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Evaluation of synergism between Cefotaxime and *Allium sativum* against Extended-spectrum beta-lactamase and Ambler class C co-producers

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ABSTRACT

The present study evaluates the synergistic association between Cefotaxime and aqueous garlic (*Allium sativum*) extract (AGE) on extended spectrum beta-lactamase (ESBL) and Ambler Class C (AmpC) co-producing *Escherichia coli* strains from skin and soft tissue infections (SSTIs). Ceftazidime-resistant *E. coli* strains were screened for beta-lactamase production by phenotypic confirmatory disc diffusion test (PCDDT) and E-test. Antibacterial activity of AGE was examined by the disc diffusion method and the minimum inhibitory concentration (MIC) of Cefotaxime and AGE was determined. The synergistic association between Cefotaxime and AGE was evaluated by calculating the fractional inhibitory concentration (FIC) index, time-kill kinetics assay (TKA), and scanning electron microscopy (SEM). The zone of inhibition by AGE against the 27 *E. coli* co-producers of ESBL and AmpC ranged from 17 to 30 mm. The average MIC of Cefotaxime and AGE was found to be 570 µg/ml and 0.86% (4.28 mg/ml), respectively. The FIC index obtained by the checkerboard method established a synergistic association between Cefotaxime and AGE in 10 (37%) test strains, which was confirmed by TKA. The SEM analysis revealed complete cell degradation at 8 hours on the treatment with Cefotaxime-AGE combination. It can be stated that the AGE may aid Cefotaxime in the treatment of beta-lactamase producing *E. coli* strains from SSTIs.

INTRODUCTION

Cefotaxime—a beta-lactam antibiotic is the most preferable antibiotic given during severe infections (Badar and Navale, 2012). Resistance toward beta-lactams in clinical strains is primarily caused due to the hydrolysis of the antibiotic by a beta-lactamase enzyme. Extended-spectrum beta-lactamases (ESBLs) are enzymes susceptible to clavulanate and they hydrolyze penicillins, extended-spectrum cephalosporins, and monobactams (Thomson, 2010). Ambler Class C (AmpC) class beta-lactamases are cephalosporinases which are poorly inhibited by clavulanic acid, and can hydrolyze cephamycins as well as other extended-spectrum cephalosporins (Sasirekha and Shivakumar, 2011). When ESBL and AmpC beta-lactamase co-exist, they mask each other; hence, their detection is difficult. They also cause an increase in the minimum inhibitory concentration (MIC) values for beta-lactam antibiotics (Sundin, 2009).

Skin and soft tissue infections (SSTIs), which lead to hospitalization, are generally caused due to Gram-negative pathogens, which can lead to sepsis, organ failure, and death (Shah and Williamson, 2018). *Escherichia coli* is one of the most common Gram-negative bacilli likely to be encountered in SSTIs from India (Patel *et al*., 2010). Due to the increase in the severity of Gram-negative infections, new antimicrobials are being analyzed in the form of natural compounds either as stand-alone or adjunctive therapy (Miyasaki *et al*., 2010).

Garlic (*Allium sativum*) exhibits a broad spectrum of antibacterial activity against pathogens (Chowdhury *et al*., 1991; Wolde *et al*., 2018). Its antibacterial activity is attributed to sulfur-containing compounds like alliin, allicin, ajoene, allyl propyl, diallyl trisulfide, s-allyl cysteine, and S-allylmercaptocysteine

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(Borlinghaus, 2014). Allicin interacts with important thiol-containing enzymes which maintain the correct redox state within microorganisms, and thus its viability. Hence, developing a resistance to garlic would require modifying these vital enzymes, which would damage the microbes, making garlic a potent antimicrobial agent (Ankri and Mirelman, 1999).

There is no documented report analyzing the association between Cefotaxime and aqueous garlic extract (AGE), against *E. coli* strains which co-produce ESBL and AmpC, from SSTIs, to the best of our knowledge. Hence, the current study aims to examine the synergistic activity of Cefotaxime and aqueous garlic extract on beta-lactamase producing *E. coli* strains isolated from SSTIs.

