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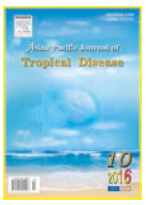

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Antibacterial effect of silver nanoparticles and capsaicin against MDR-ESBL producing *Escherichia coli*: An *in vitro* study

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ABSTRACT

Objective: To evaluate the antibacterial property of silver nanoparticles (AgNPs) and capsaicin against multidrug resistant (MDR) and extended spectrum beta-lactamase (ESBL) producing *Escherichia coli* of bovine and poultry origin.

Methods: Antibacterial efficacy of AgNPs and capsaicin was measured using broth dilution method. Five MDR-ESBL producing *E. coli* isolates of poultry (PEC4, PEC6, PEC15 and PEC16) and cattle mastitis origin (MEC2) were taken to evaluate the antibacterial effect of AgNPs and capsaicin.

Results: At 50 mmol/L AgNPs, the viability of MDR of bacterial pathogens was reduced to almost 80%–90% and at 1000 mmol/L, the viability went down to 0%–3%. The minimum inhibitory concentration (MIC_{50}) of AgNPs against these MDR-ESBL producing isolates was found to vary between 172–218 mmol/L whereas the MIC_{80} varied between 450–640 mmol/L. Capsaicin showed more prominent bactericidal effect and only at 2.5 mmol/L concentration, the viability was shown to be reduced by 20%–35% whereas at 7.5 mmol/L concentration, there was approximately 60% reduction in viability. Further at 25 mmol/L concentration, the viability was reduced to 0%–8%. The MIC_{50} and MIC_{80} of capsaicin against these MDR-ESBL producing isolates were found to vary between 4.6–7.5 mmol/L and 10.9–16.9 mmol/L, respectively.

Conclusions: The results point out that capsaicin and AgNPs could be of use in treating ESBL infection.

1. Introduction

Indiscriminate and inadvertent use of antibiotics has been often regarded as the main cause behind the rising problem of antimicrobial resistance worldwide leading to emergence of multidrug resistant (MDR) pathogens. Amongst the MDR pathogens, extended spectrum beta-lactamase (ESBL) producing *Escherichia coli* (*E. coli*) is often responsible for therapeutic failure and poor infection control programme leading to increased mortality and morbidity in human. This is due to its ability to inactivate β-lactam antibiotics including newer generation of oxyimino-cephalosporin (cefpodoxime, ceftazidime, ceftriaxone and cefotaxime) and oxyimino-monobactam (aztreonam). Although these pathogens were more commonly known for hospital-acquired infections, recent reports suggest its wide scale involvement in community borne infection as well[1]. As most of the ESBL producers are MDR and cannot be easily treated, it has become a real cause of concern for public health[2]. Furthermore, indiscriminate use of antibiotics in livestock sector often results in emergence of several antibiotic resistant strains, including ESBL producers in animals. In such cases, the animals not only act as a mere reservoir, but may in turn transmit these pathogens to human beings through direct and indirect contact[3]. The occurrence of ESBL producing MDR-*E. coli* in food-producing animals has recently been reported by this group of authors from India[4].

The emergence of MDR pathogenic organisms in human and animals has become a major cause of therapeutic failure of

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infectious diseases[5]. Although several new antibiotics have been developed in the last few decades, none of them offer improved activity against MDR bacteria[6]. This coupled with diminishing therapeutic values of different frontier antimicrobials has renewed the interest of researchers to search for some novel alternatives and to look for new antimicrobial substances.

Nanoparticles with one dimension of 100 nm or less in size are now being increasingly utilised for medical applications and are of great interest as an alternative approach to control infectious agents offering broad spectrum activities against bacteria, fungi and viruses[7]. Recent studies have shown that metal-based nanoparticles are one of the most promising therapeutic agents owing to their unique physico-chemical and biological properties[8]. Besides, the search for plant secondary metabolites including phytochemicals and extracts derived from plants has accelerated in recent years and their use can be an interesting alternative to control bacterial infections[9]. One such secondary plant metabolite is capsaicin, which is known for its multiple pharmacological and physiological properties and has recently attracted considerable attention because of its antimicrobial and anti-virulence activity[10,11].

