Pakistan Veterinary Journal

ISSN: 0253-8318 (PRINT), 2074-7764 (ONLINE)
DOI: 10.29261/pakvetj/2024.171

RESEARCH ARTICLE

Drug Resistance Modulation of Dairy MRSA through Berberine, Artesunate and Quercetin in Combination with β-Lactams

Saad Ahmad[1], Amjad Islam Aqib[2**], Muzafar Ghafoor[3], Muhammad Shoaib[4], Shahbaz ul Haq[5], Farid Shokry Ataya[6] and Li Jianxi[1*]

[1]Engineering & Technology Research Center of Traditional Chinese Veterinary Medicine of Gansu Province, Lanzhou Institute of Husbandry and Pharmaceutical Sciences of Chinese Academy of Agricultural Sciences, Lanzhou 730050, China
[2]Department of Medicine, Cholistan University of Veterinary and Animal Sciences, Bahawalpur 63100, Pakistan
[3]Department of Clinical Medicine and Surgery, University of Agriculture, Faisalabad 38000, Pakistan
[4]Key Laboratory of New Animal Drug Project, Gansu Province/Key Laboratory of Veterinary Pharmaceutical Development, Ministry of Agriculture and Rural Affairs/Lanzhou Institute of Husbandry and Pharmaceutical Sciences of the Chinese Academy of Agricultural Sciences, Lanzhou 730050, China
[5]Department of Pharmacology, Shantou University Medical College, Shantou, Guangdong 51504, China
[6]Department of Biochemistry, College of Science, King Saud University, P.O. Box 2455, 24, Riyadh 11451, Saudi Arabia
*Corresponding author: *lzjianxil@163.com; **amjadislamaqib@cuvas.edu.pk

ARTICLE HISTORY (24-070)

Received: February 5, 2024
Revised: April 14, 2024
Accepted: April 20, 2024
Published online: May 05, 2024

Key words:
Artesunate
Berberine
Quercetin
S. aureus
Drug resistance
Resistance modulation

ABSTRACT

The continued use of antibiotics is bringing extensive resistance in methicillin-resistant *Staphylococcus aureus* (MRSA) of dairy animals and demands inclusion of non-antibiotic sources in treatment protocols. The current study aims to explore antibacterial potential of berberine (BR), artesunate (AR), and quercetin (QT) and extent of their resistance modulation capacity in combination with β-lactam antibiotics against MRSA. For this study, a total of 674 milk samples from dairy cows were collected for isolation of MRSA and thereafter phytochemicals (drugs) alone and in combination with β-lactams (penicillin, oxacillin, cefoxitin, ampicillin) were evaluated against MRSA. The synergy testing showed all synergistic effects except AR with penicillin which was additive effect. The synergism of antibiotics and phytochemicals was further confirmed by time-kill assay. The killing kinetics revealed a zero bacterial count at 16h of incubation in all combinations. Additionally, the killing synergy showed a significant decline i.e. less than 60% in case of BR in combination with oxacillin and penicillin at the initial 2h of incubation followed by AR and QT in combination with ampicillin. The study thus revealed potential antibacterial effects of berberine, artesunate, and quercetin along with significant resistance modulation in terms of highly synergistic combinations with antibiotics focused on novel insights into alternatives to antimicrobials to pave the road for antimicrobial stewardship against ailments like mastitis.

To Cite This Article: Ahmad S, Aqib AI, Ghafoor M, Shoaib M, Haq SU, Ataya FS and Jianxi L, 2024. Drug resistance modulation of dairy MRSA through berberine, artesunate, and quercetin in combination with β-Lactams. Pak Vet J. http://dx.doi.org/10.29261/pakvetj/2024.171

INTRODUCTION

Methicillin-Resistant *Staphylococcus aureus* (MRSA) refers to *Staphylococcus aureus* strain that has developed resistance to methicillin and other beta-lactam antibiotics. MRSA strains have been further established as hospital-acquired (HA-MRSA), community-associated (CA-MRSA), and livestock-associated (LA-MRSA) and hence, contributing to the complexity of the challenge (Shoaib *et al*., 2023). The later has particular transmission potential at human and animal interface causing sever health issues particularly when people are working in close contact while dealing with animals (Roy *et al*., 2024). The non-judicious use of antibiotics, particularly the over-prescription, patient non-compliance, and inappropriate agricultural use has resulted in development of new resistant mechanisms in the bacterial strains leading to limited options of antibiotic treatment (Pinheiro *et al*., 2020). The genetic basis of MRSA resistance are associated with the synthesis of penicillin-binding protein 2α (PBP2α) which reduce the affinity for β-lactam antibiotics and rendering this antibiotic class largely ineffective (Alghamdi *et al*., 2023). In such scenarios, alternative to antimicrobials is a real need of the hour inclusive of which are traditional medicine.

