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Exploring the potential of *Lawsonia inermis* green nanoparticles to mitigate quorum sensing in multi drug resistant ESBL producing *E. coli*

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ABSTRACT

The green synthesis of nanoparticles (NPs) has shown promising results in targeting virulence, pathogenicity and quorum sensing (QS) of bacteria and are presently being considered as a promising anti-infective drug target. Thus, this study included synthesis of green zinc nanoparticles using *Lawsonia inermis* (heena) leaves and their characterization by UV–Vis spectroscopy, Fourier Transform Infrared Spectroscopy (FT-IR), Transmission Electron Microscopy (TEM). *In-vitro* efficacy was assessed against *E. coli* isolates confirmed by *uidA* gene primers carrying ESBL producing *blaTEM*, *blaSHV*, *ctxm*-1, *blaCTXM*-2, *blaCTXM*-9, *oxa*-48 and autoinducer *luxS* and *pfs* genes. Finally, MIC of henna nanoparticles was tested for effect on real time expression of luxS gene. UV–Vis spectra showed typical absorption peaks in around 330, 340 nm, 360 nm corresponding to ethanolic and methanolic, aqueous NPs, respectively. FT-IR analyses confirmed chemical bond formation of zinc oxide and TEM revealed the size of NPs as nearly 16nm with spherical shape. Among 42 samples, 9 were confirmed by *uidA* and out of these revealed only 8 isolates carried ESBL producing genes *blaTEM* and *blaSHV*, 4 *blaCTXM*-1 and 2 genes while one isolate had *blaOXA*- 48- like gene. The ABST revealed 5 out of nine isolates as MDR *E. coli.* The phenotypic double disc sensitivity test revealed one isolate suggestive of ESBL resistance. The 4 isolates carrying *blaCTXM*-1 and 2 genes also found positive for *luxS* and *pfs* autoinducer genes. The incubation of MDR ESBL producing and *luxS* autoinducer genes carrying isolates with Heena aqueous extract based nanoparticle at the concentration of 62.5 µg/mL down regulated the expression of *luxS* auto inducer genes up to 18 hrs of incubation. These findings are suggestive of the potential role of green nanoparticles to mitigate quorum sensing in MDR bacteria. However, validation with large number of MDR bacteria is still required.

Keywords: Antibacterial, AMR, Nanoparticles, Quorum sensing

Quorum sensing (QS) is a communication process used by bacteria to coordinate group behaviours through chemical signals called autoinducers (Miller and Bassler, 2001). As cell density increases, these signalling molecules accumulate and trigger changes in gene expression once a threshold is reached (Rutherford and Bassler, 2012). QS regulates functions that are ineffective at the single-cell level but advantageous collectively, such as biofilm formation, virulence factor production, motility, and antibiotic resistance. Both Gram-negative and Gram-positive bacteria utilize QS, often through acyl-homoserine lactones or autoinducing peptides, while *lux*S/AI-2 enables interspecies communication. This system is crucial for bacterial adaptation, survival, and pathogenicity in diverse environments (Miller and Bassler, 2001 and Rutherford and Bassler, 2012).

Zinc-oxide nanoparticles (ZNPs) synthesized via plant-mediated (green) routes have attracted considerable interest as antimicrobial agents because they combine potent biocidal mechanisms with environmentally friendly production (Raha and Ahmaruzzaman, 2022). Unlike conventional chemical or physical synthesis, green synthesis exploits phytochemicals like flavonoids, phenolics, terpenes, and quinones to reduce metal precursors and stabilize the resulting nanoparticles, avoiding toxic reagents and harsh conditions. This approach yields ZNPs with surface chemistries derived from capping phytomolecules, which can enhance dispersion, biocompatibility, and biological interactions—features particularly relevant for antimicrobial applications (Murali *et al.* 2021, Nandhini *et al.* 2024).

Lawsonia inermis (henna) plants, widely present in India and used in various cultural practices are rich in lawsone, tannins and flavonoids that act as both reducing and capping agents (Nandhini *et al.* 2024). Multiple reports have shown that henna-derived ZNPs display effective antimicrobial

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activity against Gram-positive and Gram-negative bacteria and fungal pathogens, often outperforming chemically synthesized counterparts in assays of growth inhibition and biofilm disruption (Upadhyaya *et al.* 2018; Amuthavalli *et al.* 2021). The phytochemical corona on henna-ZNPs has been proposed to facilitate closer contact with microbial cell walls and to potentiate reactive oxygen species (ROS) generation-key contributors to bactericidal action (Amuthavalli *et al.* 2021; Shnawa *et al.* 2023).