MATERIAL AND METHODS

The present study was carried out in a tertiary care hospital and was permitted by the local ethics committee of the institution (Reference no—PG/EC/40/2012).

Bacterial strains

A total of 59 non-duplicate *E. coli* strains from SSTIs of patients from our Tertiary Care Hospital were selected for the current study. The samples were cultured on MacConkey's agar, and *E. coli* strains identified by conventional tests like cultural characteristics and biochemical tests were selected (Colle *et al*., 1996).

Antimicrobial susceptibility test (AST)

The antimicrobial susceptibility of the *E. coli* strains was determined by Kirby–Bauer disc diffusion method in accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines by using commercially available antimicrobial discs (HiMedia, Mumbai, India) (CLSI, 2012). The following antibiotics were used: Ampicillin (10 µg), Amikacin (10 µg), Ciprofloxacin (5 µg), Gentamicin (10 µg), Amoxyclav (30 µg), Ceftazidime (30 µg), Cefoxitin (30 µg), Imipenem (10 µg), and Piperacillin-Tazobactam (100/10). All the strains, which were found to be resistant to Ceftazidime as per the CLSI susceptible breakpoints, were further analyzed for beta-lactamase production by the confirmatory tests.

Phenotypic confirmatory disc diffusion test (PCDDT)

The phenotypic disc diffusion test for ESBL detection was performed as per CLSI guidelines. The current CLSI guideline does not illustrate any method for detection of strains producing AmpC beta-lactamases. Hence, Cefoxitin (CX 30µg)—Cefoxitin/Cloxacillin (30/200) antibiotic discs were used for AmpC recognition (CLSI, 2012; Shah and Williamson, 2015; Tan *et al*., 2009).

E-test

ESBL and AmpC detection Ezy MIC™ strip (EM081, HiMedia, Mumbai, India) along with pure ESBL detection strips (EM079 Hi-Media, Mumbai, India) were used in the study. The strains were confirmed as ESBL and AmpC beta-lactamase co-producer in reference with the application sheet provided by the manufacturer (CLSI, 2012; Shah *et al*., 2016). The confirmed ESBL and AmpC co-producers were considered as the test strains in the study.

Preparation of aqueous garlic extract (AGE)

Fresh garlic bulbs (*A. sativum*) were purchased from the local market and its aqueous extract was prepared by the method described by Shah *et al*. (2016). To estimate the dry weight of the extract, method of Eja *et al*. (2007) was performed and the concentration was determined to be 500 mg/ml.

High-performance liquid chromatography (HPLC) analysis

The high-performance liquid chromatography of AGE was performed at Natural Remedies, Bangalore, India to determine the percentage of allicin in the sample. Stainless steel column packed with silanized octadecylsilyl silica gel was used in the analysis. The mobile phase was prepared by mixing 40 volumes of a 1% v/v solution of anhydrous formic acid and 60 volumes of methanol. Detection was performed at a wavelength of 254 nm by injecting 10 µl of the sample.

Antibacterial activity of aqueous garlic extract (AGE)

The antibacterial activity of AGE was evaluated by the disc diffusion method using Mueller–Hinton agar as described by the CLSI. Sterile paper discs (6 mm, HiMedia, Mumbai, India) were impregnated with 20 µl of the 500 mg/ml AGE and placed on the agar inoculated with the test strains. Imipenem disc (10 µg) was used as positive control, and a disc impregnated with Dimethyl sulfoxide (DMSO) as a negative control (CLSI, 2012).