Ayurvedic and Chinese medicine have a rich history of utilizing berberine, a quaternary ammonium salt derived from plants, possessing remarkable ability to combat a diverse array of microbes with antibacterial properties (Wang *et al*., 2008). The antibacterial mechanism of berberine involves damaging bacterial cell membranes, inducing the generation of reactive oxygen species (ROS), reducing intracellular adenosine triphosphate (ATP) levels and limiting biofilm production (Li *et al*., 2024). Similarly, the artemisinin, an active component of sweet wormwood has proven effectiveness against malaria and exhibits properties with anti-tumor and anti-inflammatory attributes (Dziedzic *et al*., 2015). The antibacterial mechanisms of artesunate (derivative of artemisinin) include increasing antibiotic accumulations via the inhibition of efflux pump and inhibiting the *agr* system involved in biofilm formation (Qian *et al*., 2021). Lastly, quercetin being a notable polyphenol express its antibacterial mechanism by disrupting cell walls and membranes of bacteria, inhibition of biofilm formation, reduction of virulence factors, inhibition of efflux pumps, interference with ATP synthesis, inhibition of enzyme activities and nucleic acid synthesis (Yang *et al*., 2020).

Keeping in view the increasing resistance in MRSA and proven antibacterial potential of phytomedicine candidates, it was desirable to explore the interaction of plant-based drugs with antibiotics to pave a road to formulate alternative effective therapeutic regimens. Thus, this study focuses on evaluation of antibacterial potential of berberine, artesunate, and quercetin, and their resistance modulation in combination with selected β-lactam antibiotics.

MATERIALS AND METHODS

Ingredients and their preparation: The phytochemicals; Berberine chloride (BR) (BBI Life Sciences, Shanghai, China), Artesunate (AR) (Macklin, Shanghai, China) and Quercetin (QT) (Sigma–Aldrich, USA), and antibiotics; Penicillin G sodium salt (P), Cefoxitin sodium salt (CTX), Oxacillin sodium (OXA) and Ampicillin natrium salt (AMP) were purchased from Sigma-Aldrich, USA. All antibiotics and phytochemicals were dissolved and diluted following the guidelines of Clinical and Laboratory Standards Institute.

Isolation of MRSA and confirmation of drug resistance: Milk samples were collected from 674 dairy cows (n= 2696 quarter samples) from multiple dairy farms located in Faisalabad, Pakistan. The samples were initially screened for subclinical mastitis by Surf Field Mastitis Test (SFMT) (Muhammad *et al*., 2010) and shifted to Laboratory of Veterinary Preventive Medicine and Public Health, Department of Clinical Medicine and Surgery, University of Agriculture, Faisalabad, Pakistan. The Milk samples were subjected to microbiological and biochemical examination for the isolation of *S. aureus*. The phenotypically identified *S. aureus* isolates were further confirmed by PCR amplification of 279 bp of the *nuc* gene using the forward P1: 5'-GCGATTGATGGTGATACG GTT-3' and reverse primer P2: 5′-AGCCAAGCCTTGAC GAACTAAAGC-3′ (Sahebnasagh *et al*., 2014). The clinical strains (CS) of MRSA from *S. aureus* were confirmed by targeting the *mecA* gene using the forward P1: 5′-ACTGCTATCCACCCTCAAAC-3′ and reverse primer P2: 5′-CTGGTGAAGTTGTAATCTGG-3′ with a product size of 163 bp (Sahebnasagh *et al*., 2014). For this purpose, 25 µL reaction mixture (1µL of each P1 and P2 primer, 2 µL of template DNA, 12.5 µL of Master Mix (Takara), and 8.5 µL of purified water) was run in thermocycler as per condition optimized by Aqib *et al*. (2017). PCR amplicons were run on 2% agarose gel and bands were visualized under a UV light.

Modulating the β-lactam resistance with Berberine, Artesunate, and Quercetin

Broth micro-dilution assay: The minimum inhibitory concentration (MIC) of antibiotics and phytochemicals was determined by broth micro-dilution assay following the standards of The European Committee on Antimicrobial Susceptibility Testing (EUCAST). Firstly, 100 µL of 4x Mueller Hinton broth (MHB) were added to each well of a microtiter plate. Then 100 µL aliquot of working solutions (512 µg/mL) of antibiotics and (1024 µg/mL) of phytochemicals were added in first well of the microtiter plate and subsequently two-fold serially diluted with MHB from 1st well to 10^{th} well. Afterwards, 100 µL of overnight MRSA culture at a final concentration of 1×10^5 CFU/mL were added from 1^{st} to 11^{th} well keeping 11^{th} and 12^{th} well as positive and negative controls, respectively. The plates were incubated for 24 hours at 37°C, and the minimum inhibitory concentration (MIC) was calculated according to the guidelines of EUCAST (Wojtyczka *et al*., 2014).

Well diffusion assay: The antibiotics and phytochemicals against MRSA isolates were tested through well diffusion method. For this purpose, positive control (tigecycline 16 µg/mL), negative control (normal saline), phytochemicals alone (512 µg/mL for BR, 1024 µg/mL for QT and AR), antibiotic alone (128 µg/mL), and a combination of each phytochemical and antibiotic (1/2:1/2) were prepared. Following a 24-hour incubation at 37°C, the zones of inhibitions (ZOIs) were measured (Lodhi *et al*., 2021).

Checkerboard method: A checkerboard dilution assay was performed to determine the antibiotic and phytochemical combination effects. The findings were interpreted using a fractional inhibitory concentration index. The summation of the FICs (ΣFICI) in each well (ΣFICI = FICA + FICB) was used to classify the combination of antimicrobial agents at given concentrations as synergistic (ΣFICI≤0.5), additive (0.5<ΣFICI<1.0), indifferent (1<ΣFICI ≤4) and antagonistic (ΣFICI≥4) (Trifan *et al*., 2022). Unit change in increase or decrease in MIC of antibiotics in combination with phytochemicals was calculated as per previous study (Haq *et al*., 2022).

