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Antimicrobial and synergistic activity of thiazoline derivatives in combination with conventional antibiotics against multidrug resistant *Staphylococcus aureus* isolated from abscess drainage samples

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Abstract: Emergence and spread of multidrug resistant (MDR) *Staphylococcus aureus* strains is becoming major challenge in treatment of soft tissue infections. This study aimed to explore antimicrobial and synergistic antimicrobial potential of three commercially available thiazoline derivatives (2-amino-2-thiazoline, 2-thiazoline-2-thiol and 2-acetyl-2-thiazoline) against MDR *Staphylococcus aureus* strains isolated from abscess drainage samples (n=20). MDR *Staphylococcus aureus* isolates were identified by Kirby-Bauer disk diffusion assay and were further subjected to molecular identification by 16srRNA amplification and DNA sequencing. Minimum Inhibitory Concentration (MIC) values of test compounds and antibiotics (0.25-512µg/mL) were measured and subsequently, synergism assay was performed to calculate Fractional Inhibitory Concentration (FIC) index. Out of twenty *Staphylococcus aureus* isolates, sixteen (80%) were found to be MDR whereas four (20%) were Non-MDR. Moxifloxacin and vancomycine were found most effective antibiotics, inhibiting 100% (n=20) and 95% (n=19) strains respectively. Antimicrobial activity of 2-amino-2-thiazoline (MIC: 32µg/mL), 2-thiazoline-2-thiol (MIC: 64µg/mL) and 2-acetyl-2-thiazoline (MIC: 32µg/mL) was found significant against all ten tested MDR strains. Synergistic combinations of thiazoline derivatives with test antibiotics reduced MIC values significantly. Therefore, combination of tested thiazoline derivatives with antibiotics could be used as alternative therapeutic approach to treat soft tissue infections caused by MDR *Staphylococcus aureus* after further pre-clinical and clinical studies.

Keywords: Antibiotics, *Staphylococcus aureus*, Multidrug resistance, Synergism, Thiazoline derivatives.

INTRODUCTION

Multidrug resistant (MDR) *Staphylococcus aureus* (*S. aureus*), especially Methicillin-resistant *S. aureus* (MRSA), accounts for approximately half of the hospital acquired soft tissue infections globally. Untreated and poorly managed infected abscesses can lead towards bacteremia and may necessitate amputation (Stevens *et al*., 2014, Gajdacs, 2019). Reported epidemiological data from all over the world gives an insight into the most commonly occurring causative organisms of infective abscesses and associated soft tissue infections. *S. aureus* has been reported to be the most commonly associated pathogen in both complicated and uncomplicated infective abscesses and soft tissue infections (Esposito *et al*., 2016, Gajdacs, 2019).

The development of antimicrobial drug resistance and suboptimal results of antimicrobial drug therapy, particularly with regard to infective abscesses, is the critical public health issues worldwide. The occurrence of antibiotic resistant pathogens and suboptimal therapeutic options that include conventional antibiotic regimens has risen to such an extent that people may well enter into a 'post-antibiotic era' in near future (Laxminarayan *et al*., 2013). The growing trend of microbial resistance against a number of commercially available antibiotics has necessitated the development of new antibiotics. Concerted efforts are being done globally to explore multiple strategies to cope with antibiotic resistance. One of the strategies is to discover molecules from natural origin with antimicrobial activity when used alone or in combination (Cos *et al*., 2006). Extracts of different plants have been used since centuries in Chinese, African and Eastern medicines to treat different infections and have proven effective against bacterial pathogens. Due to their improved efficacy and safety, purified compounds from plant material have also been tested for their antimicrobial potential. Global research data suggest that the effectiveness and spectrum of conventionally used antibiotics can be increased by using them in combination with natural bioactive extracts against pathogenic microorganisms (Cheesman *et al*., 2017).

Synergistic combination of antibacterial agents has been demonstrated to reduce the Minimum Inhibitory Concentration (MIC) of antibiotics. This may also provide improved treatment outcomes for several microbial

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infections. Synergistic potential of naturally occurring compounds has long been a subject of research and a number of compounds, which were isolated from natural origin, have successfully been used in combinational studies. Therefore, plant based natural compounds serve an ideal candidate towards the drug discovery and development (Guo *et al*., 2015).

Current study was conducted to perform medium-through put screening of number of natural compounds for their antimicrobial potential alone and in combination with other commercially available antibiotics. Three commercially available thiazoline (TZ) derivatives namely 2-amino-2-thiazoline, 2-thiazoline-2-thiol and 2-acetyl-2-thiazoline were chosen to be screened (both alone and in combination with conventionally used antibiotics) against MDR *S.aureus* strains isolated from abscess drainage samples.

Scientific literature already reported compounds containing thiazole ring as effective bioactive agents and have been found to be active against several drug resistant microorganisms including E. coli, *S. aureus* and *Aspergillus niger* (Sadek *et al*., 2011, Althagafi *et al*., 2019). Some thiazole derivatives were found to show the synergistic effect against multidrug resistant (MDR) S. *aureus*, and increased the effectiveness of available antibiotics (Althagafi *et al*., 2019).