Determination of Minimum Inhibitory Concentration (MIC)

The MIC of AGE and Cefotaxime was evaluated by the agar dilution method. To determine MIC of AGE, dilutions were prepared by mixing AGE with molten sterile Mueller–Hinton agar to get final concentrations ranging between 0.25% and 4% (1.25–20 mg/ml). MIC of Cefotaxime was determined by mixing Cefotaxime with molten sterile Mueller–Hinton agar to get final concentrations ranging from 25 to 2000 µg/ml. A plate of Mueller–Hinton agar containing DMSO served as a control (Nakamura, 2000).

Determination of synergistic activity by checkerboard assay using agar dilution method

Checkerboard assay was used to establish the presence of synergistic interaction between Cefotaxime (stock—10 mg/ml) and AGE (stock—10 %). The Mueller–Hinton agar plates were prepared by mixing AGE in the range of 0.1%–2% (0.5–10 mg/ml) along with 10—400 µg/ml of Cefotaxime. Using the above results, fractional inhibitory concentration (FIC) indices were calculated. The FIC index (∑FIC) for a particular strain was calculated as follows:

$$\sum FIC = FIC\ A + FIC\ B$$

where FIC A = MIC of AGE in combination/ MIC OF AGE alone.

FIC B = MIC of Cefotaxime in combination/ MIC of Cefotaxime alone

A minimum FIC index of ≤0.5 indicates synergy, while a FIC index > 2 indicated antagonism. If the minimum FIC index

was found to be >0.5 and ≤1, the effect of the combination was termed as an additive interaction. If the minimum FIC index was >1 and ≤2, the effect of the combination was recorded as indifference (Nakamura, 2000; Shah *et al*., 2016).

Determination of synergistic activity by time-kill kinetics assay (TKA)

Time-kill kinetics assay validated synergy using the *E. coli* strain showing the lowest FIC index, by the broth microdilution technique. The AGE and Cefotaxime were incorporated into 50 ml of Mueller–Hinton broth (MHB) at sub-MIC values of 0.25% (1/4× MIC) and 100µg/ml (1/15× MIC), respectively. Tests consisting of MHB incorporated with AGE and Cefotaxime alone, at the test concentrations and control lacking them, were also included in the experiment. A standardized inoculum was added to test and control flasks to obtain a final density of 10^5 CFU/ml. Bacterial growth was quantitated after 0, 2, 4, 6, 8, and 24 hours incubation at 37°C by plating 10-fold dilutions. Bactericidal activity was termed as a ≥3-$\log_{10}$ reduction in bacterial count in comparison with initial inoculum at zero time. The synergy of the antimicrobial combination was defined as a ≥2-$\log_{10}$ CFU/ml decrease in the viable count, as compared with the use of a single agent in 24 hours. The interactions were classified as indifference when they exhibited a <2-$\log_{10}$ increase or decrease in colony count and as antagonism when they exhibited a ≥2-$\log_{10}$ increase in colony count, after 24 hours incubation with the Cefotaxime—AGE combination in comparison with the most active single agent alone (Pankey *et al*. 2014; Peck *et al*. 2012; Tariq *et al*., 2014).

Scanning electron microscopy (SEM) analysis of the synergistic interaction

The above *E. coli* strain, showing the lowest FIC index, was selected for SEM analysis. The analysis was carried out at IIT, Powai, Mumbai. The strain was treated with the same sub-MIC values of Cefotaxime and AGE, selected in TKA for various time intervals of 0, 4, and 8 hours. The treated samples were centrifuged at 1,500 rpm for 2 minutes. The samples were washed using Milli Q water and centrifuged again. Obtained aliquots were diluted 10-fold. 0.01 µl of the diluted sample was drop cast on cover slip and dried. Then, it was coated with silver for conductance and placed on the stobe (sample holder) for morphology examination by SEM (Diaz-Visurraga *et al*., 2010).

