of antibacterial formulations against MDR-ESBL *E. coli*

Antibacterial formulation	Conc., µg/ml	Inhibition zone (mean ± SD), mm
ceftriaxone (CRO)	10	12.0
ceftriaxone (CRO)	20	13.5
(AgO ₂ -Nps)	10	14.5
(AgO ₂ -Nps)	20	18.0
(CRO + AgO ₂ -Nps)	10 + 10	26.4
(CRO + AgO ₂ -Nps)	20 + 20	32.6

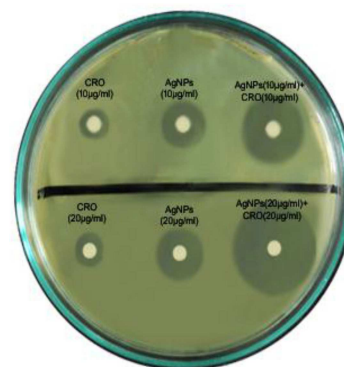


Fig. 10 Synergism between synthesised AgO₂-NPs with ceftriaxone (CRO) against ESBL *E. coli*

concentration are much larger than the disc with CRO alone and AgO₂-NPs alone. Rather than treatment with antimicrobials alone, we additionally analysed antibiotic/AgO₂-NPs combinations, our results revealed a positive synergism marked by significant increase in sizes zone of inhibition against MDR-ESBL *E. coli* strains. In AgO₂-NPs and antibiotic-treated cells, morphological changes were observed. Schreurs and Rosenberg [30] also reported changes in cell morphology in AgO₂-NPs-treated bacterial cells because of increase in membrane permeability, which influence the cells capacity to appropriately manage the transport action through the plasma membrane for example, Ag weakens the uptake and release of phosphate ions in *E. coli* [30]. The functionalisation of AgO₂-NPs using antibiotics is not only a hopeful nano-platform to combat bacterial resistance but may also trim down the dose and hence toxicity of the existing antibiotic against uro-pathogenic strains [11, 12].

3.2.5 Phenotypic changes in nanoparticle and antibiotic-treated cells of *E. coli*: Cells of ESBL enzyme producing *E. coli* were treated with Ag nanoparticles synergistic formulation with antibiotic i.e. ceftriaxone. Morphological changes become noticeably clear upon treatment with antimicrobial formulation, phase contrast microscopic examination of treated *E. coli* cells showed cell lysis, while some giant and ghost cells were also evident in Fig. 11.

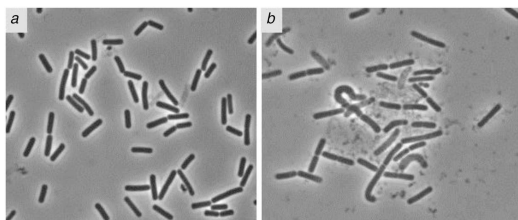


Fig. 11 Effect of AgO_2 nanoparticles with ceftriaxone on cellular morphology of *E. coli*

(a) control without treatment, (b) with treatment

4 Conclusion

AgO_2 -NPs were synthesised using Ag foil as cheaper precursor. These AgO_2 -NPs were utilised in combination with antibiotic. Their synergistic effect have been studied. Rather than treatment with antimicrobials alone, we additionally analysed antibiotic/ AgO_2 -NPs combinations. Antibiotics like ceftriaxone can be combined with AgO_2 -NPs to achieve the treatment goals. Our results revealed a positive synergism marked by significant increase in sizes zone of inhibition against MDR-ESBL *E. coli* strains. High rate of resistance was observed against ceftriaxone (100%). Treatments utilising antimicrobial nanoparticles seem to have promising additive or synergistic impacts on impending function of AgO_2 -NPs to combat ESBL producing strains. The present study also concludes that there was a rise in ESBL-producing uro-pathogenic *E. coli* with blaSHV, blaCTX-M, and both blaSHV and blaCTX-M genetic polymorphism.

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6 References

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