MATERIALS & METHODS

Test Compounds and Antibiotics

Sixteen antibiotics used in this study, were commercially obtained from Oxoid (UK) and Sigma (Germany). Antibiotics included moxifloxacin (5 µg), choramphenicol (30 µg), cefadroxil (30 µg), ofloxacin (5 µg), fusidic acid (10 µg), cefaclor (30 µg), azithromycin (15 µg), cefixime (5 µg), levofloxacin (5 µg), ciprofloxacin (5 µg), cefotaxime (30 µg), cloxacillin (5 µg), ampicillin (10 µg), amoxicillin/clavulonic acid (30 µg), vancomycin (30µg) and cefoxitin (30 µg).

Three of the test compounds (Thiazoline derivatives) (fig. 1) were utilized in this study and were commercially obtained as well. This includes *2-amino-2-thiazoline (TZ-I), 2-thiazoline-2-thiol (TZ-II),* and *2-acetyl-2-thiazoline (TZ-III)* (Sigma, Germany). Dimethyl Sulfoxide (DMSO) was used to prepare stock solutions of test compounds.

Bacterial Strains & their Characterization

Total 20 clinical isolates of *S. aureus*, obtained from surgically drained soft tissue abscesses, were collected from Dow Research Institute of Biotechnology & Biomedical Sciences, Dow University of Health Sciences, Karachi, Pakistan.

Subsequent confirmation of the cultures were done using conventional microbiological techniques including Gram staining, microscopic examination, mannitol fermentation, β-hemolysis assay using 5% sheep's blood agar, catalase test using 3% hydrogen peroxide and coagulase test (Ahaduzzaman, 2014, Niederstebruch *et al*., 2017, Tiwari and Sen 2006). Conventional characterization was further validated by PCR amplification and DNA sequencing of 16srRNA gene. Only those isolates were subjected to molecular characterizations which were confirmed as being Multidrug Resistant (MDR) after screening through Kirby-Bauer Disk Diffusion Assay.

Screening of MDR strains

Isolates tentatively identified as being *S. aureus* were subjected to Kirby Bauer Disk Diffusion assay for determination of antibiotic susceptibility pattern (CLSI, 2008). Freshly grown broth cultures were subjected to turbidity adjustment up to 0.5 McFarland standards. Test antibiotic disks were then placed on Mueller Hinton Agar plates having bacterial lawn. Plates were kept overnight in incubator at 37 °C and zones of inhibition (mm) were measured. Results were interpreted as per CLSI guidelines (CLSI, 2008, CLSI, 2019).

Determination of Minimum Inhibitory Concentration (MIC)

Minimum Inhibitory Concentration (MIC) of selected antibiotics and then the test compounds against test organisms (0.25–512µg/mL) were determined by broth microdilution method in accordance with CLSI guidelines (CLSI, 2019). Only MDR strains of *S. aureus* were selected for MIC determination assays. In this assay antibiotics and test compounds were serially diluted using Mueller Hinton Broth (MHB) (Oxoid, UK) in such a way that each well of 96 well microtiter plate (Iwaki, Japan) contained half the concentration of antibiotic present in preceding well. Finally, $5x10^6$ of bacterial cells were inoculated into each well with the exception of wells labeled negative control. After 24 hours incubation (Innova42R, USA) at 37℃, wells of microtiter plate were visually observed to check for any turbidity in the medium (CLSI, 2019). For the purpose of making the growth end points more clear, 3-4, 5dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT dye) was introduced into each well at the concentration of 0.5 µg/ml. The plates were sealed and incubated on the shaking incubator at 37 °C and 80 rpm for another 2 – 3 hours and observed for change in color (Wang *et al*., 2010).

Synergism Assessment by Fractional inhibitory concentration assay (FICI)

FICI was performed using the same protocol adopted by Farooq *et al.* (2014) (Farooq *et al*., 2014). Briefly, each well of 96 well plate was inoculated with a combination

of test compounds and antibiotics in such a way that each well contained a unique concentration of test compounds and antibiotic. The concentration of antibiotics and test compounds were used for FICI which showed no bacterial inhibition in individual antimicrobial assay. Finally, $5x10^6$ bacterial cells/ml were inoculated in each well. The plate was visually observed after incubation in order to check whether there was any turbidity in the wells. MTT dye was added in the same manner as described before, in order to make end points more visible (Wang *et al*., 2010).

Fractional Inhibitory Concentration Index (FICI) was calculated using the following formula:

$$FICI = \left(\frac{\text{MIC of agent A in combination}}{\text{MIC of agent A alone}}\right) + \left(\frac{\text{MIC of agent B in combination}}{\text{MIC of agent B alone}}\right)$$

Synergism was defined as FIC Index of ≤0.5, no interaction as having FIC Index of 0.5 to 4.0, and antagonism as having FIC Index > 4.0 (Odds FC, 2003).

STATISTICAL ANALYSIS

Assays were performed in triplicates and results were given as means ± SD. Cochrane Q test was utilized to test the hypothesis and P value of <0.05 was considered as statistically significant.