RESULTS AND DISCUSSION

Antimicrobial susceptibility test, PCDDT and E-test

In the developing countries like India, SSTIs associated with *E. coli* are marginally addressed (Ranjan *et al*., 2017). Analysis of clinical strains for antibiotic resistance is significant for epidemiological and clinical purposes. The *E. coli* strains from SSTIs showed the highest rate of resistance against Ampicillin, 89.8% (53/59). All the *E. coli* strains were susceptible to carbapenems—Imipenem and Meropenem. The lowest rate of resistance was exhibited against Amikacin (11.9%). Amongst all the strains, 78% (46/59) strains were resistant to Ceftazidime and 55.9% (33/59) to Cefoxitin (Table 1). Thus, in the current study, carbapenems were the most effective group of antibiotic followed by Amikacin, for the *E. coli* strains. The resistant rate in *E. coli* strains toward Ceftazidime was in accordance with the previous study carried out by Afroz *et al*. (2015).

PCDDT and E-test confirmed that 45.8% (27/59) strains were co-producers of ESBL and AmpC, i.e., they were multiple beta-lactamase producers, and were used as test strains in further study. All co-producers of ESBL and AmpC were also detected as multidrug-resistant, i.e., they were resistant to three or more than three groups of antibiotics. Incidences of co-expression of beta-lactamases lead to erroneous treatment choices (Pai *et al*., 2004). The co-production rate of ESBL and AmpC beta-lactamases in *E. coli* strains was in comparison to the study carried out by Mohanty *et al*. (2009). A significant observation of the current study was false susceptibility against Piperacillin-Tazobactam, *in-vitro* by ESBL and AmpC co-producers. This combination would presumably not be effective under *in-vivo* conditions for ESBL and AmpC co-producers. Similar false susceptibility was reported by another study carried out in Mumbai by Shinde *et al*. (2013). To avoid therapeutic failures, it is, therefore, suggested that the detection of AmpC along with ESBL producers should be performed routinely in the hospitals.

Antibacterial activity of AGE

The increasing rate of resistance toward antibiotics by clinical strains is a serious concern which highlights the importance of developing new strategies to limit the various mechanisms of antibiotic resistance. Previous studies have shown that a wide range of microorganisms were sensitive to crushed garlic (*A. sativum*) preparations, thus enhancing the interest for garlic as a medicinal magic potion (Ankri and Mirelman, 1999). The estimation of the active ingredient allicin was carried out by HPLC analysis and was found to be 0.20, expressed as percentage w/w. The antibacterial activity of AGE was evaluated by the disc diffusion method and the average zone of inhibition ranged from 17 to 30 mm with a mean of 23.22 ± 4.09 mm against the test strains. Thus, in this study, all the *E. coli* strains, which co-produce ESBL and AmpC, were inhibited by AGE and the results were comparable to the analysis of previous studies (Modi *et al*., 2015; Shayan *et al*., 2014).

Table 1. Rate of antibiotic resistance amongst *E. coli* strains.

Antibiotics	AK	AMC	AMP	CAZ	CIP	CTR	CX	GEN	IMP	MRP	PIT
Percentage of strains resistant	11.9	76.3	89.8	78	69.5	67.8	55.9	28.8	0	0	16.9

AK = Amikacin, AMC = Amoxyclav, AMP = Ampicillin, CAZ = Ceftazidime, CIP = Ciprofloxacin, CTR = Ceftriaxone, CX = Cefoxitin, GEN = Gentamicin, IMP = Imipenem, MRP = Meropenem, PIT = Piperacillin-Tazobactam

The table depicts the percentage of *E. coli* strains resistant against various antibiotics (n = 59).

Determination of minimum inhibitory concentration of AGE and Cefotaxime

The MIC of AGE and Cefotaxime was determined by the Agar dilution method. The MIC of AGE ranged between 0.5% and 2% (2.5–10 mg/ml) with a mean of 1.07% (5.35 ± 2.16 mg/ml) as revealed in Table 2. The MIC of Cefotaxime was found in the range of 50–1,500 µg/ml with an average of 570 µg/ml (Table 3). The MIC values estimated were comparable with the study of Shahyan *et al*. (2014). This signifies the use of garlic in controlling bacterial infections.