Mechanistically, plant-synthesized ZNPs kill microbes through a combination of processes: (1) attachment to and disruption of the microbial cell envelope, increasing membrane permeability; (2) release of Zn^{2+} ions that interfere with enzyme systems and metabolic pathways; and (3) generation of ROS (e.g., hydroxyl radicals, superoxide) that oxidize cellular components and damage nucleic acids (Gupta *et al.* 2018; Shnawa *et al.* 2023). The presence of phytochemical capping layers can modulate these effects by influencing particle size, surface charge, and dissolution kinetics—parameters that directly affect antimicrobial potency (Murali *et al.* 2021, Nandhini *et al.* 2024).

Comparative studies show that NP physicochemical features—size, shape, crystallinity, and surface functionality—determine the magnitude and spectrum of antimicrobial action. Henna-mediated Zinc Oxide nanoparticles (ZNPs) have been reported with hexagonal and spherical morphologies and sizes in the tens of nanometers, correlating with strong inhibition of *Staphylococcus aureus*, *Escherichia coli,* and *Candida species* in agar diffusion and minimum inhibitory concentration assays (Upadhyaya *et al.* 2018; Amuthavalli *et al.* 2021). Similarly, Nyctanthes-derived ZnONPs effectively reduced microbial viability and biofilm formation, and in some reports exhibited photocatalytic enhancement of antimicrobial effects under visible light—an attractive property for water treatment or surface disinfection applications (Amuthavalli *et al.* 2021; Jamdagni *et al.* 2021).

Beyond *in vitro* antimicrobial testing, emerging work examines safety and application contexts: several investigations compare green ZNPs with chemically synthesized analogs in cytotoxicity and environmental impact assays, generally finding that plant-capped particles offer improved biocompatibility at effective antimicrobial doses (Rani *et al.* 2023; Nachimuthu *et al.* 2022). Given the urgent global challenge of antimicrobial resistance, Henna derived ZNPs (HZNPs) represent promising adjuncts or alternatives to traditional antimicrobials suitable for coatings, wound dressings, water purification, and agricultural pathogen control provided that thorough toxicological and ecological evaluations accompany translational efforts (Mohideen *et al.* 2025; Nachimuthu *et al.* 2022). Moreover, in recent past, the emergence of multi drug resistant bacteria has been a major concern. Bacteria have been found to carry multiple drug resistance genes and that make the treatment more difficult leading to approximately 700,000 deaths annually (Jhalora and Bist, 2025). Further, transmission of resistance to non resistant microbes is another concern. Based on all these reports, the present study was planned to understand the mechanism of antibacterial potential of HZNPs and their efficacy against multidrug resistance ESBL producing *E. coli*.

MATERIALS AND METHODS

Ethical requirement: Samples were collected from clinical cases under the proposal number 89 approved by the Institutional Animal Ethics committee (IAEC) (IAEC/SVPUAT/2025/130 dated 27/12/2023).

Plant leaves: The present study involves collection of *Lawsonia inermis* (henna) plant leaves from the campus of SVPUA&T, Meerut during the month of March, 2024.

Sample collection: A total of 42 faecal and pus samples were collected with the help of sterile swabs from clinical cases available at Livestock Research Centre -1 and 2 and presented to Veterinary Clinical Complex of SVPUAT, Meerut and during health camps conducted at different villages by University Teamas per guidelines of WHO Laboratory Biosafety Manual for handling infectious materials (Ficociello *et al.* 2023).

Preparation of Lawsonia inermis (Henna) plant leaves extracts: Fresh leaves were thoroughly washed and shade dried for about 4 to 6 days. The fully dried crisp leaves were grounded in mixer jar and fine powder was prepared. The 10 gm dried powder was mixed in 100 mL a) deionized distilled water, b) 75% ethanol and c) 75% methanol to prepare three different extracts. This mixture was heated at 60°C for 30 minutes. After the solution was cooled it was filter sterilized, filtrate was collected and stored in 4°C for further uses (Messika *et al.* 2012).