RESULTS

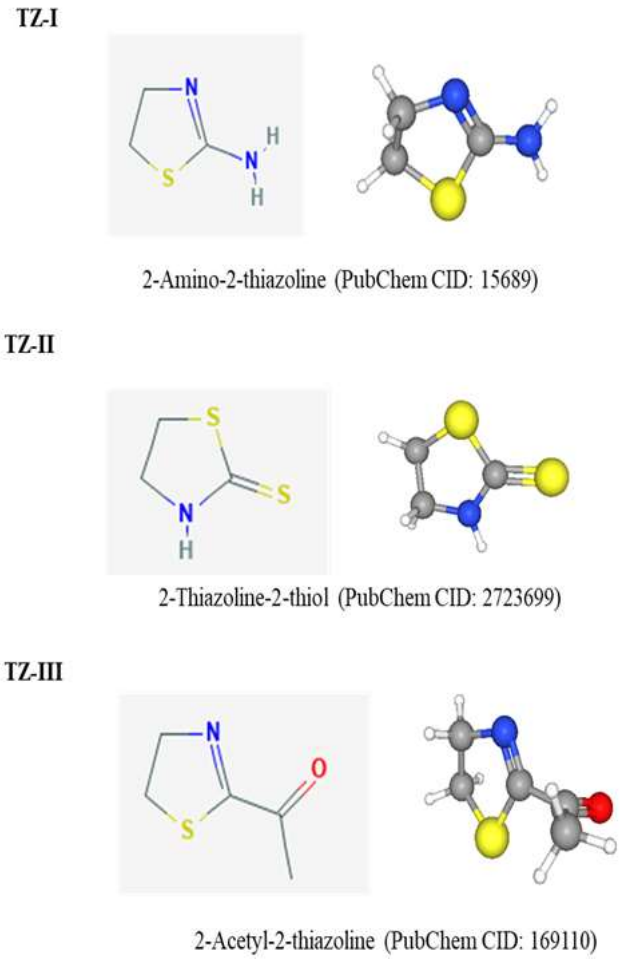
Characterization of Bacterial isolates

All of the morphological and biochemical characterization assays meant for the presumptive identification of *S. aureus* showed positive results for all 20 isolates. Gram staining and subsequent microscopy confirmed Gram positive cocci in grape like clusters. All isolates were able to withstand salt challenge test as growth was observed on Mannitol Salt Agar (MSA), one of the characteristic features of *S. aureus.* Furthermore, collected isolates were able to produce β-hemolysis on 5% sheep blood agar and showed positive catalase and coagulase tests as well (fig. 2).

For specie identification, sequence analysis of 16S rRNA gene was done (fig. 3). Obtained sequence was subjected to NCBI Basic Local Alignment Search Tool (BLAST). All the query sequences showed ≥ 97% similarity with the sequences of *S. aureus* present in the database.

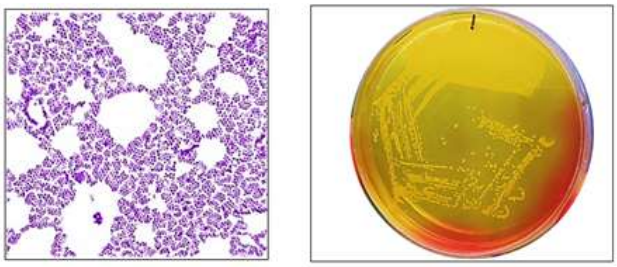
Antibiotic susceptibility pattern

Antibiotic susceptibility pattern of all twenty isolates were checked against sixteen antibiotic discs by Kirby Bauer disc diffusion assay. Out of total twenty *S. aureus* isolates, fifteen (75%) were found to be MDR-MRSA (Methicillin-Resistant *Staphylococcus aureus*), one was MDR-MSSA (Methicillin-sensitive *Staphylococcus aureus*) and four (20%) were non-MDR. All MDR-MRSA isolates were resistant to chloramphenicol, fusidic acid, ampicillin, azithromycin, ciprofloxacin, cloaxicillin, amoxicillin/calvulanic acid and cefoxitin and susceptible to Moxifloxacin and Vancomycin (fig. 4 and table 1).



The chemical structures of TZ-I (2-Amino-2-thiazoline; $C_3H_6N_2S$; MW: 102.16 g/mol), TZ-II (2-Thiazoline-2-thiol; $C_3H_5NS_2$; MW: 119.21 g/mol) and TZ-III (2-Acetyl-2-thiazoline; C_5H_7NOS; MW: 129.18 g/mol).

Fig. 1: Chemical structure of Thiazoline (TZ) derivatives tested in the study.



(a) Gram staining and microscopy showing purple colored cocci arranged in grape like clusters (b) Characteristic pin pointed golden yellowish colonies of *S. aureus* on MSA

Fig. 2: Morphological and Cultural Characterization of *Staphylococcus aureus*

Table 1: Antibiotic resistance profile of *S. aureus* isolates from abscess drainage samples (n=20) against test antibiotics employed.

