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RESEARCH ARTICLE

Antibacterial Effect of Acetylsalicylic acid on the Efficiency of Azithromycin Against Resistant *Salmonella enterica*: An in Vitro Study

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ABSTRACT

This study aimed to determine the antibacterial effect of combined acetylsalicylic acid (ASA) and azithromycin (AZM) against resistant *Salmonella enterica*. For this, the MIC and MBC of both drugs were determined, and a checkerboard test, a time-killing assay, and scanning electron microscopy were performed. The results showed that ASA had an antibacterial effect with MIC value of 16000 μg/ml and MBC value of 32000 μg/ml. Also, the combination of ASA with AZM has an additive effect with FICI interaction value 1.5 at 8000 μg/ml of ASA and 16 μg/ml of AZM, and this has a direct effect on preventing further development of resistance in *S. enterica* exposed for 21 days. The time-killing results showed that it had a concentration-dependent effect, and the combination of the two drugs gave a better result in reducing the number of bacteria at 8000 μg/ml + 16 μg/ml for ASA and AZM, respectively with $P \leq 0.05$. SEM showed concentration-dependent morphological changes, which extended from membrane rupture to loss of cell contents and death. Also, the resistance induction assay was able to keep the resistance of AZM at a lower concentration of 16 μg/ml when combined with ASA. Finally, we conclude that ASA has an antibacterial effect in vitro, and their combination is useful in achieving better therapeutic effects in infections and preventing the development of resistance.

Keywords: Checkerboard test, Interaction, MIC, Scanning electron microscopy, Time-killing assay

Introduction

Azithromycin (AZM) is a broad-spectrum macrolide antibiotic that has potent bactericidal activity against a wide range of Gram-positive and Gram-negative bacteria. AZM has been used to treat respiratory diseases, intestinal infections, gum infections, and sexually transmitted diseases worldwide.[1] In recent years, the widespread use of AZM has led to the rapid development of resistance levels among various pathogens, including *Salmonella* spp.[2] This ultimately leads to significant economic losses in animals as well as threats to public health.[3] The continuous development of bacterial resistance is considered a source of great concern to the World Health Organization,[4–6] and for this reason, several strategies have been developed to solve this problem. One of these strategies is combination therapy, which is the use of two or more ingredients in a therapeutic regimen. Combination therapy is clinically important for several reasons. Firstly, it improves treatment and gives superior therapeutic outcomes, especially when synergy is achieved. Also, it improves the response rate and reduces device toxicity, because it allows lower doses to be used while maintaining efficacy. In addition, it reduces the emergence of antibiotic resistance.[7] Hence, combination therapy has proven effective in treating many different medical infections, such as the use of acetic acid and ciprofloxacin in the treatment of chronic suppurative otitis media,

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also combination of clavulanic acid and ampicillin in the treatment of chronic obstructive pulmonary disease.[8,9] On the other hand, acetylsalicylic acid (ASA) has been widely used as an analgesic, antipyretic, anti-inflammatory, and anticoagulant drug. In addition to its role in preventing and treating cancer.[10] Nevertheless, recent studies have indicated that ASA has an antibacterial effect.[11,12] Di Bella *et al.*[13] reported that ASA has anti-viral as well as anti-biofilm properties. To overcome the resistant problem, an *in vitro* model was designed to determine the antibacterial effect of ASA in combination with AZM to combat AZM resistant *S. enterica*.

Materials and methods

An ethical approval letter (UM.VET.2022.070) was obtained before starting the experiments by the Animal Ethics Committee, College of Veterinary Medicine, University of Mosul. A total of 20 male albino Wistar rats weighing 140–180 g were used. The rats were kept in cages with adequate ventilation at a temperature of 25 ± 3°C and a 12 h light and dark cycle for 1 week to acclimatize animals, in addition, the food and water were supplied ad libitum.[14] All rats were subjected to stool culture to ensure that they were free of *Salmonella*.