Green Synthesis of Zinc Oxide nanoparticles (ZNPs): Zinc oxide nanoparticles (ZNPs) were synthesised by the previously described method (Osinska *et al.* 2020) with some minor modifications. To prepare ZNPs, 10 mL of plant extract was added to 50 mL mixture of 0.35M solution of Zinc nitrate hexahydrate. This was mixed on heating magnetic stirrer at 65°C for two hours and then titrated by the addition of 2M sodium hydroxide (NaOH) drop by drop to maintain the pH of the solution at around 8 until colour of solution changes to yellow and whitish powder settles down at the bottom. The powder is mixed with solution, centrifuged for 5 minutes at 7000 rpm, washed twice with 70% ethanol and distilled water to remove all the impurities from the synthesized HNPs. The precipitated ZNPs were dried in hot air oven overnight at 70°C, ground into a fine powder and in an airtight container and placed at cool and dark place for further use.

Characterization of ZNPs: The synthesized ZNPs were subjected to morphological, optical and chemical characterization by Transmission Electron Microscopy (TEM) (Parvekar *et al.* 2020), UV-VIS double beam spectrophotometer (Shields and Cathcart, 2010) and FTIR (Thermo Scientific Nicolet Summit X) (Singh *et al.* 2011).

Assessment of antibacterial effect of ZNPs: This assay was carried out in 96-well microtiter plate for all three types i.e., aqueous, ethanolic, and methanolic extract based

ZNPs against *E. coli* ATCC 25922 to find the most effective ZNPs. The two fold dilutions of ZNPs starting from the concentration of 1000 µg/mL to 1.9 µg/mL were used in Mueller Hinton Broth (MHB) with 50 µl of bacterial inoculums (0.5 McFarland) and incubated over night at 37°C. ZnO suspension in MHB and MHB were used as positive and negative control. The OD of the plates was taken at 630 nm.

E. coli isolation and identification: All the 42 faecal and pus samples swabs were inoculated in nutrient broth immediately after reaching the laboratory and incubated over night at 37°C for enrichment. Turbid broths were further spread on sterile MacConkey lactose agar MLA (HiMedia Laboratories, Mumbai, India) plates and incubated overnight at 37°C. The pink, round medium-sized were picked as *E. coli* suspect and were further streaked on Eosin Methylene Blue (EMB) agar (HiMedia Laboratories, Mumbai, India) and incubated overnight at 37°C thereafter, colonies having green metallic sheen further identified based on biochemical reactions. Cultures that were Gram-negative, short rods, with IMVC patterns of ++-- (Carter and Wise, 2003) were further processed for molecular confirmation by PCR targeting the *uidA* gene (Anbazhagan *et al.* 2010). Pure *E. coli* isolates were preserved as glycerol stocks and stored at −80 °C for future analysis.

Antibacterial Susceptibility Assay (ABST): All the confirmed *E. coli* isolates (n=9) were subjected for Antibacterial Sensitivity Test (ABST). Antibiotics of different classes, namely Amikacin (AK), Amoxyclav (AMC), Ampicillin (AMP), Aztreonam (AT), Cefipime (CPM), Cefotaxime (CTX), Chloramphenicol (C), Enrofloxacin (EX), Imipenem (IPM) and Vancomycin (VA) were used on Mueller Hinton Agar (MHA) plates according to CLSI guidelines (CLSI, 2020). The results were used to find multi drug resistant isolates of *E. coli*.

ESBL detection: All *E. coli* suspected of producing ESBL based on zone diameter breakpoints corresponding to resistant phenotype were included in the study. ESBL-production was confirmed by the combined disk test with cefotaxime and ceftazidime alone and in combination with clavulanic acid according to CLSI (CLSI, 2020) recommendations.

Molecular Detection of ESBL-Encoding Genes: The isolates that tested positive for ESBL production in the phenotypic detection assay were further screened of corresponding ESBL-encoding genes using multiplex PCR. Specifically, the presence of *blaOXA*-, *blaSHV*-, and *blaTEM*-type genes, as well as those from *CTX*-M groups 1, 2, and 9, was confirmed by multiplex PCR assays employing the primers and cycling conditions described by Dallenne *et al.* (2010).

Molecular detection of quorum sensing gens in E. coli isolates: All the MDR-ESBL producing *E. coli* isolates (n=5) were subjected to detection for the presence of quorum sensing *lux*S and *pfs* genes using previously prescribed sets of primer and method (Wang *et al.* 2016).