	Clinical Isolates																			
ABX (µg/disc)	SA -01	SA - 02	SA - 03	SA - 04	S A- 05	SA - 06	SA - 07	SA - 08	SA - 09	SA - 10	SA - 11	SA -12	SA -13	SA - 14	SA - 15	SA - 16	SA - 17	SA - 18	SA - 19	SA - 20
MXF (5)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
CHL (30)	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
CFR (30)	R	S	R	S	R	R	R	S	R	R	R	R	R	S	R	R	R	S	R	S
OFX (5)	R	S	R	S	R	R	R	S	R	R	R	R	R	R	R	R	R	S	R	S
FA (10)	R	S	R	R	R	R	R	S	R	R	R	R	R	R	R	R	R	R	R	S
CEC (30)	R	S	R	R	R	R	R	S	R	R	R	R	R	S	R	R	R	S	R	S
AZM (15)	R	S	R	R	R	R	R	S	R	R	R	R	R	R	R	R	R	S	R	S
CFM (5)	R	S	R	R	R	R	R	S	R	R	R	R	R	S	R	R	R	S	R	S
LVX (5)	R	S	R	S	R	R	R	R	R	R	R	R	R	R	R	R	R	S	R	R
CIP (5)	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	S	R	R
CTX (30)	R	S	R	R	R	R	R	S	R	R	R	R	R	S	R	R	R	S	R	S
CLOXA (5)	R	S	R	R	R	R	R	S	R	R	R	R	R	S	R	R	R	S	R	S
AMP (10)	R	S	R	R	R	R	R	S	R	R	R	R	R	S	R	S	R	S	R	S
AMC (30)	R	S	R	R	R	R	R	S	R	R	R	R	R	S	R	S	R	S	R	S
VAN (30)	S	S	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
FOX (30)	R	S	R	R	R	R	R	S	R	R	R	R	R	S	R	R	R	S	R	S
Status (CLSI guideline	M	NM	M	M	M	M	M	NM	M	M	M	M	M	MSSA	M	M	M	NM	M	NM

ABX=Antibiotics, MXF=Moxifloxacin, CHL=Chloramphenicol, CFR=Cefadroxil, OFX=Ofloxacin, FA=Fusidic acid, CEC=Cefaclor, AZM=Azithromycin, CFM=Cefixime, LVX=Levofloxacin, CIP=Ciprofloxacin, CTX=Cefotaxime, CLOXA=Cloxacillin, AMP= Ampicillin, AMC=Amoxicillin/clavulonic acid, VAN=Vancomycin, FOX=Cefoxitin.
SA=*S.aureus*, S=Susceptible, R=Resistant, M=MDR (Multi-Drug Resistant), NM=Non-MDR, MSSA =Methicillin Sensitive *S.aureus*

Table 2: Antimicrobial effects of compounds (Thiazoline derivatives) against multidrug resistant *S. aureus* strains (n=10).

S. No	Compounds (Thiazoline derivatives)	Minimum Inhibitory Concentration (MIC, µg/ml)
1	2-Amino-2-thiazoline	32
2	2-Thiazoline-2-thiol	64
3	2-Acetyl-2-thiazoline	32

MIC determination of antibiotics and test compounds

MIC of antibiotics and test compounds by broth microdilution assay was determined. MIC of test compounds and antibiotics was checked against ten MDR *S. aureus* strains (SA-01, 03, 05, 07, 09, 11, 13, 15, 17 and 19). It was observed that moxifloxacin and vancomycin showed complete inhibition of all tests organisms at 0.25µg/mL concentration, while chloramphenicol did not show inhibition against any test organism at higher concentration of 512µg/mL.

All three test compounds including *2-amino-2-thiazoline, 2-thiazoline-2-thiol* and *2-acetyl-2-thiazoline showed* significant inhibition against test strains at low concentration of 32µg/mL, 64µg/mL and 32µg/mL respectively (table 2).

MIC of different antibiotics alone and in combination with tested thiazoline (TZ) derivatives against multidrug resistant *S. aureus* strains was given in table 3.

Synergism Assessment by Fractional inhibitory concentration assay (FICI)

Antibacterial activity of the selected antibiotics was further evaluated in the presence of test compounds. The results of synergism assessment are summarized in table 4. It was observed that all compounds used in this study improved the antibacterial activity of antibiotics by noticeably reducing the MIC of all tested antibiotics (0.5-64µg/mL) except Ampicillin and Amoxicillin/clavulanic acid in case of strain SA-01, where no interaction has been observed as FIC index of 2 was observed.

DISCUSSION

This study was conducted with the aim to find out alternative therapeutic options to conventional antibiotics in the backdrop of increasing antibiotic resistance. MDR *S. aureus* isolated from abscess drainage samples were used as test isolates while purified natural compounds and their analogues were screened for their anti-microbial potential against test isolates.

Table 3: Minimum inhibitory concentrations (MIC, µg/mL) of different antibiotics alone and in combination with three tested thiazoline (TZ) derivatives against multidrug resistant *S. aureus* strains isolated from abscess drainage samples.