Killing kinetics: Growth kinetics was used to assess the bactericidal activity of the antimicrobial agents at ¼ MIC and the tested phytochemicals at ¼ MIC against MRSA ATCC 43300 as the results showed synergistic effect at the said concentration. Killing kinetics was found by applying 96-well microtiter plate following viable cell counts (log10 CFU/mL) against time. An inactivation curve was created for each treatment at 0h, 2h, 4h, 8h, 16h and 24h of

incubation at 37°C and plotted as a graph (Alshareef, 2021).

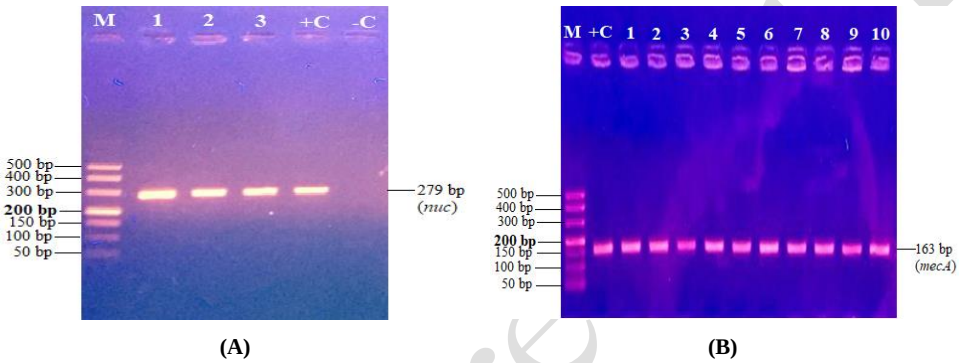
Statistical analysis: The data obtained was analyzed through t-test for statistical differences between the two groups while one-way ANOVA for ≥3 treatment groups and Tukey's test were applied with post hoc test ($p< 0.05$). The data were analyzed using SPSS version 22, while the graphs were made using GraphPad Prism version 8 for Windows.

RESULTS

Comparative Prevalence of Staphylococcus aureus and MRSA: Among the sampled isolates based on quarter level (n= 2696), 30.1% (812/2696) were identified as subclinical mastitis samples while among them, 38.2% (310/812) were identified positive for *S. aureus* (Fig. 1A) while MRSA (Fig. 1B) prevalence was noted to be 12.3% (38/310).

Antibacterial potential of phytochemicals and antibiotics: The study found a significant difference ($p<0.05$) in MIC among the tested phytochemicals within the different groups. Moreover, BR, QT and AR showed a significant difference within CS44 and CS67. Whereas in case of CS59, all the treatments showed a non-significant difference. CS110 and ATCC 43300 strain showed a non-significant difference ($p>0.05$) between berberine (BR) and quercetin (QT) having a noticeable difference with artesunate (AR) (Fig. 2A). The study also revealed greater MIC values of all the phytochemicals in clinical strains when compared to ATCC 43300 standard strain of MRSA (Fig. 2B).

The comparison of MIC values of antibiotics revealed oxacillin and cefoxitin showing no significant difference ($p>0.05$) while penicillin showed significantly lower values of MIC, and ampicillin had significantly higher values in CS44. In CS59, the MIC value of oxacillin was significantly lower than that of penicillin and cefoxitin showing non-significant difference, and that of ampicillin was higher compared to all others. Where in CS110, all the antibiotics exhibited a non-significant difference among each other except ampicillin showing higher values of MIC. CS67 and ATCC 43300 exhibited lower values for oxacillin and penicillin compared to higher values of cefoxitin and ampicillin. Overall, all the antibiotic treatments showed significant difference in their MIC values among different groups (Fig. 2C). When clinical strains compared with ATCC 43300, the results revealed a significantly higher values of MIC in clinical strains for all the antibiotics, exhibiting that oxacillin and penicillin showed the lowest MIC values followed by cefoxitin and ampicillin showing least antibacterial potential against the said bacterial isolates (Fig. 2D).



(A) **(B)**

Fig. 1: Molecular confirmation of *S. aureus* and MRSA (A) PCR products showing *nuc* gene at 279 bp for *Staphylococcus aureus*, (B) PCR products showing *mecA* gene at 163 bp for MRSA