Expression analysis of quorum sensing luxS gene in MDR ESBL producing E. coli: To investigate the expression of the quorum sensing *luxS* gene, the most resistant multidrug-resistant ESBL producing *E.coli* isolate was treated with ZNPs at their MIC. The isolate was incubated in Mueller-Hinton broth (MHB), and 150 µL of broth culture was collected at 6, 12, and 18 hours for real-time quantitative PCR (qPCR) analysis of *luxS* gene expression. RNA was extracted from the 150 µL broth culture using TRIsoln Reagent® (GeNei, Bengaluru, India) according to the manufacturer's instructions. Complementary DNA (cDNA) was synthesized using the PrimeScript®1st strand cDNA Synthesis Kit (TaKaRa, Shiga, Japan) following the manufacturer's protocol. The cDNA was used for qPCR analysis with KAPA SYBR® FAST Universal qPCR Master Mix containing SYBR Green (Roche, Basel, Switzerland). Amplification was performed using the CFX96 system (Bio-Rad, Hercules, California, USA.) Melting curve analysis was conducted to confirm the specificity of the amplified products. The relative expression levels of *luxS* were quantified using the ΔΔCt method described by (Livak and Schmittgen, 2001). The expression of *luxS* was normalized to the reference gene *gadA* and the fold change in gene expression between HZnONPs-treated and untreated isolates was calculated.

RESULTS AND DISCUSSION

Zinc oxide nanoparticles (ZNPs) are widely studied because zinc is an essential trace element, considered relatively safe for biomedical applications, cost-effective and has broad-spectrum antimicrobial activity (Sirelkhatim *et al.* 2015). Conventional physical and chemical synthesis methods often involve toxic chemicals and require high energy, whereas green synthesis utilizing natural biomolecules acting as reducing and stabilizing agents, making the process environmentally friendly and biocompatible (El-Saadony *et al.* 2024).

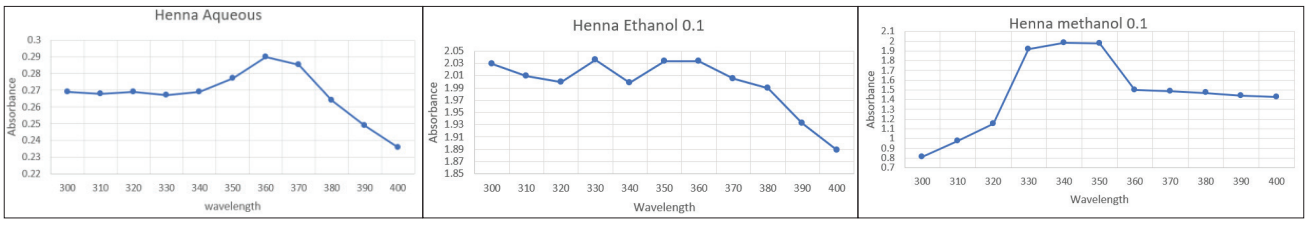

Fig. 1 In UV-vis spectrophotometry, distinct peak centered around 350 nm is specific for A- Aqueous, B- methanolic and C ethanolic extract based ZNPs due to large excitation binding energy at room temperature

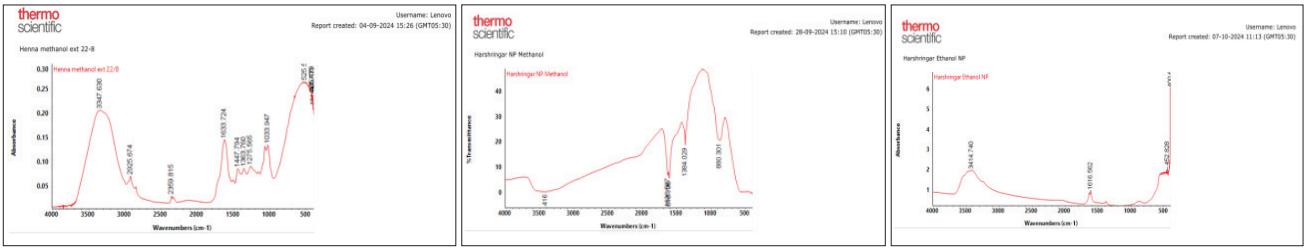

Fig. 2 FTIR based analysis of ZNPs depicting phytochemicals such as alcohols, phenols, amines, carboxylic acids and their interaction with the zinc surface and aid in the stabilization of ZNPs