ABX	MIC (µg/ml)	SA-01	SA-03	SA-05	SA-07	SA-09	SA-11	SA-13	SA-15	SA-17	SA-19
MXF	Alone	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125
	w/TZ-I	0.03125	0.03125	0.03125	0.03125	0.03125	0.03125	0.03125	0.03125	0.03125	0.03125
	w/TZ-II	0.03125	0.03125	0.03125	0.03125	0.03125	0.03125	0.03125	0.03125	0.03125	0.03125
	w/TZ-III	0.03125	0.03125	0.03125	0.03125	0.03125	0.03125	0.03125	0.03125	0.03125	0.03125
CHL	Alone	1024	1024	1024	1024	1024	1024	1024	1024	1024	1024
	w/TZ-I	4	4	4	4	4	4	4	4	4	4
	w/TZ-II	4	4	4	4	4	4	4	4	4	4
	w/TZ-III	4	4	4	4	4	4	4	4	4	4
CFR	Alone	256	256	128	256	256	256	256	256	256	128
	w/TZ-I	16	16	16	16	16	16	16	16	16	16
	w/TZ-II	16	16	16	16	16	16	16	16	16	16
	w/TZ-III	16	16	16	16	16	16	16	16	16	16
OFX	Alone	256	256	256	128	256	256	256	256	256	256
	w/TZ-I	2	2	2	2	2	2	2	2	2	2
	w/TZ-II	4	4	4	4	4	4	4	4	4	4
	w/TZ-III	32	32	32	32	32	32	32	32	32	32
FA	Alone	128	128	128	64	128	128	128	128	128	128
	w/TZ-I	1	1	4	4	1	1	1	1	1	1
	w/TZ-II	4	4	4	4	4	4	4	4	4	4
	w/TZ-III	8	8	8	8	4	8	2	8	4	8
CEC	Alone	128	128	128	64	128	128	128	128	128	128
	w/TZ-I	32	32	16	16	32	8	16	16	32	16
	w/TZ-II	32	32	32	32	32	32	32	32	32	32
	With TZ-III	64	64	64	16	64	32	64	64	64	64
AZM	Alone	128	128	128	128	128	128	128	128	64	128
	w/TZ-I	32	32	4	4	32	2	4	4	4	2
	w/TZ-II	8	32	8	4	32	32	32	8	16	4
	w/TZ-III	8	8	4	4	8	8	8	8	8	8
CFM	Alone	512	512	512	256	512	512	512	512	512	512
	w/TZ-I	64	64	16	64	32	32	32	32	16	32
	w/TZ-II	32	32	32	32	32	32	32	32	32	32
	w/TZ-III	32	128	128	128	128	128	128	128	128	32
LVX	Alone	128	128	128	64	128	128	64	128	128	128
	w/TZ-I	0.5	0.5	1	0.5	0.5	0.5	1	0.5	0.5	0.5
	w/TZ-II	4	4	4	4	4	32	4	4	4	4
	w/TZ-III	32	32	32	32	32	32	32	32	32	32
CIP	Alone	256	256	256	256	256	256	256	256	128	128
	w/TZ-I	1	1	1	1	1	1	1	1	1	1
	w/TZ-II	4	4	4	4	4	32	4	4	4	4
	w/TZ-III	32	32	32	32	32	32	32	32	32	32
CTX	Alone	256	256	256	256	256	256	256	256	256	256
	w/TZ-I	8	8	4	8	8	4	8	8	8	8
	w/TZ-II	16	16	16	16	16	16	16	16	16	16
	w/TZ-III	32	32	32	32	32	32	32	32	32	32
CLOXA	Alone	512	512	512	512	256	512	512	512	512	512
	w/TZ-I	0.5	0.5	2	8	8	8	0.5	0.5	0.5	8
	w/TZ-II	8	8	8	8	8	8	8	4	8	16
	w/TZ-III	8	64	64	64	64	64	64	64	64	64
AMP	Alone	256	256	256	256	256	256	256	256	256	256
	w/TZ-I	256	16	16	64	8	16	8	8	8	32
	w/TZ-II	256	32	32	32	32	32	32	32	32	16
	w/TZ-III	256	32	32	32	32	32	32	32	32	32
AMC	Alone	64	64	64	64	64	64	64	64	64	64
	w/TZ-I	64	8	8	8	16	8	4	8	8	16
	w/TZ-II	64	16	16	16	16	16	16	16	16	16
	w/TZ-III	64	16	16	16	16	16	16	16	16	16

TZE-I, TZE-II and TZE-III represents 2-Amino-2-thiazoline, 2-Thiazoline-2-thiol and 2-Acetyl-2-thiazoline respectively.
ABX = Antibiotics, MXF=Moxifloxacin, CHL=Chloramphenicol, CFR=Cefadroxil, OFX=Ofloxacin, FA=Fusidic acid, CEC=Cefaclor, AZM=Azithromycin, CFM=Cefixime, LVX=Levofloxacin, CIP=Ciprofloxacin, CTX=Cefotaxime, CLOXA=Cloxacillin, AMP= Ampicillin, AMC=Amoxicillin/ clavulonic acid.

