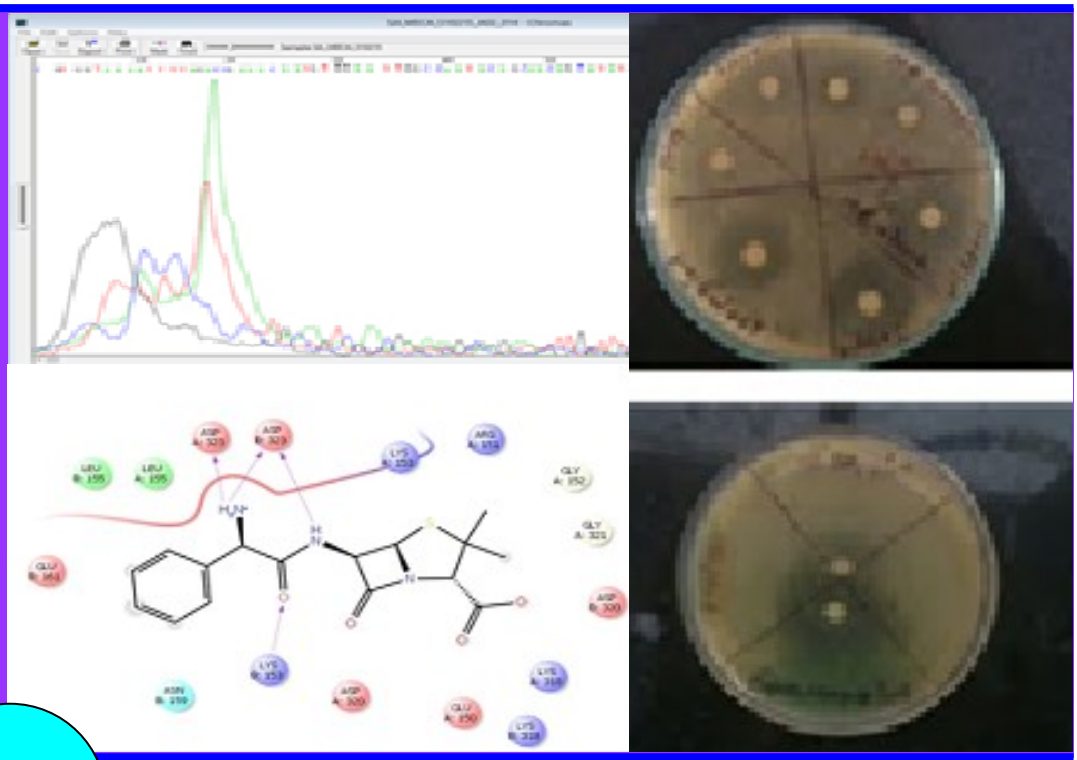

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Antibacterial potentiality of antiulcer and antispasmodic drugs with selected antibiotics against methicillin resistant *Staphylococcus aureus: In vitro* and *in silico* studies

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Abstract

In the present study, the antispasmodic drug mebeverine hydrochloride and the antiulcer drug troxipide were tested for their possible antibacterial properties *in vitro*. The antimicrobial assays of the above drugs were determined with ampicillin, penicillin and ciprofloxacin against sensitive and resistant strains and their resistance were confirmed through Polymerase Chain Reaction by identifying the presence of the *mecA* gene. A computer-aided method was used for screening the effectiveness of the drug interactions. Mebeverine and troxipide inhibited most of the sensitive and resistant strains tested *in vitro* from 32.5 to 125 µg/mL. The loss of structural alterations of the cell wall was analyzed by atomic force microscopy. In docking studies, troxipide and mebeverine were found to have substantial inhibition against penicillin binding protein 2a (IVQQ) and UDP-N-acetylglucosamine 1-carboxyvinyltransferase (2YVW) receptor proteins that seem to have interacted with most of the residues.

Introduction

Microbes such as bacteria, viruses, fungi and parasites can cause virulent and contagious diseases in individuals mainly through direct exposure to aerosols and contaminated materials (Barker and Jones, 2005). The resistance to infection depends upon the strength of the attack by the person's immune system (Carter, 2005). Antibiotics are able to control many bacterial infections like methicillin resistant *Staphylococcus aureus* (MRSA) yet they develop resistance to multiple drugs. Treatments have failed not only because of the development of resistance but also because of untoward reactions of the administered drugs in the infected individuals (Martins et al., 2008). Research reveals that there are some drugs that do not traditionally come under the category of antibacterial classification yet have moderate to powerful antibacterial action. They might have different pharmacological actions such as antihistaminic (El-Nakeeb et al., 2012), antipsychotic (Basu et al., 2005), antihypertensive (Dutta et al., 2005), antispasmodic (Karak et al., 2003), cardiovascular (Dasgupta et al., 2007; Kumar et al., 2004) and anti-inflammatory, such as the drug diclofenac sodium (Annaduri et al., 2008).