In view of the importance of MDR pathogens and the quest to search for suitable and alternative approach to control them, the present study was conducted to evaluate the antibacterial effect of silver nanoparticles (AgNPs) and a plant-derived compound-capsaicin against few MDR-ESBL producing *E. coli* isolates of bovine and poultry origin.

2. Materials and methods

2.1. Bacterial isolates

Five MDR-ESBL producing *E. coli* isolates of poultry (PEC4, PEC6, PEC15 and PEC16) and cattle mastitis origin (MEC2) were taken to evaluate the antibacterial effect of AgNPs and capsaicin. In general, organisms that were resistant to three or more classes of antibiotics were considered as MDR. In this study, these isolates were resistant to at least six antibiotics belonging to diverse groups.

2.2. AgNPs

AgNPs of 100 nm size with 107.87 molecular weight, 0.998 7 g/mL density and 0.02 mg/mL concentration were procured from Sigma-Aldrich, USA.

2.3. Capsaicin

Capsaicin [8-methyl-N-Vanillyl-trans-6-nonenamide ($C_{18}H_{27}NO_3$) of 305.41 molecular weight] was procured from HiMedia, India in powder form and the stock solution (1 mol/L) was prepared as per the manufacturer's instructions.

2.4. Determination of minimum inhibitory concentration (MIC_{50} and MIC_{80}) of AgNPs and capsaicin

The minimum inhibitory concentrations (MIC_{50} and MIC_{80}) of AgNPs and capsaicin were determined against the MDR-ESBL producing *E. coli* isolates using the broth dilution method as described previously[9]. Briefly, sterile test tubes containing 10 mL of nutrient broth were supplemented with various concentrations of AgNPs (0, 6.25, 12.5, 25, 50, 100, 250, 500 and 1 000 mmol/L) and capsaicin (0, 2.5, 5.0, 7.5, 12.5 and 25 mmol/L). Thereafter, the tubes were inoculated with each MDR- ESBL producing strains (1.5×10^6 to 3×10^6 /mL) and incubated at 37 ℃ for 24 h. The optical density at 650 nm (OD_{650}) of the tubes was recorded at 0 h (beginning of lag phase) and once again after 24 h of incubation at 37 ℃. The viability of the organism was calculated in terms of turbidity and the difference between final and initial OD_{650}. Specific OD_{650} was interpreted as the growth of the bacteria. The percent viability of bacteria at any specific concentration was calculated as per the following formula:

Percent viability = {[Specific OD_{650} (Final OD_{650} – Initial OD_{650}) for any concentration of AgNPs or capsaicin]/[Specific OD_{650} (Final OD_{650} – Initial OD_{650}) of negative control]} × 100. Further, the MIC_{50} and MIC_{80} were calculated for both AgNPs and capsaicin using Graphpad prism software version 5.0.

3. Results

The antibacterial activity of AgNPs against five ESBL producing MDR-*E. coli* isolates was determined by broth dilution assay using AgNPs at concentrations varying from 0 to 1 000 mmol/L (0.00, 6.25, 12.5, 25, 50, 100, 500 and 1 000 mmol/L) and was presented in Figure 1. The bacterial viability was reduced to 80%–90% using AgNPs at 50 mmol/L concentration, whereas at 100 mmol/L concentration, there was approximately 40% reduction in viability. The bacterial viability was reduced to 0%–3% only at 1 000 mmol/L. The MIC_{50} of AgNPs against these MDR-ESBL isolates was found to vary between 172–218 mmol/L, whereas the MIC_{80} varied between 450–640 mmol/L.