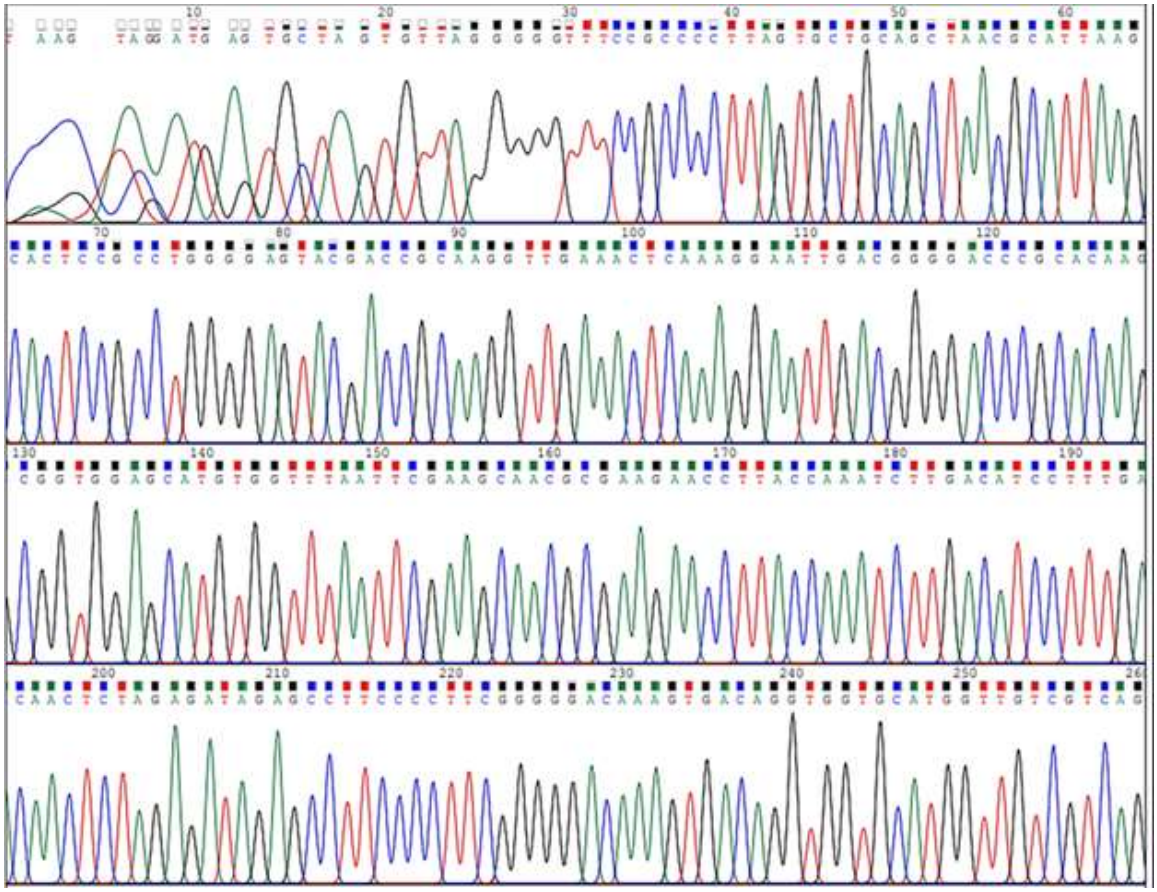
Table 4: Antimicrobial synergistic activity of thiazoline (TZ) derivatives against multidrug resistant *S. aureus* strains isolated from abscess drainage samples.