In the present study, we evaluated the antibacterial activity of troxipide and mebeverine *in vitro* and discovered a synergism between the selected drugs with antibiotics ampicillin, penicillin, and ciprofloxacin against a clinical isolate of *S. aureus*. In addition, we used a computer program for screening the existing drugs and the effectiveness of the drug interactions was tested with an *in silico* docking model with various receptor protein found on *S. aureus* strains. Nearly all

these assumed targets were involved in more than one metabolic pathways of MRSA.

Materials and Methods

Bacterial strains

S. aureus NCIM 2079, *K. pneumoniae* NCIM 2719, and *Enterobacter cloacae* NCIM 2164 were obtained from NCIM, Pune. Clinical strain *S. aureus*, *Escherichia coli* and *Enterococcus faecalis* were obtained from KAP Viswanathan Medical College, Tiruchirappalli, Tamil Nadu, India. The strains were confirmed and stored at 4°C until use.

Drugs

Troxipide, mebeverine hydrochloride, metaclopropamide and aceclofenac were obtained as pure drugs from Sigma Aldrich, India and kept under refrigeration until use.

Media

Nutrient broth, Muller-Hinton broth, nutrient agar and Muller-Hinton agar were prepared and steam sterilized at 15 psi for 15 min by autoclaving.

Standardization of inocula

The selected strains were grown in Muller-Hinton broth overnight at 37°C in an incubator under standard conditions. The harvested cells of the exponential phase culture were suspended in sterile distilled water and adjusted to a turbidity of 0.5 McFarland standard using a spectrophotometer (Cary-60 UV-Visible, Agilent Technologies) at 625 nm.

Molecular identification of mecA gene using gene-specific primers

Total DNA isolation and PCR analysis

Total DNA was extracted from overnight cultures of the selected bacterial isolates using a DNA isolation kit (Qiagen) and suspended in 100 µL of elution buffer (10 mM Tris-HCl, pH 8.5) and quantified by measuring optical density at 260 nm UV-Thermo Scientific

Box 1: Minimum Inhibitory Concentration of Drug

Requirement

Sterile test tubes, test tube rack, cotton, broth, bacteria, troxipide, incubator, Eppendorf micropipette with microtips

Procedure

Step 1: Sterilization of test tubes, test tube stands, water, glassware were sterilized by autoclave at 15 psi for 15 min

Step 2: Collection of bacterial samples(*S. aureus* NCIM 2079, *K. pneumoniae* NCIM 2719, *E. cloacae* NCIM 2164 and clinical strains *S. aureus, E. coli* and *E. faecalis).* Identification of specific activity of non-antibiotic troxipide against *S. aureus* NCIM 2079 is shown in the video

Step 3: Usage of antibacterial promoter agents (non-antibiotic): Troxipide, mebeverine, metaclopropamide and aceclofenac were used). Troxipide was used in this experiment

Step 4: Marking of test tubes one by one

Step 5: Addition of Muller-Hinton broth to all the test tubes, and then add troxipide with serial dilution and solutions were mixed by vortex mixture. Finally add inoculums to the respective test tubes

Step 6: Plugging all the test tubes tightly with sterile cotton for incubation at 37°C for 36 hours

Step 7: Results were observed after incubation and compared the tubes with the inoculum control (visual observation)

Step 8: Usage of spectrophotometer for estimating the percentage of inhibition

Drugs for determination of MIC value

Ciprofloxacin, ampicillin, penicillin, troxipide, mebeverine, metaclopropamide and aceclofenac

Comment

The lowest concentration of drug in a tube that failed to show any visible macroscopic growth was considered as its MIC

Test tube	Broth	Troxipide (1000 µg/mL)	Drug concentration (µg/mL)	Inoculum (*S. aureus*)
1	2 mL	2 mL	1000	200 µL
2	2 mL	2 mL- vortexted for 2 min, then transfer 2 mL to 3rd test tube	500	200 µL
3	2 mL	2 mL- vortexted for 2 min, then transfer 2 mL to 4th test tube	250	200 µL
4	2 mL	2 mL- vortexted for 2 min, then transfer 2 mL to 5th test tube	125	200 µL
5	2 mL	2 mL- vortexted for 2 min, then transfer 2 mL to 6th test tube	62.5	200 µL
6	2 mL	2 mL- vortexted for 2 min, then transfer 2 mL to 7th test tube	31.25	200 µL
7	2 mL	2 mL- vortexted for 2 min, then transfer 2 mL to 8th test tube	15.625	200 µL
8	2 mL	2 mL- vortexted for 2 min, then transfer 2 mL to 9th test tube	7.8125	200 µL
9	2 mL	2 mL- vortexted for 2 min, then transfer 2 mL to 10th test tube	3.90625	200 µL
10	2 mL	2 mL- vortexted for 2 min	1.953125	200 µL
Broth	2 mL			
Inoculum	2 mL			200 µL
Drug		2 mL		

References

Cappuccino and Sherman, 2002; Karak et al., 2003.