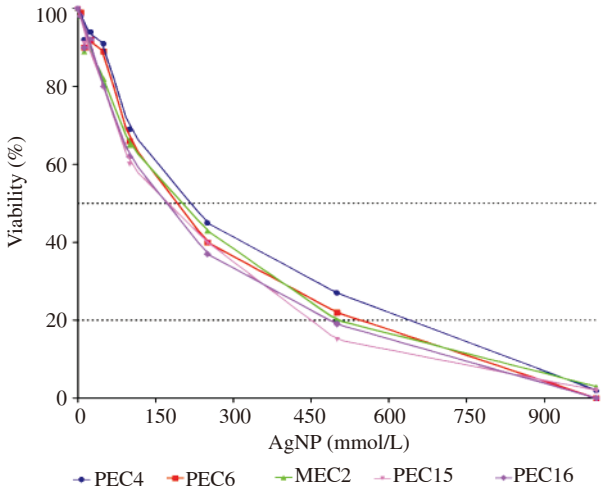

Figure 1. Antibacterial effect of AgNPs towards ESBL producing *E. coli.*

Likewise, the antibacterial effect of capsaicin determined by broth dilution assay was also tested against the isolates of ESBL producers at concentrations ranging from 0 to 25 mmol/L (Figure 2). At 2.5 mmol/L concentration, the bacterial viability was found to be reduced by 20%–35%, whereas at 7.5 mmol/L concentration, there was approximately 60% reduction in viability. At 25 mmol/L concentration, the viability was reduced to 0%–8%. The MIC_{50} of capsaicin against these MDR-ESBL producing isolates was found to be 4.6–7.5 mmol/L. The MIC_{80} was also detected to vary between 10.9–16.9 mmol/L.

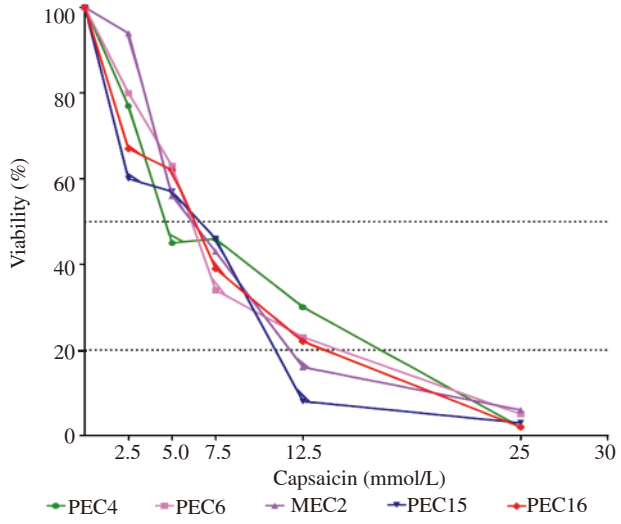

Figure 2. Antibacterial effect of capsaicin towards ESBL producing *E. coli*.

4. Discussion

Nowadays, antimicrobial resistance is a serious concern in hospitals, public healthcare systems and veterinary practices as well worldwide. In livestock sector, emergence of antimicrobial resistance not only complicates the therapeutic options and clinical outcome of important animal diseases but also has a huge impact over farming economy. To overcome this burgeoning problem, scientific efforts are on in pursuit of alternative therapeutics to tackle these MDR strains.

In recent years, researchers have witnessed metal-based nanoparticles as an effective alternative for disease control. Studies have also shown that metal nanoparticles, in particular silver ions exert strong inhibitory and bactericidal effects as well as possess a broad spectrum of antimicrobial activities[12,13]. Various modes of action for antibacterial effects of AgNPs are because of the change of bacterial cell membrane permeability and interaction of AgNPs with sulfhydryl group of essential enzymes thereby facilitating release of K^+ ion from bacterial cells[14,15].