Bacteria and inoculum preparation

Salmonella enterica ATCC (14028) was obtained from the Media Diagnostic Center, Erbil, Iraq. Before experiments, the bacteria were subjected to an antibiotic sensitivity test)AST(using the disc diffusion method according to the Clinical and Laboratory Standards Institute (CLSI) to ensure that the bacteria had resistance properties against AZM.[15] After that, different doses of inoculation were prepared. Briefly, the bacteria were subcultured on Mueller- Hinton agar (Neogen, UK) and incubated at 37°C for 24 h. On the second day, different doses of bacteria were prepared using normal saline and then adjusted according to various McFarland standards to obtain a final density of approximately 1.5×10^6, 1.5×10^7, and 1.5×10^8 CFU/ml.[16]

Animal inoculation and verification of bacterial pathogenicity

Twenty rats used in this study were randomly distributed into 4 groups (5 rats/group). The rats were subjected to fasting for 18 h before inoculation, and then different doses of previously prepared bacteria were given at a rate of 0.5 ml/animal orally using a special sterile dosing syringe. G1 was inoculated with 1.5×10^6 CFU/ml, G2 with 1.5×10^7, and G3 with 1.5×10^8 CFU/ml, G4 was given 0.9% NaCl normal saline (Pioneer, Iraq) as negative control. All rats were monitored for 72 h and clinical signs were recorded. The rats were euthanized according to Institutional Animal Care Guidelines after clinical signs appeared.[17] Standard microbiology methods were used to re-isolate and identify *S. enterica* from the liver of the infected rats using Xylose Lysine Deoxycholate agar (XLD agar) (Neogen, UK), while VITEK®was used to confirm the re-isolation. Also, AST was done to confirm the resistance properties of *S. enterica* isolate using Azithromycin 15 μg disc.[15]

Determination of minimal inhibitory concentration (MIC)

Minimum inhibitory concentrations (MIC) of AZM and ASA were determined by the broth macrodilution method.[15] AZM was obtained from Jamjoom Pharma, Saudi Arabia, and ASA was obtained from Bella France Healthcare, France. Briefly, different concentrations of AZM 0.125–128 μg/ml and ASA 500–128,000 μg/ml were prepared using serial dilution in double-strength Brain heart infusion broth (BHI) (Neogen, UK) (the range of these values was determined using a previous pilot study). *S. enterica* was subcultured on the Mueller-Hinton agar (Neogen, UK), and then 0.5 McFarland 1.5×10^8 CFU/ml bacterial inoculum was prepared using a normal saline solution. After that, 100 μl of the prepared bacterial suspension was added to all tubes and then incubated at 37°C for 24 h. The tubes were visually inspected for any turbidity at the end of the incubation period. The MIC was determined as the highest dilution of drug that inhibits the bacteria growth.

Determination of minimum bactericidal concentrations (MBC)

MBC was determined based on the MIC value. Three tube dilutions before the MIC value were chosen for both AZM and ASA subcultured on MHA and incubated at a temperature of 37°C for 24 h.[15]

Evaluation of the combined activity of AZM and ASA using the checkerboard test

A checkerboard test was used to determine the interaction between AZM and ASA according to the previously described method by Bich Hanh *et al.*[18] Briefly, AZM 256 μg/ml and ASA 128000 μg/ml stocks were used to prepare a series of two-fold dilutions of each drug in a 96-well microplate. Then,

100 μl of 0.5 McFarland 1.5 $\times$ 10^8 CFU/ml bacterial inoculum was prepared. After that, the plate was incubated at 37°C for 24 h and the results were determined visually based on the presence or absence of turbidity. The values for the fractional inhibitory concentration index (FICI) for the two drugs were calculated as follows: FICI = (MIC of AZM in combination/MIC of AZM alone) + (MIC of ASA in combination/MIC of ASA alone). The values of interaction between the two drugs were determined as follows. FICI $\leq$ 0.5 indicates synergy interaction, 0.5–4 indicates additive interaction, and finally, FICI $>$ 4 indicates antagonism interaction.

Time-killing assay

Time-killing assay was performed to determine the time-dependent antibacterial effect for both AZM and ASA. The assay was performed using the values obtained from the previously determined MIC for each of AZM 32 μg/ml and ASA 16000 μg/ml, and also the values of checkerboard titration results for the combination of both AZM 16 μg/ml and ASA 8000 μg/ml and finally the positive control group *S. enterica* was included in this study. The assay was done in 3 replicates for each of the mentioned groups according to the microdilution method of Scoffone *et al.*[19] Briefly, BHI broth was prepared and different drug concentrations were added, then 100 μl of prepared bacterial inoculum at 0.5 McFarland 1.5 $\times$ 10^8 CFU/ml was added before the tubes were incubated at 37°C for different periods 0, 2,4,6,8 and 24 h. The results were recorded by counting the bacteria using the standard spread plate method.[15] The broth was serially diluted using the microdilution method, and then 100 μl of each dilution was spread on XLD plates and incubated at 37°C for 24 h. The results were recorded as log 10 CFU/ml.