Determination of synergistic activity by checkerboard assay, TKA and SEM

Cefotaxime was selected to study synergistic interactions with AGE, as it the most preferable antibiotic prescribed during severe infections (Badar and Navale, 2012). Synergy, if demonstrated, is indicative for the successful combination of antimicrobial therapy. If a combination of AGE and Cefotaxime can cause the reversal of the Cefotaxime resistance or lower its dosage in therapy, then this combination could potentially improve the outcome for patients with severe infections. Checkerboard assay was used to determine the combined activity of various concentrations of Cefotaxime and AGE on test strains. Based on the FIC index, 37% (10/27) test strains showed synergism, 33.3% (9/27) strains exhibited additive, and 29.6% (8/27) exhibited indifference effect. None of the strains showed an antagonistic effect between AGE and Cefotaxime (Tables 4 and 5). Thus, the combination of AGE and Cefotaxime showed at least a fourfold reduction in their respective MICs in 37% of the test strains and a twofold reduction in 33.3% of the test strains. The antagonistic effect was not observed in any of the strains, indicative of a positive association between the two antimicrobial agents.

The time-kill kinetics assay was performed to confirm the synergistic association with the strain of *E. coli* having the lowest FIC index. Bactericidal activity was defined as a ≥3-$\log_{10}$ reduction in the bacterial count when compared with initial inoculum at zero time. Bactericidal activity was not observed for AGE or Cefotaxime when used alone at sub-MIC values at the end of 24 hours, in comparison with inoculum at zero time. AGE showed a reduction of 2-$\log_{10}$ CFU/ml, and Cefotaxime did not show any reduction when used alone. The absence of bactericidal activity in the above test may be due to the application of sub-MIC values of AGE (1/4× MIC) and Cefotaxime (1/15× MIC) against the test strain. The sub-MIC values may not be effective in killing the bacteria, but they are capable of modifying their physico-chemical characteristics and the structural design of their surface. It may also interfere with some vital bacterial cell functions such as adhesiveness, surface hydrophobicity, fimbriation, motility, and host-bacteria interactions. The final result of the treatment may be a reduction in bacterial virulence factors (Braga *et al*., 2000).

Table 2. MIC of AGE against *E.coli* strains co-producing ESBL and AmpC.

Concentration of AGE (mg/ml)	1.25	2.5	5	10	20
No. of strains inhibited	-	4	19	4	-
Percentage of strains inhibited	-	14.8	70.4	14.8	-

The table depicts the number and percentage of *E.coli* strains inhibited by various minimal concentrations of AGE. (n = 27).

Synergy of antimicrobial combination was defined as a ≥2-$\log_{10}$ CFU/ml decrease in the viable count, as compared with the use of a single agent in 24 hours. It was observed that when the test strain was exposed to Cefotaxime - AGE combination; in which the concentration of each antibacterial agent was at sub-MIC value, then after 24 hours of incubation, a 3-$\log_{10}$ CFU/ml reduction in the cell count was observed in comparison to Cefotaxime alone and a decrease of 2-$\log_{10}$ CFU/ml in colony count, in comparison to AGE tested alone (Fig. 1). These results indicated a synergistic association between Cefotaxime and AGE against the strain and indicated that the use of garlic was enhancing the bactericidal activity of Cefotaxime. Thus, the present study reaffirms the previous findings that AGE can cause some irreversible damage to the bacterial cells, which may be attributed to a myriad of sulfur compounds present like allicin, which makes it lethal for the cells (Ankri and Mirelman, 1999).