Synthesis and characterization of green ZNPs: The study was designed to evaluate the role of green ZNPs in addressing the quorum sensing based transmission of antimicrobial resistance in multi drug resistant bacteria. For the purpose the Zinc oxide nanoparticles were synthesized using three different plant extracts of *Lawsonia inermis* (henna) leaves. These were characterized by three different methods. UV–Vis spectral analysis confirmed the biogenic synthesis of ZNPs. Ethanolic, methanolic and aqueous extract based ZNPs showed highest peak at 330, 340 and 360nm, respectively (Figure 1). This indicates nanoparticles in excitation form from ground state to excited state with narrow size distribution leading to sharp absorption peak in UV region 200-400 nm suitable for medical applications (AL-Asady *et al.* 2020).

FT-IR based Infrared studies were carried out to check the purity of nanoparticles and presence of functional groups (Figure 2). The various peaks observed in FTIR are detailed in Table 1.

The phytochemicals such as alcohols, phenols, amines, carboxylic acids, etc. interact with the zinc surface and aid in the stabilization of ZNPs (Shnawa *et al.* 2023). The peaks that were observed at 1634 and (600, 450) cm^{-1} correspond to Zn–O stretching and deformation vibration, respectively. Metal oxides generally give absorption peaks in the regions between 600 and 400 cm^{-1}. The Zn–O frequencies observed for the synthesized ZNPs are in accordance with literature (Al-asady *et al.* 2020; Song and Xie, 2025). The broad peak observed at about 3300 cm^{-1} indicates the OH stretching vibrations.

TEM uses an electron beam to image a nanoparticle sample, providing much higher resolution than is possible with light-based imaging techniques. TEM was used to have an idea of size of synthesized green Heena extract based ZNPs and TEM imaging showed the presence of nanoparticle of approximately 25 nm size which was spherical in shape (Figure 3). Phytochemicals present in the extract drive the reduction of metal ions and stabilize the nanoparticles on the surface (Amuthavalli *et al.* 2021). The size and morphology of NPs is decided by multiple factors including extract concentration, calcination temperature, and precursor type and most of the studies reported ZNPs in the range of 5–100 nm with spherical or hexagonal morphology (Bhatt *et al.* 2024; Ajitha *et al.* 2016).

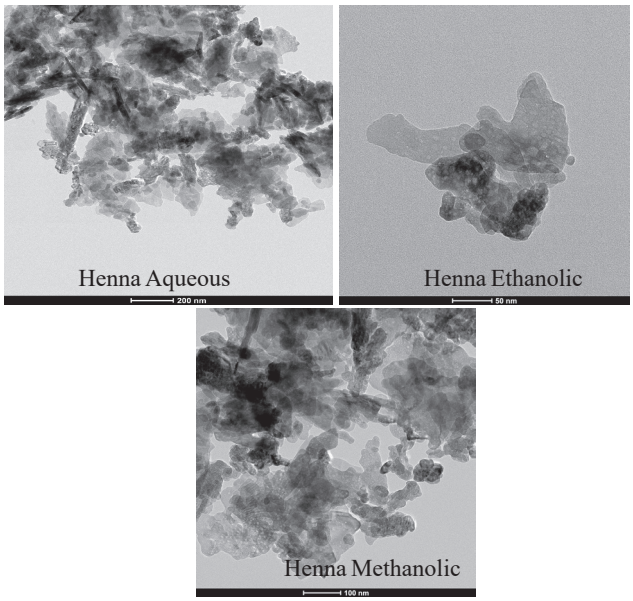

Fig. 3 TEM images of Heena aqueous, ethanolic and methanolic extract based ZNPs

Table 1. The peaks observed in FTIR based analysis of ZNPs and corresponding functional groups

S. No.	Peak position	Functional Group	Possible interpretation
1.	402–475	Zn–O stretching vibration	Confirms formation of ZnO nanoparticles (metal–oxygen bond).
2.	857–861	C–H bending (aromatic)	Indicates aromatic ring structures, likely from flavonoids/phenolics in Lawsonia inermis.
3.	1393–1398	COO^- symmetric stretch	Presence of carboxylate groups from phenolic acids; suggests phytochemicals act as capping/stabilizing agents.
4.	1974–2183	Combination/overtone bands	Associated with aromatic systems, supporting adsorption of polyphenolic compounds.
5.	3375–3399	O–H / N–H stretching	Broad peak due to hydroxyl groups (phenols, alcohols) and amines/proteins; confirms role of biomolecules in reduction and stabilization.