Click the Video clip
1. Set up
2. Broth and drug
3. Inoculation

BIOMATE 35. PCR amplification was performed using a 50 µL reaction mixture containing 100 ng of template DNA, 20 µmol of gyrA and gyrB primers, 200 µM of dNTPs, 1.5 mM of $MgCl_2$, 1U of *Taq* DNA polymerase (MBI Fermentas) and 10 µL of 10X *Taq* polymerase buffer. The sequences of the methicillin resistance gene (*mecA*) primers used were as follows:

mecA-F 5'-AAAATCGATGGTAAAGGTTGGC-3'

mecA-R 5'-AGTTCTGCAGTACCGGATTTGC-3'

Amplification was carried out with an initial denaturation at 94°C for 5 min followed by 35 cycles of denaturation at 94°C for 30 sec, annealing at 55°C for 30 sec, extension at 72°C for 1 min and final extension at 72°C for 5 min using a thermocycler (Eppendorf Personal Cycler, Germany). The amplified gene was analyzed on a 1% agarose gel for *mecA* amplicons in 1X TAE buffer at 50 V and was further confirmed by DNA sequencing with ABI PRISM 3730 Genetic Analyzer (Applied Biosystems) (Towner KJ et al., 1998, Trindade PA et al., 2003).

Checker-board method

To determine the interactions between the identified methicillin resistant bacteria and a combination of two drugs, the most frequently used procedure is the checker-board method. In this technique, aliquots of log -phase bacterial cultures (0.5 McFarland standard) were transferred to microtiter plates containing earlier-tested concentrations of drugs— ampicillin 25 to 800 µg/mL, troxipide and mebeverine 6.25 to 200 µg/mL. In the first row, ampicillin alone was distributed, whereas, in first column troxipide was added, and similarly, the other drugs were dispensed in micro well plates. The inoculated micro titer plates were incubated at 37°C for 36 hours (Kumar et al., 2004). The growth inhibition was measured by determining the absorbance at 530 nm in a multi-mode plate reader (Enspire, Perkin Elmer). From these readings, the synergistic and additive interactions were calculated (Mazumdar et al., 2003).

In vitro disc diffusion tests between the non-antibiotic drugs and the antibiotics

The drug-antibiotic combinations were tested by the disc diffusion technique with sterile filter paper discs (7.25 mm, Whatman No. 1) (Mazumdar et al., 2003). The filter paper was soaked in different concentrations (10, 20, 30, 40 and 50 µg) of drug. Sensitive and resistant strains were grown in sterile liquid media for 18 hours. Plates were prepared with Muller-Hinton agar and inoculated on the surface of which drug-soaked sterile disks were placed. The plates were incubated at 37°C for 36 hours and the zone of inhibition for each bacterial strain was measured. The experiments were performed in triplicates.

Atomic Force Microscopy (AFM) analysis

Atomic force microscopy was used to analyze the surface morphology and topology of the prepared samples mebeverine loaded MRSA cultures (Tyagi and Malika, 2010; Li et al., 2007). The images were taken using a Park Systems XE 100 (Germany). The samples were measured in the non-contact mode.

Methodology of docking

The chemical structures of troxipide, mebeverine and the reference drugs ampicillin and ceftrioxone were drawn using ChemSketch software version 12.01. Ceftriozone was selected for the docking studies as it is best drug of choice that effectively acts against MRSA. The target Protein Data Bank (PDB) structure with the IDs: Penicillin binding protein **2a** from MRSA strain (1VQQ), UDP-N-acetylglucosamine 1-carboxyvinyl-transferase (2YVW), fructose 1,6-bisphosphate aldolase (4LV4) and potassium transporter gating component ktrA as a c-di-AMP receptor (4J7C**)** (Corrigan et al., 2013; Yadav et al., 2012) were downloaded from PDB and the ligand structures were imported to ChemSketch. The target was given as an input, and the binding site was specified and prepared. The ligand was docked with the target. Population size was set to 50, generations to 10 and the number of solutions to 1. The docking analysis was performed and the docked poses were further examined. The software includes GLIDE module version 5.9, Mastero 9.4, Quik prop - 3.6 -Schrodinger, LLC, New York, NY, 2013- docking, Swiss PDB viewer-4.04 – protein viewer, Pymol viewer 1.3 – image viewer, Marvin sketch 5.5 – ligand structure (Tomasz and Alexander, 2005; Parasuraman et al., 2014; Perumal et al., 2014; Sabitha and Rajkumar, 2012).

Results

Table I shows the inhibitory activity of the antibiotics and drugs used. The MIC of ciprofloxacin is 3.95 to 15.625 µg/mL, and mebeverine and troxipide, 15.625 to 62.5 µg/mL against sensitive and resistant bacterial strains. The MIC of penicillin and ampicillin were 125-500 µg/mL against sensitive strains whereas it showed resistance against clinical strain *S. aureus* at 1000 µg/mL and its resistance was further identified with PCR analysis.