Scanning electron microscopy (SEM) examination

Cellular morphological changes of the treated *S. enterica* were observed using a scanning electron microscope (Inspect F50 Scanning Electron Microscope (SEM)/FEI Company, Holland) according to Yi *et al.*[20] Initially, the bacterial cells were cultured on MHA plate and incubated at 37°C for 24 h. Then, the bacteria were subcultured in 10 ml of BHI broth for 3 h to reach the log phase of growth. After that, the bacteria were washed 3 times with PBS solution pH = 7.4, and adjusted to obtain 1 $\times$ 10^7 CFU/ml. Equal volumes of the suspended bacteria were added to each group as follows. The negative control group has only the suspended bacteria with an equal volume of BHI broth. The combination group has AZM 16 μg/ml and ASA 8000 μg/ml, ASA group MIC 16000 μg/ml and MBC group 32000 μg/ml. All groups were incubated at 37°C for 3 h. After that, the bacteria were centrifuged at 1200 rpm for 5 min and the supernatant was discarded. The pellet was washed 3 times with PBS pH = 7.4, and then 50 μl of diluted bacteria were placed on a microscopic slide with 1 $\times$ 1 cm dimensions. The bacteria were fixed with 2.5% glutaraldehyde at 4°C for 4 h. After fixation, the bacteria were dehydrated in gradient ethanol 30, 50, 70, and 90% and finally washed twice with 100% ethanol. After that, it was covered with gold and examined by scanning electron microscope.

Resistance induction assay

The macro dilution tube method was used to determine the effect of ASA on the development of resistance in *S. enterica*. Briefly, the bacteria were subcultured in successive concentrations of AZM, and ASA using the values obtained from previously determined MIC, and their combination using the previously done checkerboard test as a starting point. Also, *S. enterica* was inoculated as a positive control. The experiment was performed with 3 replicates for 21 days according to Navarro *et al.*[21] The OD was measured at 0, 4, 14, and 21 days of exposure using a spectrophotometer at 600 nm. The concentration of the drug at which *S. enterica* acquired resistance was determined if the breaking point rises 4 times, this means that the bacteria became resistant.

Statistical analysis

Statistical analysis using the SPSS-ASA program was performed with a two-way analysis of variance (ANOVA). The results were subjected to the least significant difference (LSD) test at $P < 0.05$.

Results and discussion

The results for the determination of bacterial pathogenicity indicated that G3 with 10^8 CFU/ml has induced prominent clinical signs of salmonellosis in rats within 48 h of dosing. The clinical signs include depression, anorexia, dehydration, roughness, rapid breathing, and diarrhea. On the other hand, the other groups developed mild clinical signs. The results of re-isolation showed characteristic black center colonies on XLD Fig. 1.A, while VITEK®results showed 98% identity as *S. enterica*. Additionally, the AST confirmed the resistance of the isolate to AZM with an inhibition zone of 10 mm Fig. 1.B.

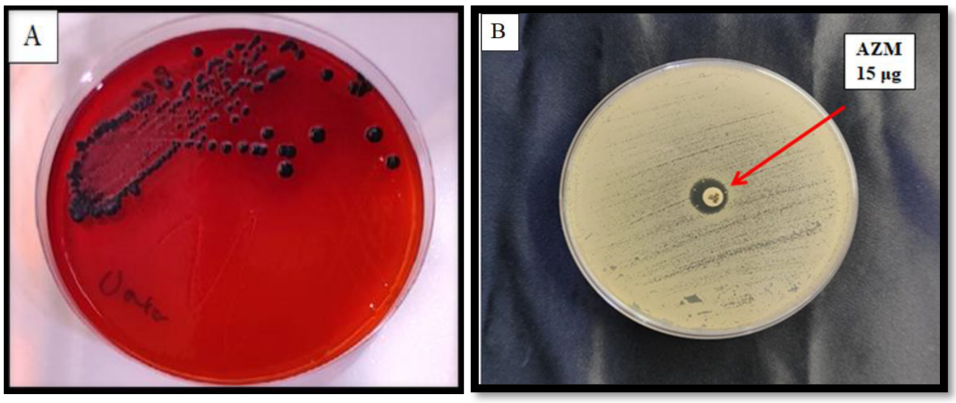

Fig. 1. A. *Salmonella enterica* re-isolated from the liver of rat infected with bacteria at a density of 10^8 CFU/ml (G3) using XDL agar. B. AST using AZM with 10 mm zone of inhibition (arrow).