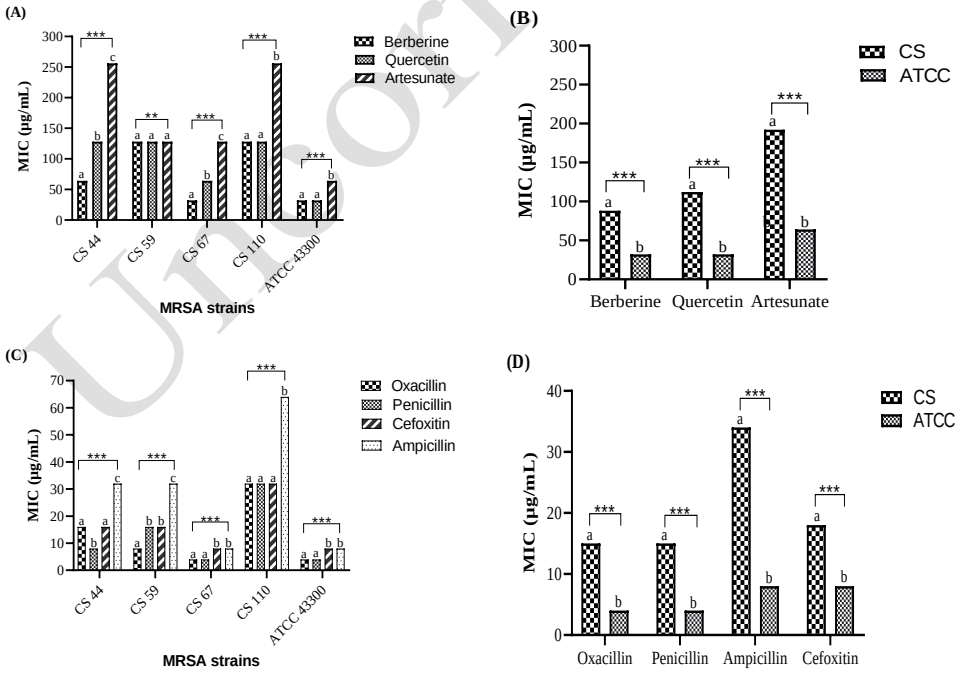


Fig. 2: Drug resistance profile of MRSA (A) Phytochemical's MIC within different isolates of MRSA (B) Comparative MIC of phytochemicals between clinical strains and ATCC 43300 (C) Antibiotics' MIC within different isolates of MRSA (D) Comparative MIC of antibiotics between clinical strains and ATCC 43300: (*** Significant difference ($p<0.05$), ** no significant difference ($p>0.05$), a,b,c showing significant difference within the group.)

Interaction responses of phytochemicals with antibiotics: The results obtained from broth micro-dilution and well diffusion assays demonstrated a significant decrease in the mean MIC of antibiotics and phytochemicals when used in combination. Among the antibiotics tested, all antibiotics consistently exhibited the most favorable outcomes with BR. Apart from BR, oxacillin and cefoxitin yielded superior results when combined with AR, whereas penicillin and ampicillin showed enhanced efficacy when combined with QT. The mean MIC of phytochemicals also significantly decreased in combination with antibiotics including the best combination of phytochemicals with ampicillin followed by combination with cefoxitin (Table 1). The FIC index of all the combinations revealed that combinations showed synergistic effect except that of the penicillin with artesunate, whose effect was found to be additive (Table 1).

Change in MIC of antibiotics in combination with BR, AR and QT: When comparing the drug interaction assay to the antibiotics used alone, the analysis of unit changes in MIC of antibiotics in combination with phytochemicals revealed a non-significant difference ($p>0.05$) between them, indicating a consistent response. Among all, cefoxitin showed the greatest reduction with BR (-0.989), AR (-0.980), and QT (-0.961) (Table 1). The same is true for other antibiotics in this experiment, who's MICs were significantly reduced in combination with the phytochemicals.

Killing synergy of antibiotic and phytochemical combinations: The killing synergy analysis revealed distinct patterns for each antibiotic. BR exhibited a substantial decline (<60%) in viable cell count when combined with oxacillin and penicillin within the first 2 hours of incubation. By the 4-hour mark, significant reductions (<50%) were observed in combination with cefoxitin and ampicillin. Notably, no viable bacterial cells were detected by the 16-hour reading (Fig. 3A). In contrast, QT displayed a non-significant decline at 2 and 4 hours, followed by a significant decrease at 8 hours, with a subsequent 90% reduction by 16 hours and complete elimination by 24 hours (Fig. 3B). A smooth decline was seen in case of AR in each time interval. AR demonstrated a consistent decline in viable cell count over time intervals (Fig. 3C), with reductions to 80%, 60%, and 20% at 2, 4, and 8 hours, respectively and a further decrease to 5% by the 16-hour reading (Table 2).

DISCUSSION

Methicillin-resistant *Staphylococcus aureus* (MRSA) has emerged as a global health concern due to its high resistance to multiple antibiotics and ability to cause severe infections. The complexity of MRSA strains lies in their potential to adapt and evolve in response to antibiotic and host immune pressures through the factors like antibiotic resistance, biofilm formation, and virulence factors (Roy *et al*., 2024). The emergence of such multidrug-resistant (MDR) bacteria highlights the need for alternative therapies. One promising approach to combat antimicrobial resistance (AMR) involves dual drug-delivery strategies that showed its success by targeting multiple pathways involved in bacterial resistance mechanisms. This approach in turn, enhances the efficacy of existing antibiotics while minimizing drug concentrations and target multiple resistance mechanisms, thus offer promise in the fight against antibiotic-resistant infections like MRSA (Alghamdi *et al*., 2023).