The SEM micrographs demonstrated the differences in appearances of *E. coli* cells which were untreated and treated with sub-MIC values of antibacterial agents. SEM micrographs revealed the unusual morphological changes in the *E. coli* cell which co-produced ESBL and AmpC, indicating partial cell damage in 8 hours, on exposure to both the antibacterial agent alone. An uneven cell surface was observed when exposed to Cefotaxime (Fig. 2A) and a slight rupture in the cell wall of the strain was visible when it was treated with AGE alone (Fig. 2B). However, complete cell degradation was observed at 8 hours when the cell was treated with Cefotaxime—AGE combination (Fig. 2C). The surfaces of all untreated cells were intact and smooth (Fig. 2D). The analysis confirmed the synergistic association between Cefotaxime and AGE when the complete cell damage of the *E. coli* strain was observed at 8 hours, in comparison to a partial cell wall damage that was observed when Cefotaxime or AGE was used alone as an antibacterial agent. The micrographs suggested that AGE and Cefotaxime affected the structure of the cell wall of the *E. coli* strain. Similar results were observed in a previous study, which reported cell wall lysis for *Listeria monocytogenes* when exposed to garlic shoot juice. Cell wall degradation has been suggested due

Table 3. MIC of Cefotaxime against *E.coli* strains co-producing ESBL and AmpC.

Concentration of Cefotaxime (µg/ml)	50	100	200	400	800	1,000	1,250	1,500
No. of strains inhibited	1	4	4	5	8	3	1	1
Percentage of strains inhibited	3.7	14.8	14.8	18.5	29.6	11.1	3.7	3.7

The table depicts the number and percentage of *E.coli* strains inhibited by various concentrations of Cefotaxime. (n = 27).

Table 4. Combined effect of Cefotaxime and AGE *E.coli* strains co-producing ESBL and AmpC by checkerboard method.

Type of Interaction	ESBL+ AmpC
No. of strains exhibiting Synergy (FIC ≤ 0.5)	10
No. of strains exhibiting Additive (0.5 <FIC ≤ 1)	9
No. of strains exhibiting Indifference (1< FIC ≤ 2)	8
No. of strains exhibiting Antagonism (FIC >2)	-

The table depicts the number of the *E.coli* strains inhibited by combining concentrations of AGE and Cefotaxime. (n = 27).

Table 5. MIC of Cefotaxime and AGE in combination and FIC index against *E.coli* strains co-producing ESBL and AmpC by checkerboard method.

Strain no.	MIC of AGE (mg/ml)	MIC of AGE in combination with sub-MIC of Cefotaxime (mg/ml)	MIC of Cefotaxime (µg/ml)	MIC of Cefotaxime in combination with sub-MIC of AGE (µg/ml)	∑FIC (FIC Index)	Interpretation
1	5	5	800	400	1.5	I
2	5	2.5	400	100	0.75	AD
3	5	2.5	1,000	400	0.9	AD
4	5	5	800	200	1.25	I
5	5	2.5	400	100	0.75	AD
6	5	5	800	200	1.25	I
7	2.5	0.5	100	25	0.45	S
8	5	2.5	800	400	1	I
9	5	1.25	1,000	200	0.45	S
10	5	2.5	1,250	50	0.54	S
11	5	5	800	400	1.5	I
12	2.5	0.5	100	25	0.45	S
13	5	2.5	400	100	0.75	AD
14	10	5	1,000	400	0.9	AD
15	5	5	800	400	1.5	I
16	5	2.5	400	200	1	I
17	10	2.5	200	100	0.75	AD
18	5	0.5	200	50	0.35	S
19	2.5	0.5	50	25	0.7	AD
20	5	1.25	1,500	100	0.316	S
21	10	5	800	200	0.75	AD
22	5	0.5	100	25	0.35	S
23	2.5	0.5	200	25	0.325	S
24	10	2.5	200	100	0.75	AD
25	5	1.25	400	100	0.5	S
26	5	5	800	100	1.125	I
27	5	0.5	100	25	0.35	S

S = Synergistic association, I = Indifference, AD = Additive association.