In the present study, AgNPs used were spherical with a particle size of 100 nm. MIC_{50} and MIC_{80} of AgNPs for the tested MDR isolates of *E. coli* ranged from 172 to 218 mmol/L and 450 to 640 mmol/L, respectively. The antimicrobial effect of AgNPs and its activity against the MDR pathogens have been evaluated by various researchers. In a study, Amirulhusni *et al.* recorded MIC_{50} at a concentration of 15 μg/mL and reported total inhibition potential of AgNPs (≥ 50 μg/mL concentration) against MDR strain of *Pseudomonas aeruginosa (P. aeruginosa)*[16]. Lara *et al.* determined the antibacterial effect and found concentrations of AgNPs between 30 and 100 mmol/L effective against various resistant and drug susceptible bacterial strains[17]. Paredes *et al.* evaluated the antibacterial effect of AgNPs against MDR-*E. coli* and recorded MIC value at 25 μg/mL[18]. AgNPs were also found effective against ESBL producing bacteria at 100 mg/mL concentration[19]. Further, green AgNPs synthesized by *Streptomyces* sp. (VITSJK10) were found to have significant antimicrobial activity against MDR-ESBL pathogens like *Klebsiella pneumoniae*, *E. coli*, *etc.*[20]. Few researchers have also compared antibacterial activity of AgNPs against MDR bacterial pathogens employing agar well diffusion and turbidometric assays[21,22].

As a wide variety of methods are employed to study the antibacterial effect of AgNPs by various researchers, it makes the comparison of results to be a difficult task. Nevertheless, the variation in size, shape, stability and concentration could also have influenced the efficacy of AgNPs against microorganisms. In fact, particle size plays a central role in antimicrobial activity, as small particles exhibit higher antimicrobial activity than the big particles[23]. Again, shape of the AgNPs also determines the antibacterial effectivity of AgNPs and displays a shape-dependent interaction with the bacterial cells. Previous studies indicated that truncated triangular AgNPs display the strongest biocidal action against *E. coli* as compared to spherical and rod-shaped nanoparticles[24,25]. So, *in vivo* studies to define a safe range for the application of AgNPs are required.

Since long, chile peppers are known to show antimicrobial property[26]. Capsaicin, the secondary plant metabolite used in this study is an active component and pungent ingredient found in a variety of peppers of the genus *Capsicum*[27]. Reports suggest that capsaicin is effective against a number of ailments including respiratory problems, and even displays inhibitory potential against foodborne pathogens like *Salmonella typhimurium* and *P. aeruginosa*[28].

In the present study, capsaicin was also found to have an appreciable antibacterial effect against ESBL producing MDR *E. coli* isolates with the MIC_{50} and MIC_{80} ranging between 4.6–7.5 mmol/L and 10.9–16.9 mmol/L, respectively. Capsaicin was previously reported to mediate antibacterial effect by changing the membrane fluidity and efflux pump inhibition against NorA thereby conferring resistance on *Staphylococcus aureus* to norfloxacin[29,30]. *In vitro* bactericidal activity of capsaicin against *Helicobacter pylori* has also been documented at 50 μg/mL[31]. Further, the antibacterial effect of this flavonoid was also reported against *Vibrio cholera*, *Salmonella typhimurium*, *P. aeruginosa* and *Bacillus subtilis* in the recent past[28,32,33].

Although several studies have demonstrated that capsaicin possesses antimicrobial activity against various MDR Gram-positive and Gram-negative bacteria, none of them reported the effects of capsaicin against MDR *E. coli* to substantiate the findings of this study[34,35]. However, before its clinical trial, the carcinogenic potential of capsaicin should be investigated thoroughly, as it modulates xenobiotic metabolizing enzymes, particularly microsomal cytochrome P450-dependent monooxygenases, which are involved in activation of various chemical carcinogens and mutagens[36,37].

In conclusion, AgNPs and capsaicin could effectively be used and act as tool to inhibit the growth of MDR-ESBL producing *E. coli* pathogen. However, to explore more possible uses of AgNPs and capsaicin as antimicrobial agents, research involving *in vivo* study with combination therapy is suggested which may help to reduce the MDR burden and prevent its further transmission into different clinical environments.

Conflict of interest statement

We declare that we have no conflict of interest.

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