To identify the methicillin resistance of *S. aureus*, gene-specific primers were designed for amplification of the *mecA* gene by Polymerase Chain Reaction. The amplified region has been sequenced and confirmed that the 436 bases were found to be similar to the *mecA* gene of *S. aureus* strain S10215. This was done to confirm that the amplified sequence of the given sample was indeed, that of the *mecA* gene. The obtained sequences were searched by BLAST on the NCBI

Table I

Minimum inhibitory concentrations (MICs) of the selected drugs and antibiotics by broth dilution method

Drugs	*Staphylococcus aureus* NCIM 2079 (µg/mL)	Clinical strain *Enterococcus faecalis* (µg/mL)	*Klebsiella pneumoniae* NCIM 2719 (µg/mL)	*Enterobactor clocea* NCIM 2164 (µg/mL)	Clinical strain *Staph aureus* (µg/mL)
Ciprofloxacin	15.6	7.8	3.95	3.95	15.6
Penicillin	No activity	500	125	125	1000
Ampicillin	No activity	125	125	125	1000
Metaclopropamide	No activity	No activity	No activity	No activity	No activity
Mebeverine	62.5	31.25	62.5	15.6	62.5
Troxipide	62.5	62.5	62.5	62.5	62.5
Aceclofenac	No activity	No activity	No activity	No activity	No activity

website, and they clearly belong to the taxa *S. aureus* penicillin binding protein (*mecA*) (details shown in Figure 1: A,B,C) which plays an important role in β-lactamase resistance. Therefore, the resistive nature of the antibiotic was studied by using both disc diffusion and minimum inhibitory concentration methods with specific β-lactamase antibiotics penicillin and ampicillin.

Table II. In checker board method the MRSA strain was used. When used alone, penicillin and ampicillin have an MIC of 1000 µg/mL against MRSA but when combined with troxipide 50 µg/mL and mebeverine 50 µg/mL the MIC reduced to 50 µg/mL showing the synergistic effect.

In Table III and Figure 1D, the disc diffusion tests demonstrated the inhibitory effects of troxipide and mebeverine against MRSA and *E. faecalis*. Ciprofloxacin (10 µg/mL) alone shows an inhibitory zone of 36 mm, but when combined with troxipide (50 µg/mL) the inhibitory zone was increased to 48 mm, whereas with mebeverine (50 µg/mL) the diameter of zone of inhibition was 52 mm. Thus, the synergistic effects of combination of drugs were observed to some extent.

In Figure 2, the atomic force microscopy observation of MRSA cells demonstrates structural alterations characterized by the loss of regular shape that might be possibly attributed to the inhibition of cell wall biosynthesis by mebeverine. The cell wall degradation is more pronounced in drug-treated MRSA cells.

Analyzing the docking results

The docking analysis was done for the selected drugs with target receptors penicillin binding protein 2a from MRSA strain (1VQQ), the UDP-N-acetylglucosamine 1-carboxyvinyltransferase (2YVW), the fructose 1,6-bisphosphate aldolase (4LV4), and the potassium transporter gating component ktrA as a c-di-AMP receptor (4J7C) (Fuda et al 2004; Corrigan et al., 2013; Tomasz et al., 2005) using docking software. The structures docked by GLIDE are generally ranked according to the glide scoring function. From the output folder, the binding energy for the best hydrogen pose of the ligand was noted and the docked images are shown (Figure 3). The hydrogen bond interactions between the ligand and binding site residues were analyzed.

Table II

Synergistic interaction of combinations of antibiotics and non-antibiotics against methicillin-resistant *S. aureus* by checker board method

Drugs	MIC (µg/mL)	Type of interaction
Penicillin	-	No activity
Penicillin + Troxipide	50	Synergy*
Penicillin + Mebeverine	50	Synergy*
Ampicillin	-	No activity
Ampicillin + Troxipide	50	Synergy*
Ampicillin + Mebeverine	50	Synergy*

Penicillin and ampicillin from 25-800 µg/mL combined with troxipide and mebeverine from 6.25 to 200 µg/mL; *Penicillin 200 µg/mL and troxipide 50 µg/mL showed synergistic effect; *Penicillin 100 µg/mL and mebeverine 50 µg/mL showed synergistic effect; *Ampicillin 200 µg/mL and troxipide 50 µg/mL showed synergistic effect; *Ampicillin 100 µg/mL and mebeverine 50 µg/mL showed synergistic effect