Estimation of green ZNPs Minimum Inhibitory Concentratio (MIC): All three extracts based green ZnONPs were tested for their antibacterial potential by estimation of their MIC against *E. coli* ATCC 25922. Aqueous extract based ZNPs showed lowest MIC value at 62.5µg/mL followed by ethanolic and methanolic extracts based ZNPs as 125µg/mL and 500µg/mL. Based on this aqueous extract based ZNPs were further used to evaluate their role in quorum sensing. *L. inermis* leaves have been reported with the antibacterial potential against *Staphylococcus aureus, Staphylococcus epidermidis* (Co-agulase negative staphylococci or CONS), ß-hemolytic Streptococci and *Pseudomonas aeruginosa* (Hourieh *et al.* 2000). Further, alcoholic and oily extracts of *Lawsonia inermis* leaves were found more effective than the water extracts against common skin affections causing pathogens (Al-Rubiay *et al.* 2008). The present study revealed aqueous extract based ZNPs with lowest MIC suggesting the better efficacy probably due to activation of different combinations of chemical bonds (Song and Xie, 2025).

Isolation and identification of E. coli: Further, to establish the antibacterial potential of ZNPs against clinical isolates 42 samples were collected from clinical cases and subjected to isolation and confirmation of *E. coli* isolates (Figure 4). Samples showing pinkish colonies on MLA, green metallic sheen on EMB were confirmed for Gram negative bacteria by Gram staining (Hucker and Conn, 2023). For the confirmation of *E. coli*, the *E. coli* specific *uid*A gene was amplified and out of 16 presumptive *E. coli* only 9 isolates revealed amplicon of 556 bp (Figure 4). The *uidA* gene encodes beta-glucuronidase enzyme specific to *E. coli* and is commonly used for the confirmation of *E. coli* (Feng *et al.* 1991; Martins *et al.* 1993).

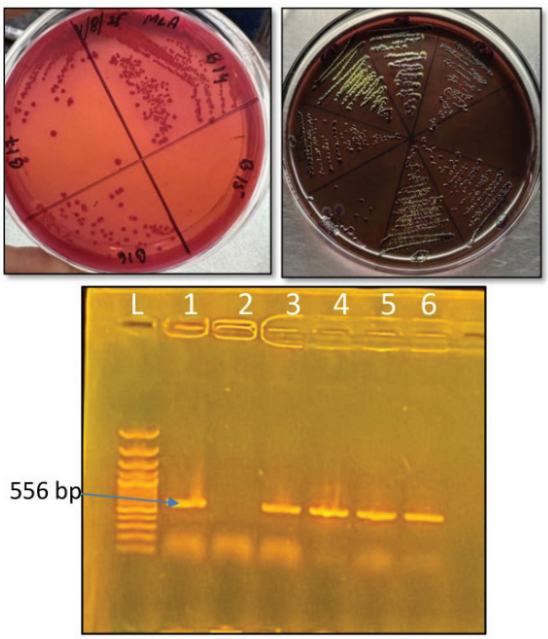

Fig. 4 Lactose fermenting pink color colonies on MLA. B. Metallic sheen on EMB agar. C. Showing 556 bp uidA gene amplification with 100 base pair adder in lane "L"

Identification of multi drug resistant E. coli: All the confirmed isolates were subjected to antibacterial susceptibility assay against different groups of antibiotics as per guidelines of CLSI (2020) to identify MDR strains of *E. coli*. Amikacin was found resistant in 88.8% isolates, amoxyclav in 44.4%, ampicillin in 11.1%, aztreonam in 88.8%, cefipime in 44.4%, cefoperazone + sulbactam in 88.8%, cefotaxime in 88.8%, chloramphenicol in 33.3%, ciprofloxacin in 100%, 33.3%. Five out of 9 isolates were found to be MDR strains showing resistance against more than 3 groups of antibiotics (Bharadwaj *et al.* 2022). These were also tested for ability to produce ESBL by Double Disk Synergy Test (Kaur *et al.* 2013). Out of 5 MDR *E. coli* isolates, only one isolate showed more than 5 mm difference with respect to the antibiotic used i.e., Ceftazidime (CAZ) (30 µg), Ceftazidime/Clavulanic acid (CAC) (30/10 µg), Cefotaxime (CTX) (30 µg), Cefotaxime/Clavulanic acid (CEC) (30/10 µg) suggestive of ESBL producing ability.