Therefore, the re-isolated *S. enterica* in G3 was kept in 20% glycerol stock and used as standard inoculum for subsequent experiments. In this study, ASA was used to overcome the resistance of S. *enterica* against AZM. The results of bacterial pathogenicity were confirmed after re-isolation of resistance *S. enterica* with prominent clinical signs in G3 with 10^8 CFU/ml. This was consistent with clinical signs obtained by other studies such as anorexia, high temperature, pulse, respiratory rate, diarrhea, dehydration, and recumbancy.[17,22,23]

The results of the broth macrodilution method showed that the MIC values were 32 μg/ml and 16000 μg/ml for AZM and ASA, respectively. On the other hand, the results of MBC for AZM confirm that it has bacteriostatic properties at 4096 μg/ml with the presence of bacterial growth up to 7 folds concentration of the MIC. However, the MBC value for ASA was raised only one fold with a value of 32000 μg/ml and this confers its bactericidal property Fig. 2. Additionally, the results of the broth macrodilution method indicated that ASA has an antibacterial effect. Other studies also reported that ASA possessed an antibacterial effect. Chan *et al.*[24] reported that ASA has antibacterial activity against strains of *Staphylococcus aureus*. Also, Di Bella *et al.*[13] suggested that ASA has anti-infective properties. The MIC 16000 μg/ml of ASA against resistant *S. enterica* revealed an antibacterial property at high concentrations. Also, the same results were reported by Al-Bakri *et al.*[25] at high concentrations1200-2700 μg /ml. On the other hand, AZM has a lower MIC of 32 μg/ml against resistant *S. enterica* when compared with CLSI.[15] Also, BhAt *et al.*[26] confirmed that some *S. enterica* isolates were resistant to AZM at a lower threshold $\geq$32 μg/ml. In our study, ASA showed its evident bactericidal effect on MBC 32000 μg/ml at only one fold compared with its MIC at 16000 μg/ml. However, AZM remains bacteriostatic up to 7-fold concentrations of the MIC 4096 μg/ml. Radhakrishnan *et al.*[27] reported that the drug is considered bactericidal when the difference between the MIC and MBC

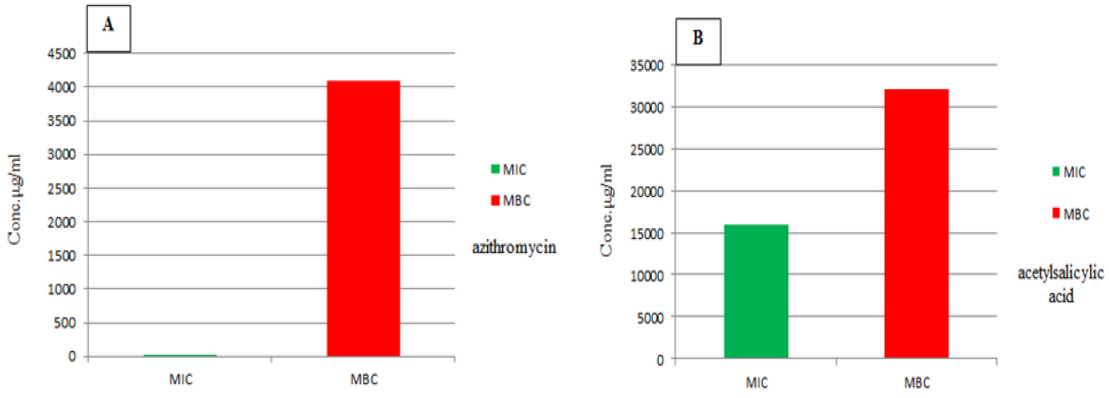

Fig. 2. A. MIC 32 μg/ml and MBC 4096 μg/ml for AZM against resistant *S. enterica*. B. MIC 16000 μg/ml and MBC 32000 μg/ml for ASA against resistant *S. enterica.*