The docking score was exhibited between troxipide, mebeverine, ceftriaxone and ampicillin with penicillin binding protein 2a from MRSA strain (1VQQ), the UDP -N-acetylglucosamine 1-carboxyvinyltransferase (2YVW), the fructose 1,6-bisphosphate aldolase (4LV4), and the potassium transporter gating component ktrA as a c-di-AMP receptor (4J7C). These results can be correlated to the optimized binding of drugs to the active site of the receptor protein. Troxipide and mebeverine seem to have interacted with most of the residues with an optimum energy. The docking outcome of these ligands is given in Table IV. The interaction energy including van der Waals and electrostatic forces as well as intermolecular hydrogen bonding was calculated. The docking score widely

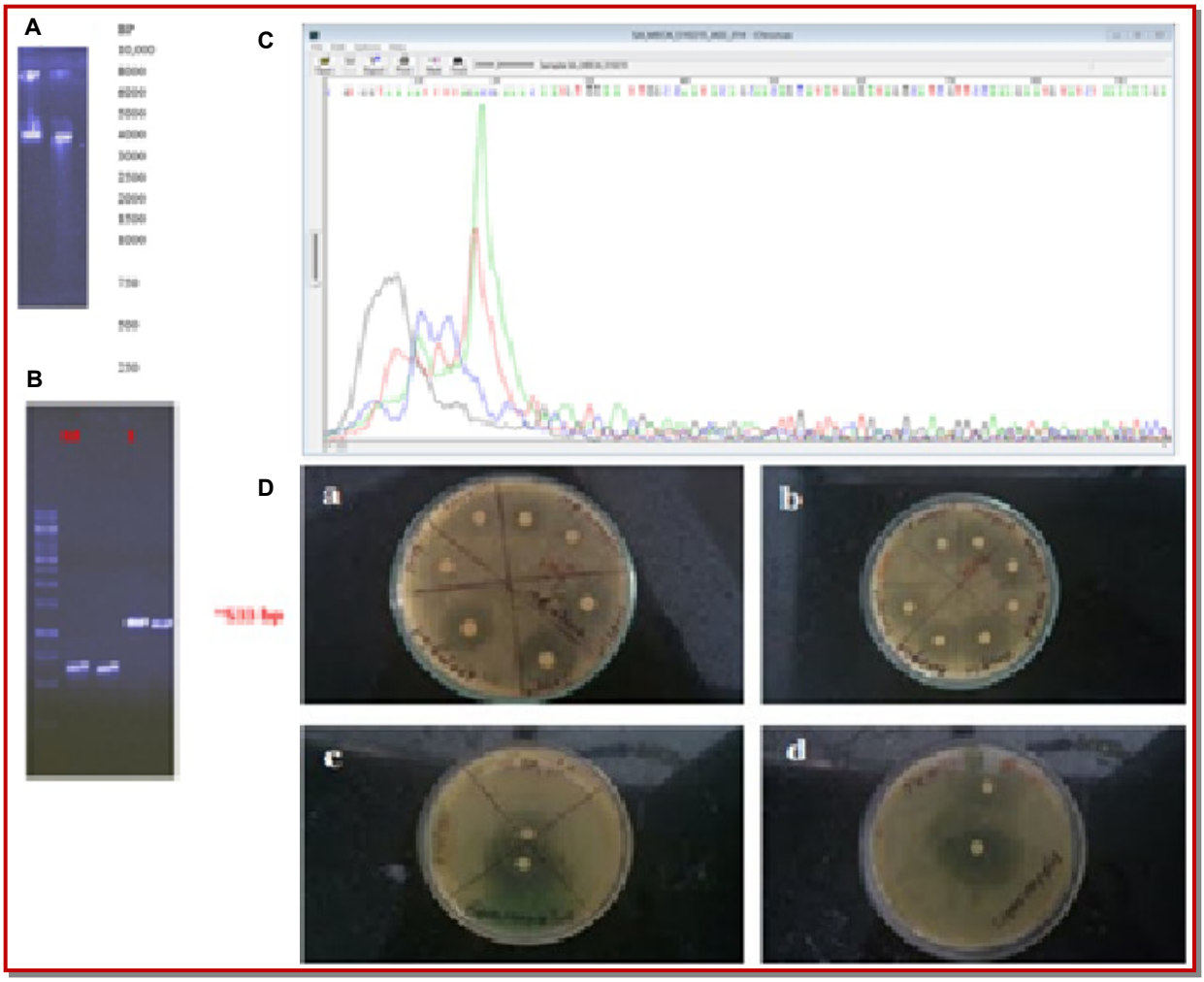

Figure 1: Bacterial genomic DNA (A); PCR amplification of *mec*A gene from *Staphylococcus aureus* (B), Lane M: 1 kBP DNA ladder; Lane 1: *mec*A gene from *S. aureus*; Sequence of mecA gene (C); Zone of inhibition of bacterial growth for various drugs and combinations of antibiotics and drugs (D), a. Antispasmodic drug mebevereine against *E. faecalis*, b. Antiulcer drug troxipide alone against *E. faecalis*, c. Antibiotics with antispasmodic drug against *E. faecalis*, d. Antibiotics with antiulcer drug against *E. faecalis*