Identification of ESBL producing E. coli: In recent past extended-spectrum β-lactamases (ESBLs)-producing *E. coli* have been reported to be a significant public health threat due to their ability to produce ESBL- class A β-lactamases that hydrolyze and evolve resistance to the monobactams (aztreonam) and oxy-imino cephalosporins that includes antibiotics like cefotaxime, ceftazidime, ceftriaxone, cefuroxime, and cefepime, making such multidrug-resistant infections difficult to treat with commonly recommended antibiotics (Kayastha *et al.* 2020). All confirmed 9 isolates were examined for the presence of ESBL using previously prescribed six ESBL primers namely *blaTEM*, *blaSHV*, *blaCTXM*-1, *blaCTXM*-2, *blaCTXM*-9, and *blaOXA*-48-like (Dallenne *et al.* 2010). Eight out of 9 isolates showed presence of *blaTEM* and *blaSHV* having amplicon size 816 bp and 4 isolates showed the presence of *blaCTXM*-1 & 2 having amplicon size 688 bp and 561 bp, respectively. Only one isolate showed presence of *blaOXA*- 48- like having amplicon size 281 bp (Figure-5). This MDR isolate was used for their further expression analysis.

Confirmation of quorum sensing genes in E. coli: Prior to real time expression studies all the isolates were confirmed for the presence of *luxS* and *pfs* genes as per previously described method (Wang *et al.* 2016). Out of 5 MDR *E. coli* isolates, 4 revealed the presence of both *luxS* and *pfs* genes with the amplification of 477 and 675bp amplicons, respectively (Figure 6). *Pfs* a 5′-methylthioadenosine/S-adenosylhomocysteine nucleosidase gene encodes an enzyme that act with *luxS* to produce autoinducer-2 (AI-2). AI-2 acts as a signaling molecule and *luxS*/AI-2 based quorum sensing system. AI-2 allows bacteria to sense population density and accordingly coordinate virulence and biofilm formation (Kim *et al.* 2006). The presence of these two genes indicated the possible role of AI-2 in activation of quorum sensing system to induce resistance by increasing virulence or biofilm formation.

*lux*S, a transcript of structural operon *lux*CDABF is

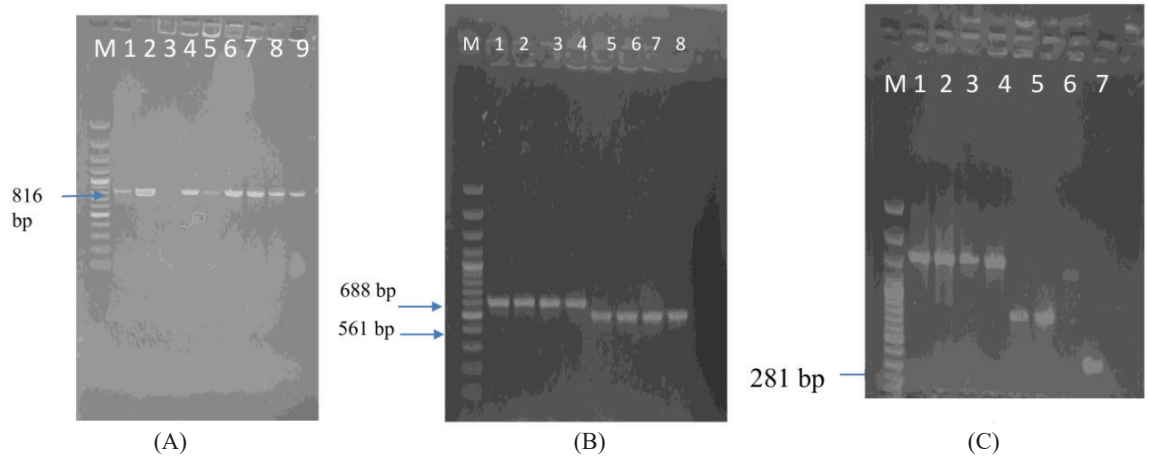

Fig. 5 Molecular detection of ESBL producing genes in MDR *E. coli.* (A) Amplification of *blaTEM* and *blaSHV* genes having amplicon size 816 bp, (B) Amplification of *blaCTXM*-1 and 2 having amplicon size 688 bp and 561 bp, respectively. (C) Amplification of *blaOXA*- 48- like having amplicon size 281 bp.