	FIC Index									
ABX+TZ derivatives	SA-01	SA-03	SA-05	SA-07	SA-09	SA-11	SA-13	SA-15	SA-17	SA-19
MXF+TZ-I	0.2505	0.251	0.2505	0.251	0.2505	0.2505	0.251	0.2505	0.2505	0.2505
MXF+TZ-II	0.2505	0.251	0.2505	0.251	0.2505	0.2505	0.251	0.2505	0.2505	0.2505
MXF+TZ-III	0.2505	0.251	0.2505	0.251	0.2505	0.2505	0.251	0.2505	0.2505	0.2505
CHL+TZ-I	0.0352	0.035	0.0352	0.035	0.0352	0.0352	0.035	0.0352	0.0352	0.0352
CHL+TZ-II	0.0352	0.035	0.0352	0.035	0.0352	0.0352	0.035	0.0352	0.0352	0.0352
CHL+TZ-III	0.0352	0.035	0.0352	0.035	0.0352	0.0352	0.035	0.0352	0.0352	0.0352
CFR+TZ-I	0.0645	0.065	0.126	0.065	0.0645	0.0645	0.065	0.0645	0.0645	0.126
CFR+TZ-II	0.0645	0.065	0.126	0.065	0.0645	0.0645	0.065	0.0645	0.0645	0.126
CFR+TZ-III	0.0645	0.065	0.126	0.065	0.0645	0.0645	0.065	0.0635	0.0645	0.126
OFX+TZ-I	0.0234	0.023	0.0234	0.023	0.0234	0.0234	0.023	0.0234	0.0234	0.0234
OFX+TZ-II	0.0234	0.023	0.0234	0.035	0.0234	0.0234	0.023	0.0234	0.0234	0.0234
OFX+TZ-III	0.126	0.126	0.126	0.251	0.126	0.126	0.126	0.126	0.126	0.126
FA+TZ-I	0.0234	0.023	0.0352	0.065	0.0352	0.0352	0.035	0.0234	0.0234	0.0234
FA+TZ-II	0.0352	0.035	0.0352	0.065	0.0352	0.0352	0.035	0.0352	0.0352	0.0352
FA+TZ-III	0.0645	0.065	0.0645	0.126	0.0352	0.0645	0.023	0.0645	0.0352	0.0645
CEC+TZ-I	0.2505	0.251	0.126	0.251	0.2505	0.0645	0.126	0.126	0.2505	0.126
CEC+TZ-II	0.2505	0.251	0.2505	0.502	0.2505	0.2505	0.251	0.2505	0.2505	0.2505
CEC+TZ-III	0.5002	0.5	0.5002	0.251	0.5002	0.2505	0.5	0.5002	0.5002	0.5002
AZM+TZ-I	0.2505	0.251	0.0352	0.035	0.2505	0.0234	0.035	0.0352	0.0645	0.0234
AZM+TZE-II	0.0645	0.251	0.0645	0.035	0.2505	0.2505	0.251	0.0645	0.2505	0.0352
AZM+TZ-III	0.0645	0.065	0.0352	0.035	0.0645	0.0645	0.065	0.0645	0.126	0.0645
CFM+TZ-I	0.126	0.126	0.0352	0.251	0.0645	0.0645	0.065	0.0645	0.0352	0.0645
CFM+TZ-II	0.0645	0.065	0.0645	0.126	0.0645	0.0645	0.065	0.0645	0.0645	0.0645
CFM+TZ-III	0.0645	0.251	0.2505	0.5	0.2505	0.2505	0.251	0.2505	0.2505	0.0645
LVX+TZ-I	0.0352	0.035	0.0234	0.023	0.0352	0.0352	0.023	0.0352	0.0352	0.0352
LVX+TZ-II	0.0352	0.035	0.0352	0.065	0.0352	0.2505	0.035	0.0352	0.0352	0.0352
LVX+TZ-III	0.2505	0.251	0.2505	0.5	0.2505	0.2505	0.251	0.2505	0.2505	0.2505
CIP+TZ-I	0.0352	0.035	0.0352	0.035	0.0352	0.0352	0.035	0.0352	0.0234	0.0234
CIP+TZ-II	0.0234	0.023	0.0234	0.023	0.0234	0.126	0.023	0.0234	0.0352	0.0352
CIP+TZ-III	0.126	0.126	0.126	0.126	0.126	0.126	0.126	0.126	0.2505	0.2505
CTX+TZ-I	0.0352	0.035	0.0234	0.035	0.0352	0.0234	0.035	0.0352	0.0352	0.0352
CTX+TZ-II	0.0645	0.065	0.0645	0.065	0.0645	0.0645	0.065	0.0645	0.0645	0.0645
CTX+TZ-III	0.126	0.126	0.126	0.126	0.126	0.126	0.126	0.126	0.126	0.126
CLOXA+TZ-I	0.126	0.126	0.0352	0.023	0.0352	0.0234	0.126	0.126	0.126	0.0234
CLOXA+TZ-II	0.0195	0.023	0.0234	0.023	0.0352	0.0234	0.023	0.0234	0.0234	0.0352
CLOXA+TZ-III	0.0234	0.126	0.126	0.126	0.2505	0.126	0.126	0.126	0.1255	0.126
AMP+TZ-I	2	0.065	0.0645	0.251	0.0352	0.0645	0.035	0.0352	0.0352	0.126
AMP+TZ-II	2	0.126	0.126	0.126	0.126	0.126	0.126	0.126	0.126	0.0645
AMP+TZ-III	2	0.126	0.126	0.126	0.0645	0.126	0.126	0.126	0.126	0.126
AMC+TZ-I	2	0.126	0.126	0.126	0.2505	0.126	0.065	0.126	0.126	0.2505
AMC+TZ-II	2	0.251	0.2505	0.251	0.2505	0.2505	0.251	0.2505	0.2505	0.2505
AMC+TZ-III	2	0.251	0.2505	0.251	0.2505	0.2505	0.251	0.2505	0.2505	0.2505

Synergistic activity is assessed as fractional inhibition concentration (FIC) index. Synergism was defined as: FIC index ≤0.5, Synergism; >0.5to≤4.0, No interaction; >4.0, Antagonism.

TZ-I, TZ-II and TZ-III represents 2-Amino-2-thiazoline, 2-Thiazoline-2-thiol and 2-Acetyl-2-thiazoline respectively.

ABX = Antibiotics, MXF=Moxifloxacin, CHL=Chloramphenicol, CFR=Cefadroxil, OFX=Ofloxacin, FA=Fusidic acid, CEC=Cefaclor, AZM=Azithromycin, CFM=Cefixime, LVX=Levofloxacin, CIP=Ciprofloxacin, CTX=Cefotaxime, CLOXA=Cloxacillin, AMP= Ampicillin, AMC=Amoxicillin/clavulonic acid.

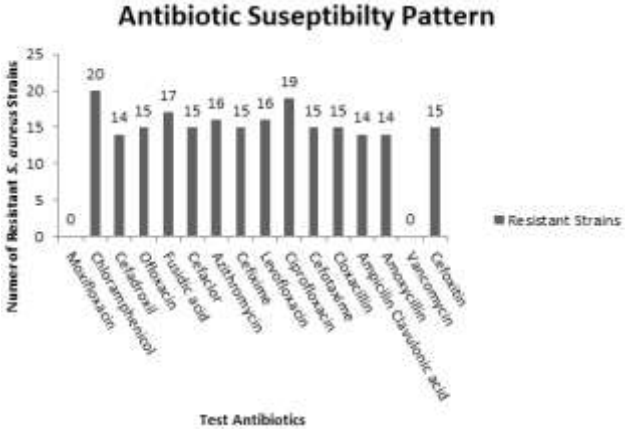


Peaks of different colours represent base calls of different bases at corresponding positions.