Table 1: Interaction analysis of different antibiotics with Berberine, Artesunate and Quercetin

Antibiotics	Phytochemicals	Mean MIC		Mean Change	Unit	Mean MIC		Mean Change	Unit	FICI	Interaction Type
		Antibiotic Alone	Antibiotic Combination			Phytochemical Alone	Phytochemical Combination				
Oxacillin	BR	15	0.843	-0.943		88	28	-0.681		0.37	Synergistic
	AR		0.937	-0.937		192	72	-0.625		0.43	Synergistic
	QT		1.40	-0.906		112	32	-0.714		0.37	Synergistic
Penicillin	BR	15	0.937	-0.937		88	29	-0.670		0.39	Synergistic
	AR		1.875	-0.875		192	72	-0.625		0.5	Additive
	QT		1.062	-0.929		112	32	-0.714		0.28	Synergistic
Ampicillin	BR	18	0.937	-0.948		88	18	-0.795		0.25	Synergistic
	AR		2.375	-0.868		192	36	-0.812		0.31	Synergistic
	QT		1.781	-0.901		112	18	-0.839		0.25	Synergistic
Cefoxitin	BR	34	0.375	-0.989		88	21	-0.761		0.24	Synergistic
	AR		0.656	-0.980		192	64	-0.667		0.35	Synergistic
	QT		1.312	-0.961		112	20	-0.821		0.21	Synergistic

Minimum Inhibitory Concentration (MIC), Fractional Inhibitory Concentration Index (FICI), Berberine chloride (BR), Artesunate (AR), Quercetin (QT).

Table 2: Comparison of time-kill assay (bacterial count) under the effect of BR, AR and QT.

Antibiotic	Phytochemical	0h	2h	4h	8h	16h	24h
Oxacillin	BR	6696 ± 144.66	3850 ± 79.43	2842 ± 62.86	358 ± 53.71	28 ± 8.0	0 ± 0.0
	AR	6421 ± 20.13	5432 ± 52.45	3856 ± 66.9	1360 ± 80.0	326 ± 41.63	0 ± 0.0
	QT	4426 ± 61.1	4065 ± 52.2	3818 ± 89.23	1272 ± 88.22	477 ± 20.13	0 ± 0.0
Penicillin	BR	6546 ± 61.1	2406 ± 48.22	949 ± 66.61	134 ± 16.16	18 ± 15.87	0 ± 0.0
	AR	6733 ± 61.1	5334 ± 61.1	3346 ± 61.1	1644 ± 66.0	313 ± 41.63	0 ± 0.0
	QT	4453 ± 83.26	3862 ± 68.85	2860 ± 91.65	1346 ± 61.1	456 ± 26.22	0 ± 0.0
Ampicillin	BR	6933 ± 61.1	5722 ± 33.3	2024 ± 85.72	656 ± 56.86	0 ± 0.0	0 ± 0.0
	AR	4840 ± 80.0	3820 ± 40.0	2334 ± 61.1	913 ± 30.55	162 ± 16.16	0 ± 0.0
	QT	5213 ± 61.1	3634 ± 61.1	2766 ± 41.63	1330 ± 56.86	381 ± 18.03	0 ± 0.0
Cefoxitin	BR	3558 ± 62.0	2818 ± 54.45	1593 ± 30.55	160 ± 20.0	0 ± 0.0	0 ± 0.0
	AR	6866 ± 140.47	5374 ± 90.36	3646 ± 94.51	1360 ± 80.0	429 ± 50.01	0 ± 0.0
	QT	4413 ± 61.1	3670 ± 49.27	3080 ± 87.76	1426 ± 94.51	326 ± 41.63	0 ± 0.0

Berberine chloride (BR), Artesunate (AR), Quercetin (QT).