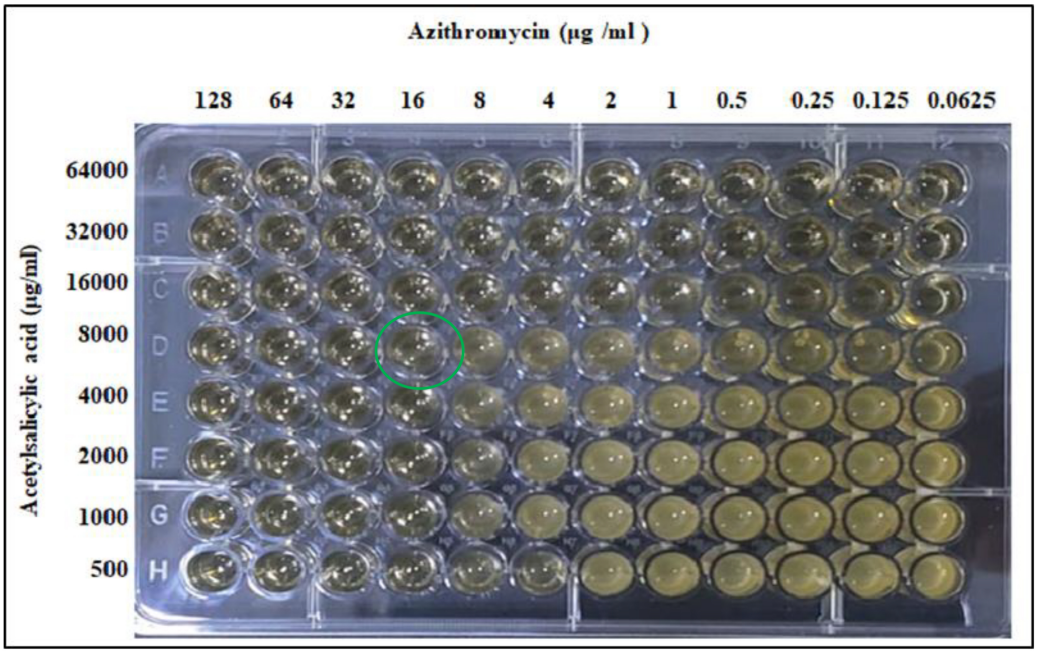

Fig. 3. Checkerboard test, the circle represents the combination of AZM 16 μg/ml and ASA 8000 μg/ml against antibiotic resistant *S. enterica*.

is less than 4 folds, while the drug is considered bacteriostatic when the difference is greater than 4 folds.

The checkerboard test results showed that the combination of AZM and ASA at 16 μg/ml and 8000 μg/ml, respectively was able to inhibit the bacterial growth with a 1.5 FICI value Fig. 3 and Table 1. The result of the checkerboard test indicated that the interaction between the two drugs is additive. This result could have clinical importance in terms of reduction of the therapeutic dose of each drug which improves the therapeutic index. Also, it can reduce the therapeutic cost.[28]

FIC, fractional inhibitory concentration; FICI, fractional inhibitory concentration index

The results of the time-killing assay for a combination of AZM and ASA against resistant *S. enterica* showed a significant reduction in the number of viable bacteria count 6.28 $\pm$ 0.02 CFU/ml. compared to other groups. This result is consistent with the result of Shrivastava *et al.*,[29] who indicated that the drug combination of tobramycin and ceftazidime has more inhibitory properties for bacterial growth than tobramycin and ceftazine alone. Also, significant differences were recorded at different time intervals within each group $P \leq 0.05$ Table 2. Our results obtained from the time-killing assay confirmed the interaction observed in the checkerboard test, and the combination of the two drugs gave a better effect than of each drug alone with a concentration-dependent antibacterial effect that appeared early after 2 h of incubation. This can give better therapeutic effects at lower concentrations, fewer side effects, and improved therapeutic efficacy by improving the sensitivity of *S. enterica* to AZM. Also, Zhou *et al.*[30] reported that ASA increases the activity of amphotericin B in *Candida albicans* and *C. parapsilosis*. Another study by Chan *et al.*[31] also indicated that ASA improves the effectiveness of cefuroxime and chloramphenicol against methicillin-resistant *Staphylococcus aureus* (MRSA). The time-killing assay for AZM showed that the antibacterial effect appeared after 2 h followed by bacterial regrowth, and this was consistent with the time-kill kinetics recorded by Kim *et al.*[32]

The results of electron microscopy showed that ASA had an antibacterial effect in all groups compared to the negative control group. However, combined AZM 16 μg/ml with ASA 8000 μg/ml induce cellular

Table 1. Checkerboard test showing the effect of the combination of AZM with ASA against resistant *S. enterica*.