Fig. 3: Representative electropherogram showing a part of 16S rRNA sequence from isolates.

In the current study, out of twenty *S. aureus* isolates, fifteen isolates were characterized as MDR (MRSA) and one as MDR (MSSA). It would be important to mention that MRSA isolates are showing resistance not only to methicillin but to a number of β-lactam antibiotics like oxacillin, cefoxitin, cloxacillin, whereas by definition MDR isolates are the ones which are either resistant to atleast one member or 2 or more classes of antibiotics or resistant to more than 2 antibiotics of a single class. Thus, all the isolates confirmed as being MRSA shall be considered as MDR, whereas an MSSA isolate may or may not be MDR (Pantosti and Venditti, 2009, Gurung *et al*., 2020).

The susceptibility pattern of test organisms, including the MIC values, showed little or no variations when tested against test antibiotics and test compounds. Similar results, in terms of similarity of antimicrobial susceptibility have been reported by researchers in literature. The similarity of results in terms of antibiotic susceptibility can be accredited to the fact that the clinical isolates were collected from the same geographical location, thus exhibiting similar phenotype with very little variation. This is evident from a number of research studies. Ullah *et al.* (2016) in a single centered local study conducted at Rehman Medical & Teaching Institute Peshawar, reported the prevalence of MRSA isolates. Their results showed a high degree of similarity in terms of antibiotic susceptibility pattern and MIC values (Ullah et al., 2016).



Total twenty *S. aureus* strains (n=20) were screened against sixteen test antibiotics.

Fig. 4: Number of resistant *S. aureus* strains against test antibiotics used to determine antibiotic susceptibility pattern.

Another noteworthy outcome of the current study is the uniform sensitivity of all cultures against the antibiotics Vancomycin and Moxifloxacin. Test strains showed maximum inhibition at a minimum concentration of mentioned antibiotics.

The test compounds, in addition to antibiotics, were also subjected to antimicrobial susceptibility assay. Yurttas *et al.* (2015) demonstrated the significant antimicrobial activity of different thiazoline derivatives against a Gram positive organism *Enterococcus faecalis,* and Gram negative organisms including *Pseudomonas aeruginosa, Klebsiella pneumoniae and Escherichia coli* (Yurttaş *et al*., 2015). Not only this, but the researchers also demonstrated antifungal activities of thiazoline derivative against *Candida albicans, Candida krusei, Candida glabrata,* and *Candida parapsilosis* (Yurttaş *et al*., 2015). In another similar study Ghasemi *et. al.* (2015) demonstrated the antibacterial activity of several novel thiazoline derivatives against *Proteus mirabilis, Shigella dysenteriae,* and *Listeria monocytogenes* (Ghasemi *et al*., 2015). The biological activity of these thiazoline derivatives can be accredited to the thiazole moiety which is responsible for the reported antimicrobial, anti-inflammatory and anti-tumor activity (Gill *et al*., 2015). Also, the presence of *1-phenyl-1H-tetrazole* moiety in certain thiazoline derivatives is also responsible for their biological activities (Altintop *et al*., 2014).

For all the assays performed to determine antimicrobial activity of test compounds, Kirby Bauer disk diffusion assay was not performed since there were no interpretive criteria for zone diameters. Thus only broth microdilution assay was performed and results were recorded in terms of MIC values. The results showed no variations in terms of MIC values among all 10 microbial cultures.

MIC data of test antibiotics and test compounds were later utilized in antibiotic synergism assay. There was a significant reduction of MIC values observed when antibiotics were tested in combination with studied compounds against isolated strains of *S. aureus*. Similar synergistic potential of natural compounds have been reported in literature in which the compounds reduces the MIC of antibiotics more than 100 fold when used in combination. Mohammad *et al.,* (2015) reported a reduction of MIC of glycopeptide antibiotic Vancomycin by 512 fold when used in combination with certain thiazoline derivatives, thereby rendering the otherwise antibiotic resistant isolates to antibiotic sensitive ones (Mohammad *et al*., 2015).

CONCLUSION

Multidrug resistant *Staphylococcus aureus* poses a great challenge for the effective treatment of infections, as very limited antibiotics are effective against them. In the present study conducted in Karachi Pakistan, we reported that out of conventional antibiotics used for treatment of soft tissue infections and infective abscesses by physicians, Vancomycin and Moxifloxacin still possess effective antimicrobial activity against MDR *S. aureus.* This is evident from the fact that 100% (n=20) test organisms were found sensitive towards Vancomycin and Moxifloxacin. Moreover, we hereby report a total of three compounds, namely *2-amino-2-thiazoline, 2-thiazoline-2-thiol and 2-acetyl-2-thiazoline* having a potential to be used as antimicrobial agents, both alone as well as synergistically with commercially used antibiotics, thus proposing some novel combinations of conventional antibiotics and natural compounds for treatment strategies.

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