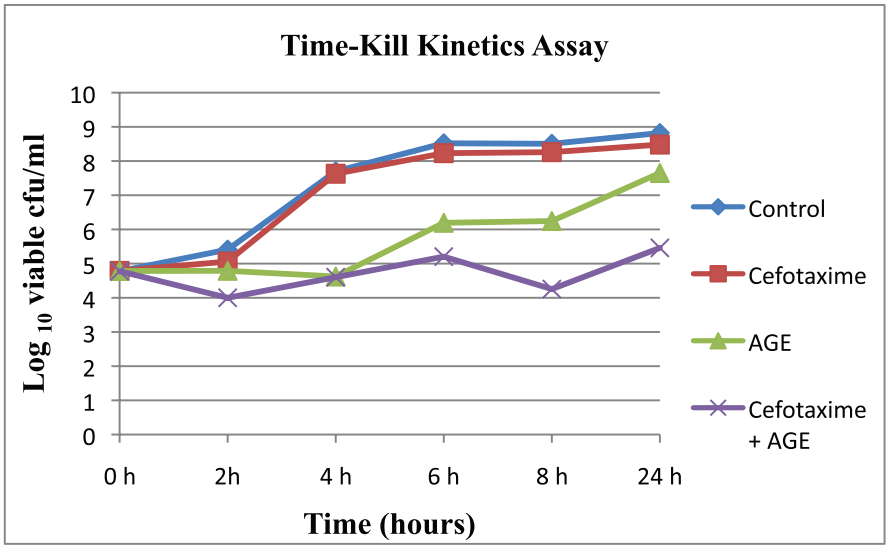

Figure 1. Synergistic effect study of Cefotaxime and AGE on *E.coli* strain co-producing ESBL and AmpC by Time-kill kinetic assay ($n = 3$).