	MIC μg/ml of AZM			MIC μg/ml of ASA				
Bacteria	Alone	Combination	FIC of AZM	Alone	Combination	FIC of ASA	FICI	Outcome
Resistant *S. enterica*	32	16	0.5	16000	8000	1	1.5	Additive or indifference

Table 2. Time-killing assay of AZM and ASA and their combination at different time periods expressed as $\log_{10}$ CFU/ml.

Time (h)	Control positive	AZA 32 μg/ml	ASA 16000 μg/ml	AZA 32 μg/ml + ASA 16000 μg/ml
0	6.53 ± 0.03^{Ae}	6.56 ± 0.02^{Ab}	6.55 ± 0.01^{Ad}	6.52 ± 0.03^{Ab}
2	8.21 ± 0.03^{Ad}	5.51 ± 0.02^{Bb}	5.51 ± 0.02^{Be}	5.62 ± 0.08^{Ce}
4	8.54 ± 0.01^{Ac}	6.73 ± 0.00^{Ba}	6.71 ± 0.01^{Bc}	6.41 ± 0.01^{Cc}
6	8.67 ± 0.01^{Ab}	6.75 ± 0.01^{Ca}	7.71 ± 0.01^{Bb}	6.67 ± 0.11^{Ca}
8	8.89 ± 0.00^{Aa}	6.81 ± 0.00^{Ca}	7.70 ± 0.01^{Bb}	6.53 ± 0.03^{Db}
24	8.81 ± 0.00^{Aa}	6.62 ± 0.01^{Cb}	7.26 ± 0.01^{Ba}	6.28 ± 0.02^{Dd}
LSD		0.0912		

The values represent the mean ± standard error of 3 replicates for each group.
Different capital letters refer to significant differences among groups at $P \leq 0.05$.
Different small letters refer to significant differences among time intervals within the group at $P \leq 0.05$.

changes at low concentrations compared with higher values of MIC 16000 μg/ml and MBC 32000 μg/ml for ASA, respectively Fig. 4. The results of electron microscopy showed that ASA had an antibacterial effect in all groups compared to the negative control group. However, combined AZM 16 μg/ml with ASA 8000 μg/ml induce cellular changes such as cell membrane disturbance at low concentrations, while higher values of MIC 16000 μg/ml and MBC 32000 μg/ml for ASA induce cellular changes like damaged cell wall, loss of cell contents and remains flaccid Fig. 4. Other studies reported that ASA causes cell wall damage through the formation of reactive oxygen species or affecting membrane-bound enzymes of the lipid bilayers or may cause changes in the membrane protein structure.[33–35] The presence of disruptions in the membrane or pores leads to the loss of the integrity of the membrane and the ability to act as a permeable barrier, and this allows the passage of small molecules and ions such as K^+, Na^+, $Cl^-$and H^+ which leads to disturbance in the pH, cell metabolism and cytoplasm, extravasation, thrombosis, and cell death.[36]

Finally, the results of the resistance induction assay showed that combined ASA with AZM was able to prevent further development of resistance compared