ranges from -6.1727 to -5.42268 against IVQQ protein, -8.20542 to -4.6481 against 2YVW, -5.864 to -3.43802 against 4J7C and -3.36156 to -3.28715 against 4LV4. Table IV shows that the penicillin binding protein receptor (IVQQ) interactions with selected ligands and docking score values of -6.1727 for mebeverine and -5.42268 for troxipide were compared to the commercial antibiotics -5.73339 for ceftriazone and -5.76686 for ampicillin. These scores indicate that mebevereine has more interactions than ampicillin and ceftriazone. Ceftriaxone and ampicillin score value -8.2, -4.79 respectively against 2YVW were compared with mebeverine -6.297 indicates more interaction than ampicillin, troxipide exhibits similar interaction compared with ampicillin.

Discussion

Troxipide, which has a 3-(3,4,5-trimethoxybenzamido) piperidine chemical structure, is used in the treatment of gastroesophageal reflux disease. ME, 4-[ethyl-[1-(4-methoxyphenyl) propan-2-yl]amino]butyl 3,4-dimethoxybenzoatehydrochloride, which is used in the treatment of irritable bowel syndrome (IBS) and the associated abdominal cramping. Both were found to possess antibacterial activity against sensitive and resistant strains (Karak et al., 2003). The MIC was observed from 9 to 125 µg/mL. Treatment of *S. aureus* infection is a serious task due to wide spread resistance to beta lactam antibiotics.

In the present study, mebeverine and troxipide exhibited inhibitory action against MRSA especially mebeverine shows higher synergistic interaction with ampicillin than troxipide. Earlier studies revealed synergistic interaction between no antibiotic with non-antibiotic. When these drugs were used in combination there was an enhancement of the antibacterial capability against Gram (+)ve and Gram (-)ve micro organism. On the

Table III

Zone of inhibition of bacterial growth with combinations of antibiotics and drugs

Name of the organism	Antibiotics and Drugs	Zone of inhibition (mm)				
		Concentration (µg/10 µL)				
		10	20	30	40	50
MRSA	Ampicillin	No activity	No activity	No activity	No activity	No activity
	Mebeverine	14 ± 0.1	14 ± 0.2	16 ± 0.2	12 ± 0.1	12 ± 0.1
	Ampicillin + Mebeverine	14 ± 0.1	14 ± 0.5	16 ± 0.1	16 ± 0.2	16 ± 0.2
	Troxipide	12 ± 0.3	14 ± 0.5	12 ± 0.2	15 ± 0.5	15 ± 0.5
	Ampicillin + Troxipide	12 ± 0.2	15 ± 0.2	12 ± 0.4	15 ± 0.5	15 ± 0.2
Enterococcus faecalis	Ciprofloxacin	28 ± 0.3	30 ± 0.5	32 ± 0.2	33 ± 0.5	32 ± 0.5
	Mebeverine	18 ± 0.3	20 ± 0.6	23 ± 0.3	19 ± 0.5	19 ± 0.5
	Ciprofloxacin (10 µg/10 µL) + Mebeverine	43 ± 0.2	ND	ND	43 ± 0.3	52* ± 0.7
	Troxipide	18 ± 0.3	20 ± 0.5	22 ± 0.2	24 ±0.5	26 ± 0.5
	Ciprofloxacin (10 µg/10 µL) + Troxipide	44 ± 0.3	ND	ND	ND	48 ± 0.5
	Mebevereine + Troxipide	23 ± 0.5	17 ± 0.4	22 ± 0.5	16 ± 0.3	15 ± 0.7