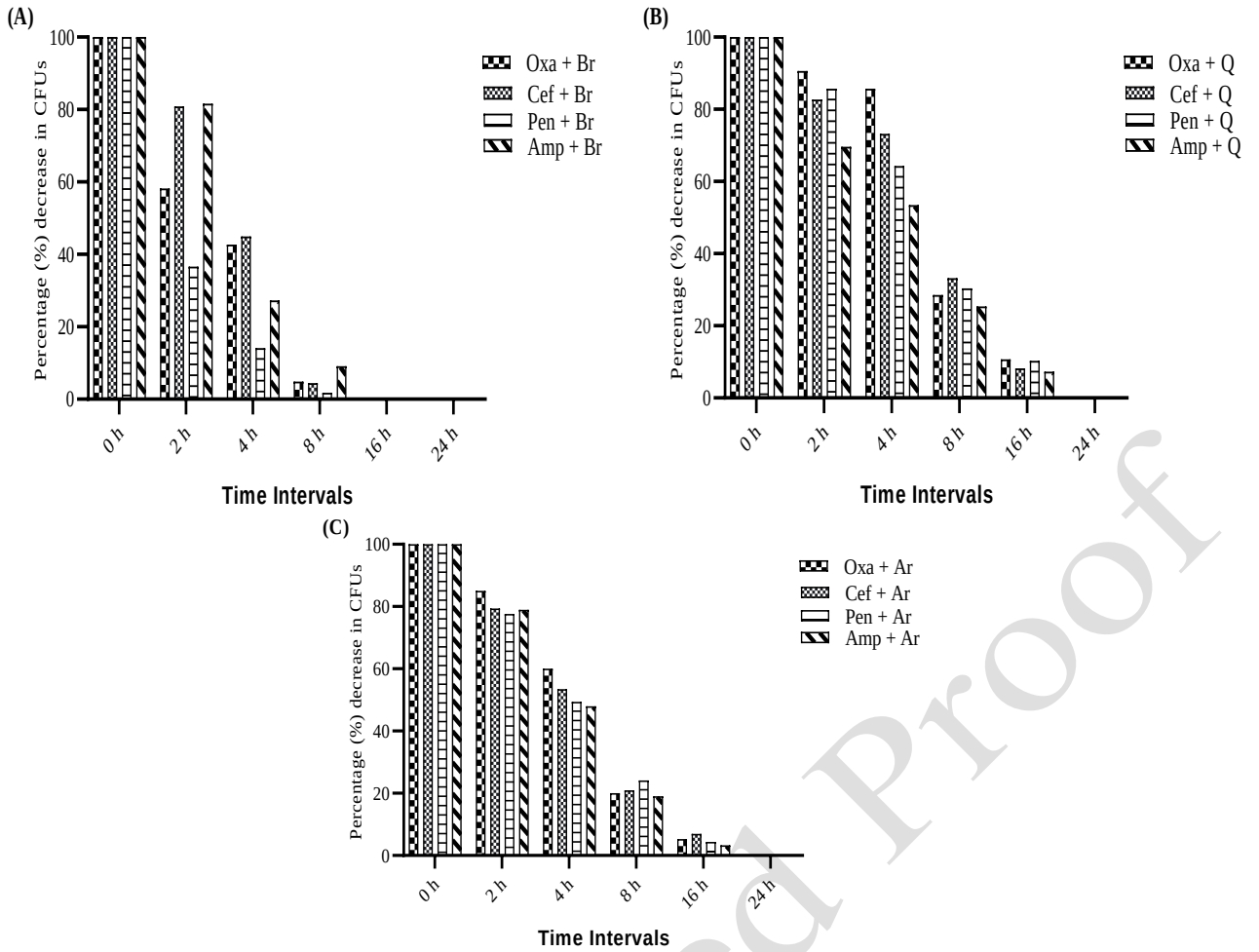


Fig. 3: Percent decrease in CFU/mL with respect to incubation time (A) Berberine chloride (BR) combinations, (B), Quercetin (QT) combinations, (C) Artesunate (AR) combinations,

The antibacterial response of BR in this study among different strains of MRSA is in line with the findings of Wu *et al*. (2022), who found its MIC to be 51 µg/mL indicating that BR was involved in inhibiting cell wall biosynthesis, inducing oxidative damage, reducing stress resistance, and inhibiting the synthesis of aromatic amino acids. Additionally, BR with the MIC range of 78 µg/mL, was found to damage the structure of the bacterial cell membrane and inhibit protein and DNA synthesis, as observed in studies against *Streptococcus agalactiae* by Peng *et al*. (2015). QT exhibited a gradual decline in MIC, contrasting with the findings of Adeyemi *et al*. (2020) who suggested that QT restricts bacterial growth through lipid peroxidation and kynurenine pathway activation. Researchers (Nguyen and Bhattacharya, 2022) mentioned a contradicting MIC of QT with our studies against *S. aureus* to be 20 µg/mL with proposed antimicrobial mechanisms including cell membrane damage, alteration of membrane permeability, inhibition of nucleic acid and protein synthesis, reduced expression of virulence factors, mitochondrial dysfunction, and prevention of biofilm formation., Further, our results are aligned with the studies conducted by other researchers (Pal and Tripathi, 2020) who observed disruption of cell wall and membrane integrity in various bacteria treated with quercetin showing MIC of 128 µg/mL. AR stood last in the list with the highest MIC among the phytochemicals, contradicting with the findings of Li *et al*. (2011) and Wei *et al*. (2020) who demonstrated its MIC to be 8000 µg/mL and 512 µg/mL with a proposed the antibacterial mechanism of AR through the inhibition of multidrug efflux pump systems Acr-AB-TolC.

The synergy analysis of BR exhibited significant synergistic interactions with oxacillin and penicillin against MRSA leading to a substantial reduction in microbial growth which was corroborated by several studies, including those by Yu *et al*. (2005) highlighting its effectiveness against different bacterial strains. Similarly, QT displayed complete synergism with various antibiotics against MRSA and other strains, supported by other studies (An *et al*., 2011; Siriwong *et al*., 2015; Pal and Tripathi, 2020). However, some studies, like Su *et al*. (2014), found additive interactions of QT with different antibiotics against MRSA. AR exhibited an additive effect with penicillin but showed synergistic interactions with other antibiotics, consistent with findings from Wei *et al*. (2020), Le-Tien *et al*. (2023), Jiang *et al*. (2013), and Haq *et al*. (2022). These results underscore the potential of BR, QT, and AR combinations against a range of bacterial strains, providing valuable insights for effective antibiotic therapy.

The time-kill kinetics study offered additional support for the synergistic impact of BR, QT, and AR when combined with the tested antibiotics. There was a notable reduction in CFU within 4 hours of incubation in the case

of BR, consistent with the observations made by Dash *et al*. (2020). Moreover, the studies conducted by Wojtyczka *et al*. (2014) documented a complete inhibition of bacterial growth within 24 hours aligning with our findings. The delayed and strong bactericidal effect of QT when combined with antibiotics is reinforced by the research findings of Odabaş *et al*. (2023). Conversely, AR exhibited a smooth reduction in CFU with all antibiotics over the 24h incubation period, with no growth observed after 24h, a trend also observed in the studies by Jiang *et al*. (2011).