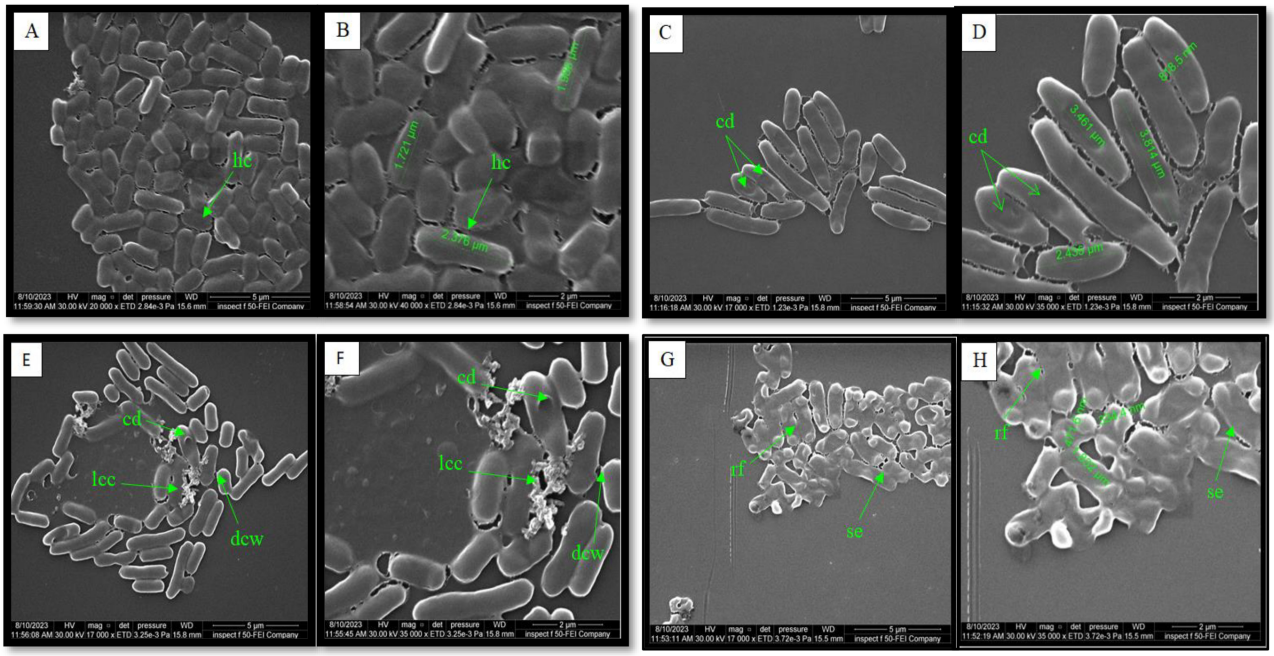

Fig. 4. SEM micrographs of ASA showing morphological changes in resistant *S. enterica*. A and B (negative control) at 200000X and 40000X respectively; C and D (combination of AZM 16 μg/ml and ASA 8000 μg/ml) at 17000X and 35000X, respectively, E and F (MIC of ASA 16000 μg/ml) at 17000X and 35000X, G and H (MBC of ASA 32000 μg/ml) at 17000X and 35000X, respectively. healthy cell (hc) cell membrane disturbance (cd), damaged cell wall (dcw) with formation of holes on the cell surface, loss of cell contents (lcc), cell shrinkage and vacuolization (se), remains flaccid (rf).

Table 3. Resistance induction assay for AZM, ASA and their combination at different days of exposure.

	Positive control *S. enterica*	AZM		ASA		Combination of AZM and ASA	
Days	OD	Conc. μg /ml	OD	Conc. μg /ml	OD	Conc. μg /ml	OD
0	$0.991 \pm 0.00^{A\,a}$	32	0.037 ± 0.04^{Bd}	16000	0.079 ± 0.00^{Ba}	16 + 8000	0.040 ± 0.00^{Ba}
4	1.004 ± 0.01^{Aa}	32	0.512 ± 0.05^{Bb}	16000	0.063 ± 0.00^{Ca}	16 + 8000	0.046 ± 0.01^{Ca}
8	$1.002 \pm 0.00^{A\,a}$	64	0.493 ± 0.09^{Bb}	16000	0.050 ± 0.01^{Ca}	16 + 8000	0.054 ± 0.01^{Ca}
14	$0.924 \pm 0.00^{A\,b}$	256	0.148 ± 0.02^{Bc}	16000	0.079 ± 0.00^{Ca}	16 + 8000	0.063 ± 0.00^{Ca}
21	$0.990 \pm 0.01^{A\,a}$	512	0.769 ± 0.03^{Ba}	16000	0.058 ± 0.01^{Ca}	16 + 8000	0.058 ± 0.01^{Ca}
LSD				0.0551			

The values represent the mean ± standard error of 3 replicates for each group.
Different capital letters refer to significant differences among groups at $P \leq 0.05$.
Different small letters refer to significant differences among time intervals at $P \leq 0.05$.

with AZM alone during the experiment. The AZM alone developed further resistance up to 512 μg/ml at day 21 and recorded OD 0.769 ± 0.03, while the combination of ASA 8000 μg/ml with AZM was able to keep the resistance against AZM at a lower concentration of 16 μg/ml at day 21 of induction and recorded 0.058 ± 0.005 $P \leq 0.05$ Table 3.