Data are mean ± standard error

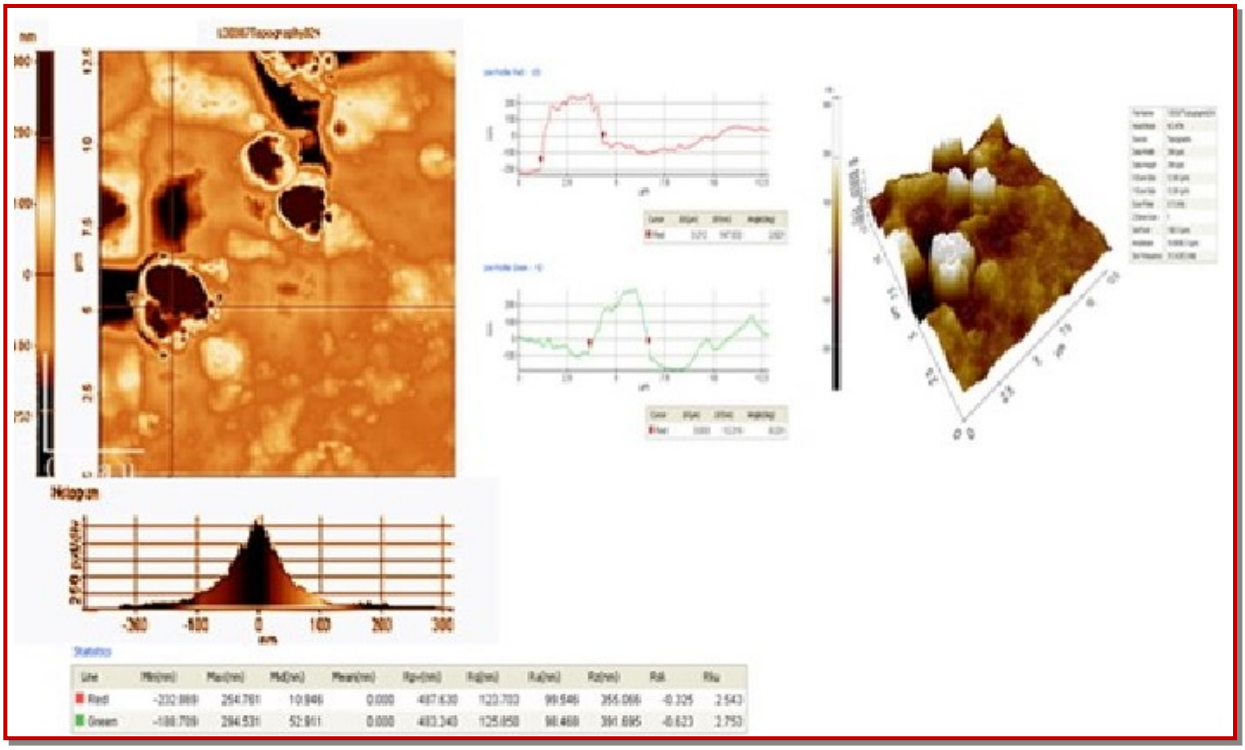

Figure 2: The influence of mebeverine hydrochloride on methicillin-resistant *S. aureus* (MRSA) as studied by atomic force microscopy

basis of MIC and disc diffusion method the antibacterial activity of mebeverine and troxipide and their following synergistic effect with an antibiotic ampicillin indicated that they act like non-antibiotics diclofenac sodium (Dutta et al., 2008) oxyfedrine (Mazumdar et al., 2003) omeprazole, ranitidine (Alkuraishy, 2011) dicyclomine (Karak et al., 2003), azelastin (El-Nakeeb et al., 2012; Akilandeswari et al., 2015).