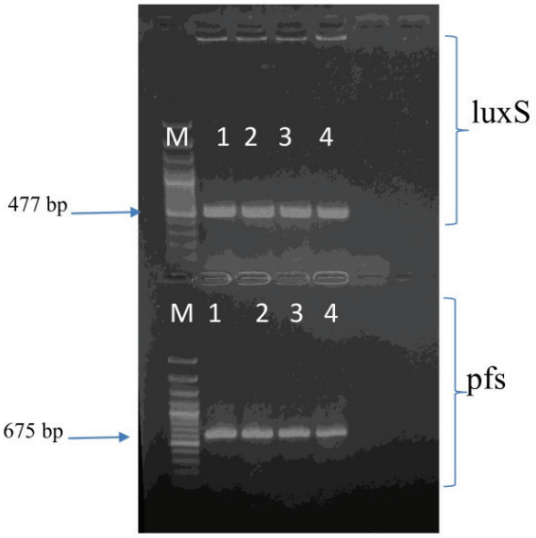

Fig. 6 Detection of quorum sensing gene *luxS* and *pfs* in MDR ESBL producing *E. coli* isolates

responsible for production of AI-2 that predominantly involves in cell to cell signaling in bacteria. AI-2 produced by a bacteria cast signals that are responsible to sense it's own density in multi microbial population and also perform inter species interaction. Thus, AI-2 play critical role in inter species interactions including transmission of AMR (Chu and Yung *et al.* 2024; Sharma *et al.* 2024; Keizers *et al.* 2025). The MDR ESBL producing *E. coli* isolate carrying both *luxS* and *pfs* quorum sensing genes was incubated ZNPs at MIC to evaluate the potential of NPs in expression of *luxS* gene. qRT-PCR revealed major down regulation (Figure 7) in *lux*S expression after exposure to ZNPs suspension compared to normal gene expression in bacterial broth without NPs. Fold change in gene expression revealed time dependent *luxS* down regulation with the highest down regulation at 6 hr and subsequently ideclined after 12 and 18 hrs of incubation.

Down regulation of *luxS* can directly affect the synthesis of AI-2 and reduce or even stop inter species interaction with failure to detect it's own density that directly control the intend of bacteria to adopt or develop resistance. The deletion of *luxS* affect multiple genes involved in the process of iron uptake, utilization of carbon, biosynthesis of methyl transfer reactions and methionine leading to death of bacteria (Wang *et al.* 2005).

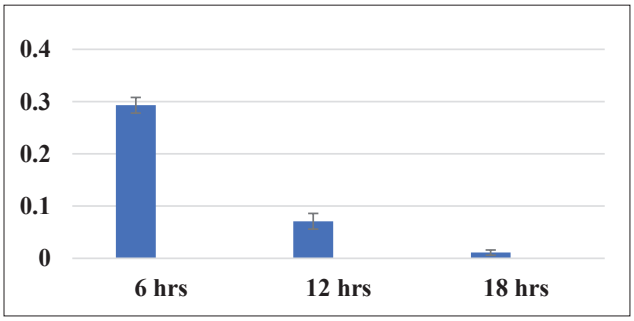

Fig. 7 Depicting time dependent fold change in the expression of *luxS* gene

The study revealed the potential ability of Heena ZNPs to inhibit quorum sensing *luxS* gene against multi drug resistance ESBL producing *E. coli*. However, this potential need to be further explored in therapeutics directly or as an adjunct to antibiotic to reduce the chances of AMR transmission.

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AUTHOR CONTRIBUTION

SC conducted trial, written manuscript, SU and RA assisted in study and trials, VJ, AV, AM performed writing,

editing and formatting, AK design the study, revised manuscript and submitted.

DECLARATION OF INTERESTS

The authors affirm that there are no known competing interests that might have influenced the work presented in this paper.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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