Interestingly, the results of the resistance induction assay showed that ASA can prevent further development of resistance in *S. enterica* exposed either alone or in combination with AZM. This finding is consistent with Wang *et al.*,[37] who found that ASA significantly inhibits the growth of *Helicobacter pylori in vitro* by increasing the sensitivity of these bacteria to amoxicillin, clarithromycin, and metronidazole. Also, our results are consistent with Malla *et al.*[38] who indicated that ASA was able to reverse colistin resistance and make *Enterobacteriaceae* and *Pseudomonas aeruginosa* more sensitive to this antibiotic by changing their ability to dissolve in fats.

Conclusion

ASA has shown antibacterial properties against *S. enterica* and has a concentration-dependent antibacterial effect. It also causes morphological changes in the cell wall and effectively prevents the emergence of additional AZM resistance in azithromycin-resistant *S. enterica*. *in vitro*, AZM and ASA exhibit an additive interaction. This combination could provide a new and effective alternative method in the clinical treatment of resistant *S. enterica*.

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Authors' declaration

- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are ours. Furthermore, any Figures and images, that are not ours, have been included with the necessary permission for republication, which is attached to the manuscript.
- No animal studies are present in the manuscript.
- Author(s) signed on ethical consideration's approval.
- Ethical Clearance: The project was approved by the local ethical committee at University of Mosul.

Authors' contribution statement

A.N.A. contributed to the design of the research. S.M.A, I.M.A and A.N. A. and contributed in implementation of the research, data analysis. S.M.A draft the manuscript. I.M.A and A.N. A. revised the final manuscript.

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التأثير المضاد البكتيري لحمض أسيتيل ساليسيلك على كفاءة الأزيثرومايسين في السالمونيلا المعوية المقاومة له: دراسة مختبرية

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الخلاصة

كان الهدف من الدراسة هو تحديد التأثير المضاد للبكتريا لحمض اسيتيل ساليسيلك على فعالية الازيثروماسين في السالمونيلا المقاومة له. لأجل ذلك تم تحديد التركيز المثبط الادنى لنمو البكتريا والتركيز الادنى لقتل البكتريا لكلا العقارين . ايضا تم تنفيذ اختبار رقعة الشطرنج واختبار قتل الوقت بالإضافة الى فحص المجهر الالكتروني . اظهرت النتائج ان لحمض اسيتيل ساليسيلك تأثير مضاد للبكتريا حيث بلغت قيمة التركيز المثبط الادنى لنمو البكتريا 16000 ميكروغرام /مل و والتركيز الادنى لقتل البكتريا 32000 ميكروغرام /مل وان مزيج الازيثروماسين وحمض اسيتيل ساليسيلك عند تركيز16 +8000 ميكروغرام /مل على التوالي له تأثير اضافي حيث بلغت قيمة FICI 1.5. وان لحمض اسيتيل ساليسيلك تأثير مباشر في منع تطور مزيد من المقاومة في السالمونيلا المعرضة له لمدة ٢١يوم . واظهر اختبار قتل الوقت ان حمض اسيتيل ساليسيلك تأثير يعتمد على التركيز واعطى مزيج الازيثروماسين وحمض اسيتيل ساليسيلك نتيجة افضل في تقليل عدد البكتريا عند تركيز16 +8000 ميكروغرام /مل على التوالي . واظهر فحص المجهر الالكتروني تغيرات شكلية تعتمد على التركيز ، حيث تراوحت من تمزق الغشاء الى خروج محتويات الخلية والموت . نستنتج من ذلك ان حمض اسيتيل ساليسيلك له تأثير مضاد للبكتريا في المختبر وان الجمع بين العقارين مفيد في تحقيق تأثيرات علاجية افضل في مكافحة العدوى ومنع تطور المقاومة .

الكلمات المفتاحية: فحص رقعة الشطرنج ، التداخل، التركيز المثبط الأدنى، مجهر الكتروني ماسح، فحص قتل الوقت.