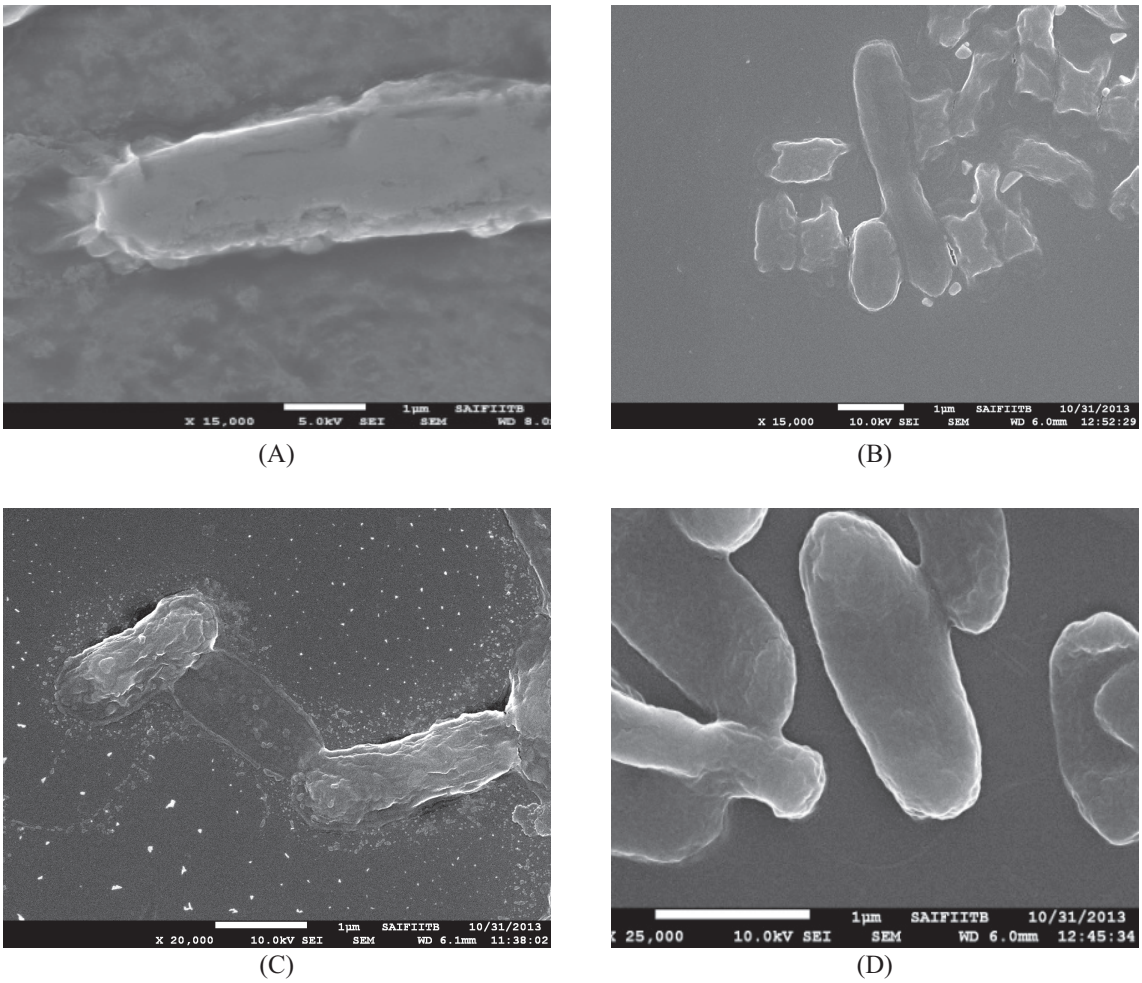

Figure 2. (A) Effect of Cefotaxime on *E. coli* co-producing ESBL and AmpC beta-lactamase. The micrograph shows uneven cell wall surface with spikes at the end of 8 hours indicating damage caused by Cefotaxime. (B) Effect of Aqueous Garlic extract on *E. coli* co-producing ESBL and AmpC beta-lactamase. The micrograph shows a cell wall rupture and uneven cell surface at the end of 8 hours, indicating damage caused by Aqueous Garlic extract. (C) Effect of Cefotaxime—Aqueous Garlic extract combination on *E. coli* co-producing ESBL and AmpC beta-lactamase. The micrograph shows complete cell degradation at the end of 8 hours, indicating damage caused by combined effect of Cefotaxime and Aqueous Garlic extract, thus confirming Synergism. (D) Control: *E. coli* co-producing ESBL and AmpC beta-lactamase at 0 hour without Cefotaxime or Aqueous Garlic extract. The micrograph shows intact *E. coli* cells, indicating no damage and a growing bacterial cell.

to the weakening of the peptidoglycan layer after garlic extract exposure (Kim *et al*., 2007). In the current study, the micrographs indicated deformed cell shape as well as rupture, lysis, and damage of the cell wall which may be attributed to the combined action of Cefotaxime and antimicrobial compounds present in AGE.

There is no documented study showing a synergistic effect between Cefotaxime and AGE, against *E. coli* strains which co-produce ESBL and AmpC, from SSTIs. However, a study by Guoliang *et al*. (2015) reports a synergistic association between AGE and Cefotaxime against non-beta-lactamase producing *Pseudomonas aeruginosa*, *Candida albicans*, and *Methicillin-resistant Staphylococcus aureus*. Previous study carried out by Shah *et al*. (2016) also reported synergistic activity between Cefotaxime and AGE against *Acinetobacter* strains which co-produce ESBL and AmpC.

CONCLUSION

Due to the increased abuse of synthetic antibiotics and the increasing spread of strains with multi-drug resistance, there is a need for an alternative antibacterial agent. The current study is the first to reveal the synergism between Cefotaxime and AGE against *E. coli* strains co-producing ESBL and AmpC from SSTIs. The synergistic association between the two agents demonstrates the potential application of garlic and antibiotic combinations as a tool for the treatment of infections caused by the drug-resistant bacteria, especially beta-lactams. Further studies are needed to elucidate the molecular mechanisms causing the synergistic effect between Cefotaxime and AGE *in vitro.*

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CONFLICT OF INTEREST

The authors declare that they have no competing interests with whomsoever.

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