Conclusion: The study found higher prevalence of MRSA from dairy animals with severe concern of emerging resistance against antibiotics. They phytochemicals, berberine (BR), quercetin (QT), and artesunate (AR), on the other demonstrated significant antibacterial activity against MRSA in that sense, the former showed highest efficacy followed by QT and AR. The interaction of these phytochemicals with selected beta-lactam antibiotics (penicillin, ampicillin, oxacillin, and cefoxitin) was all synergistic except that of the penicillin with artesunate, which was additive. The salient effective combinations of antibiotics with phytochemicals included AR and QT with ampicillin, BR with oxacillin and penicillin. The combination of phytochemicals with antibiotics showed a significant reduction in minimum inhibitory concentration at early stages of incubation indicating effective therapeutic regimen available to cover sudden infections. The study thus concludes greater room available for combination therapy consisting of phytochemicals and antibiotics to tackle emerging drug resistance and demands further studies with immediate focus on exploration of molecular mechanisms and field trials to formulate products ready to use against infections.

Author's contribution: Li Jianxi, Saad Ahmad and Amjad Islam Aqib conceived and designed the study. Saad Ahmad and Muzaffar Ghafoor executed the experiments. Muhammad Shoaib, Amjad Islam Aqib, Shahbaz ul Haq and Farid S Ataya analyzed the data and revised manuscript. All authors interpreted the data, critically revised the manuscript for important intellectual contents and approved the final version.

Acknowledgment: The authors extend their gratitude to Dr. Muhammad Saqib, Chairman, Department of Clinical Medicine and Surgery, University of Agriculture, Faisalabad, Pakistan for allowing the use of laboratory facilities at Laboratory of Veterinary Preventive Medicine and Public Health. We acknowledge role of China Agriculture Research System (CARS-36-04), Tongliao Science and Technology Project (TLCXYD202102-02), the Science and Technology Innovation Project of CAAS Collaborative Innovation (CAAS-XTCX2016011-01-09) and the Innovation Project of Traditional Chinese Veterinary Medicine and Clinical Science (CAAS-ASTIP-2015-LIHPS) for this study. The authors also extend their appreciation to researchers supporting Project number (RSPD2024R693), King Saud University, Riyadh, Saudi Arabia.

Declaration of Competing Interest: The authors declare that they have no competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability: All data supporting the conclusions of this article are included in the article.

Ethics approval and consent to participate: Not applicable.

Consent for publication: Not applicable.

REFERENCES

Adeyemi OS, Ebugosi C, Akpor OB, *et al*., 2020. Quercetin caused redox homeostasis imbalance and activated the kynurenine pathway. Biology 9:219. https://doi.org/10.3390/biology9080219.

Alghamdi BA, Al-Johani I, Al-Shamrani JM, *et al*., 2023. Antimicrobial resistance in methicillin-resistant *Staphylococcus aureus*. Saudi J Biol Sci 30:103604. doi: 10.1016/j.sjbs.2023.103604

Alshareef FJASM, 2021. Protocol to evaluate antibacterial activity MIC, FIC and time kill method. Acta Sci Microbiol 4:2581-3226.

An J, Zuo GY, Hao XY, *et al*., 2011. Antibacterial and synergy of a flavanonol rhamnoside with antibiotics against clinical isolates of methicillin-resistant *Staphylococcus aureus* (MRSA). Phytomedicine 18:990-993. https://doi.org/10.1016/j.phymed.2011.02.013.

Aqib AI, Ijaz M, Anjum AA, *et al*., 2017. Antibiotic susceptibilities and prevalence of methicillin resistant *Staphylococcus aureus* (MRSA) isolated from bovine milk in Pakistan. Acta Trop 176:168-172.

Dash S, Kumar M and Pareek N, 2020. Enhanced antibacterial potential of berberine via synergism with chitosan nanoparticles. Mater Today Proc 31:640-645.

Dziedzic A, Wojtyczka RD and Kubina R, 2015. Inhibition of oral *Streptococci* growth induced by the complementary action of berberine chloride and antibacterial compounds. Molecules 20:13705-13724.

Haq SU, Wang L, Guo W, *et al*., 2022. Enhancing activity of β-lactam and fluoroquinolones antibiotics by artemisinin and its derivatives against MDR *Escherichia coli*. Front Vet Sci 9:1048531. https://doi.org/10.3389/fvets.2022.1048531

Jiang W, Li B, Zheng X, *et al*., 2011. Artesunate in combination with oxacillin protect sepsis model mice challenged with lethal live methicillin-resistant *Staphylococcus aureus* (MRSA) via its inhibition on proinflammatory cytokines release and enhancement on antibacterial activity of oxacillin. Int Immunopharmacol 11:1065-1073.

Jiang W, Li B, Zheng X, *et al*., 2013. Artesunate has its enhancement on antibacterial activity of β-lactams via increasing the antibiotic accumulation within methicillin-resistant *Staphylococcus aureus* (MRSA). J Antibiot 66:339-345.

Le-Tien C, Blemur L and Baltzis D, 2023. Artesunate Dry Emulsion Formulation Combined with Antibiotics for Treatment of *Helicobacter pylori* Infections: In Vitro/In Vivo Evaluation. Int J Mol Sci 24:11008.