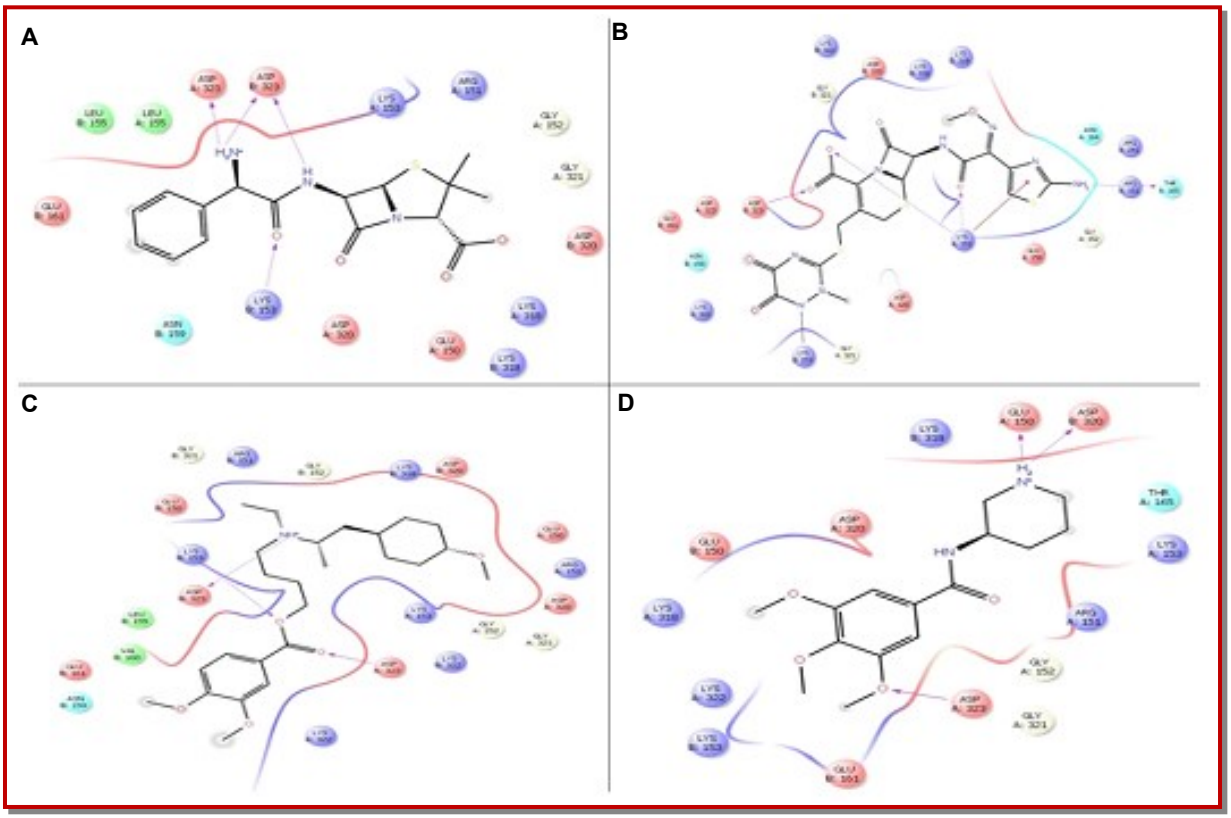

Figure 3: Ampicillin (A), ceftrioxone (B), mebeverine (C), troxipide (D) with IVQQ (PE binding protein 2a from MRSA strain)

However, bacterial growth inhibition for both troxipide and mebeverine was achieved after 36 hours compared with the conventional antibiotics. Though the mechanism of bacterial inhibition of non-antibiotics is not well -known, it could be due to multiple factors interfering with bacterial cell wall synthesis. Specifically multiple receptors found on the MRSA strain could be responsible for the reduction of MIC of two drugs in combination which were confirmed by docking analysis (Tomasz and Alexander, 2005; Parasuraman et al., 2014; Perumal et al., 2014; Sabitha and Rajkumar, 2012). We confirmed the efficacy of drug by performing an *in silico* docking study to obtain results with several receptor proteins responsible for the resistance of *S. aureus*. From these investigations, we have found that the MRSA strain has multiple receptors for resistance and if any one of the receptors interacts with the non-antibiotic compound, then it is seen to have antibacterial potency. When the compounds were docked against microbial receptors, the drugs exhibited stronger interactions with the potential target of MRSA receptors than the FDA-approved antibiotics ampicillin. Ligand interaction with the active site of the MRSA receptor protein with lower energy reveals higher binding affinity towards the active site of the receptor. These ligands might prove to be moderate inhibitors for MRSA infections, thus proving to be potentially beneficial for creating a novel antibacterial therapeutic molecule and the compounds mebeverine and troxipide were found to have substantial inhibition against IVQQ and 2YVW receptors.

Conclusion

The increasingly widespread emergence of bacterial resistance to multiple antibiotics might be overcome partially by utilizing these non-antibiotics troxipide and mebeverine hydrochloride as alternative approaches, thereby reducing the additional usage of antibiotics.

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Conflict of Interest

The authors declare no conflicts of interest

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Table IV

Docking studies of troxipide and mebeverine hydrochloride with various receptor protein*

Ligand	Receptor protein	Docking score	Glide energy	Interaction with H-bond side chain and backbone
Ampicillin	IVQQ(PBP)	-5.8	-45.5	Asp A323, Asp B323, Lys B153
Ceftriaxone	IVQQ(PBP)	-5.7	-62.8	Lys A153, Arg A151,Thr A165, Asp B323
Troxipide	IVQQ(PBP)	-5.4	-36.0	Glu A150, Asp B320, Asp A323
Mebeverine	IVQQ(PBP)	-6.2	-49.8	Lys B153, Asp B323, Asp A323
Ceftriaxone	2YVW	-8.2	-64.1	Arg 329, Arg 129, Gln 303, Gln 133,Thr 172
Ampicillin	2YVW	-4.8	-33.5	Lys 31, Asn 32, Arg 129
Mebeverine	2YVW	-6.3	-40.7	Thr 170, Asp 311, Lys 31, Arg 100
Troxipide	2YVW	-4.6	-31.0	Asp 311
Ceftriaxone	4J7C	-5.9	-56.9	Asn A143, Lys B211, Lys B131, Asp B146,Tyr B144, Tsp B146, Tyr B144
Troxipide	4J7C	-4.0	-33.2	Asn B143, Asp A127
Ampicillin	4J7C	-3.9	-37.2	Tyr B144, Leu A142
Mebeverine	4J7C	-3.4	-40.1	Arg B120, Asn A143, Asp B127
Ceftriaxone	4LV4	-3.4	-26.6	Asp 276, Ser 53, Asn 274, Gly 253
Troxipide	4LV4	-4.7	-33.2	Hip 96 Glu 178
Ampicillin	4LV4	-2.0	-31.8	Gly 253
Mebeverine	4LV4	-3.3	-34.6	Glu 178, Gly 253

*Penicillin binding protein 2a from MRSA strain (1VQQ), UDP-N-acetylglucosamine 1 carboxyvinyltransferase (2YVW), potassium transporter gating component ktrA as a c-di-AMP receptor (4J7C) and fructose 1, 6-bisphosphate aldolase (4LV4) Use one digit after dot

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