Li B, Yao Q, Pan XC, *et al*., 2011. Artesunate enhances the antibacterial effect of β-lactam antibiotics against *Escherichia coli* by increasing antibiotic accumulation via inhibition of the multidrug efflux pump system AcrAB-TolC. J Antimicrob Chemother 66:769-777.

Li J, Hou X, Xiao J, *et al*., 2024. Synthesis of new derivatives of berberine canagliflozin and study of their antibacterial activity and mechanism. Molecules 29:273 https://doi.org/10.3390/molecules29010273

Lodhi FL, Saleem MI, Aqib AI, *et al*., 2021. Bringing resistance modulation to epidemic methicillin resistant *S. aureus* of dairy through antibiotics coupled metallic oxide nanoparticles. Microb Pathog 159:105138. doi:10.1016/j.micpath.2021.105138

Muhammad G, Naureen A, Asi MN, *et al*., 2010. Evaluation of a 3% surf solution (surf field mastitis test) for the diagnosis of subclinical bovine and bubaline mastitis. Trop Anim Health Prod 42:457-464.

Nguyen TLA and Bhattacharya D, 2022. Antimicrobial activity of quercetin: an approach to its mechanistic principle. Molecules 27:2494. doi:10.3390/molecules27082494

Odabaş KE, Koyuncu ÖÖ, Bilmen S, *et al*., 2023. Quercetin: synergistic interaction with antibiotics against colistin-resistant *Acinetobacter baumannii*. Antibiotics 12:739. doi:10.3390/antibiotics12040739.

Pal A and Tripathi A, 2020. Demonstration of bactericidal and synergistic activity of quercetin with meropenem among pathogenic

carbapenem resistant *Escherichia coli* and *Klebsiella pneumoniae*. Microb Pathog 143:104120. doi:10.1016/j.micpath.2020.104120.

Peng L, Kang S, Yin Z, *et al*., 2015. Antibacterial activity and mechanism of berberine against *Streptococcus agalactiae*. Int J Clin Exp Pathol 8:5217-5223.

Pinheiro REE, Chaves TP, Melo ES, *et al*., 2020. Modulatory-antibiotic activity of the essential oil from *Eucalyptus citriodora* against MDR bacterial strains. Cell Mol Biol 66:60-64.

Qian Y, Xia L, Wei L, *et al*., 2021. Artesunate inhibits *Staphylococcus aureus* biofilm formation by reducing alpha-toxin synthesis. Arch Microbiol 203:707-717.

Roy MC, Chowdhury T, Hossain MT, *et al*., 2024. Zoonotic linkage and environmental contamination of Methicillin-resistant *Staphylococcus aureus* (MRSA) in dairy farms: Int J One Health 18:100680. https://doi.org/10.1016/j.onehlt.2024.100680.

Sahebnasagh R, Saderi H and Owlia P, 2014. The prevalence of resistance to methicillin in *Staphylococcus aureus* strains isolated from patients by PCR method for detection of mecA and nuc Genes. Iran J Public Health 43:84-92.

Shoaib M, Aqib AI, Muzammil I, *et al*., 2023. MRSA compendium of epidemiology, transmission, pathophysiology, treatment, and prevention within one health framework. Front Microbiol 13:1067284. doi:10.3389/fmicb.2022.1067284

Siriwong S, Thumanu K, Hengpratom T, *et al*., 2015. Synergy and mode of action of ceftazidime plus quercetin or luteolin on *Streptococcus pyogenes*. Evid Based Complement Alternat Med 2015:759459. doi:10.1155/2015/759459.

Su Y, Ma L, Wen Y, *et al*., 2014. Studies of the in vitro antibacterial activities of several polyphenols against clinical isolates of methicillin-resistant *Staphylococcus aureus*. Molecules 19:12630-12639.

Trifan A, Zengin G, Sinan KI, *et al*., 2022. Unveiling the phytochemical profile and biological potential of five artemisia species. Antioxidants 11:1017. doi:10.3390/antiox11051017

Wang D, Yu L, Xiang H, *et al*., 2008. Global transcriptional profiles of *Staphylococcus aureus* treated with berberine chloride. FEMS Microbiol Lett 279:217-225.

Wei S, Yang Y, Tian W, *et al*., 2020. Synergistic activity of fluoroquinolones combining with artesunate against multidrug-resistant *Escherichia coli*. Microb Drug Resist 26:81-88.

Wojtyczka RD, Dziedzic A, Kępa M, *et al*., 2014. Berberine enhances the antibacterial activity of selected antibiotics against coagulase-negative *Staphylococcus* strains in vitro. Molecules 19:6583-6596.

Wu S, Yang K, Hong Y, *et al*., 2022. A new perspective on the antimicrobial mechanism of berberine hydrochloride against *Staphylococcus aureus* revealed by untargeted metabolomic studies. Front Microbiol 13:917414. doi:10.3389/fmicb.2022.917414.

Yang D, Wang T, Long M, *et al*., 2020. Quercetin: its main pharmacological activity and potential application in clinical medicine. Oxid Med Cell Longev 2020: 8825387. doi:10.1155/2020/8825387

Yu HH, Kim KJ, Cha JD, *et al*., 2005. Antimicrobial activity of berberine alone and in combination with ampicillin or oxacillin against methicillin-resistant *Staphylococcus aureus*. J Med Food 8:454-461.