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IN-VITRO AND *IN-VIVO* SYNERGISM BETWEEN VALSPODAR (PSC833) AND ANTIBIOTICS AGAINST *STAPHYLOCOCCUS AUREUS* STRAINS

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ABSTRACT: Here, we report the *in-vitro* and *in-vivo* effects of Valspodar (PSC833), a second-generation mammalian efflux pump inhibitor on the susceptibility of methicillin-resistant and methicillin-sensitive *Staphylococcus aureus* (MRSA and MSSA) towards two antibiotics, Oxacillin (OX) and Cefoxitin (FOX). Checkerboard microdilution assays revealed various degrees of strain-dependent synergy demonstrated in the form of major reductions in antibiotic Minimum Inhibitory Concentrations (MIC) and Valspodar Minimum Effective Concentration (MEC). Fractional Inhibitory Concentration (FIC) for OX-PSC833 tested against MRSA and MSSA were 0.125 and 0.048, respectively, and for FOX-PSC833 was 0.5 for the MSSA. *In-vivo* studies showed that using a combination of an antibiotic and Valspodar to treat bacteremia induced by the different strains of S. aureus in a mouse model did not achieve complete killing of the MRSA strains, but resulted in a significant reduction in bacterial counts. MEC of PSC833 achieving synergy with antibiotics was as low as 5.2 µg/ml. Valspodar is a good candidate for antibiotic combination therapy at concentrations that are considered safe for human application.

INTRODUCTION: As the threat of antibiotic resistance is growing, pharmaceutical investment in antibiotic research and development must be revived. An approach to the problem would be modifying existing antibiotics by combining a resistance mechanism inhibitor to a conventional antibiotic. Among the most common modalities of resistance in bacteria are direct antibiotic inactivation and reduction of intracellular drug concentrations by either decreasing cellular permeability or increasing the activities of a variety of efflux pumps [1].

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Hence, combining a pre-existing antibiotic with another drug that acts as an efflux pump inhibitor (EPI), a membrane permeabilizer [2, 3], or an inhibitor of antibiotic inactivating enzymes such as β-lactamase is expected to restore and potentiate its therapeutic activity.

Inhibition of efflux pumps is a promising strategy to restore the antimicrobial action of effective antibiotics deemed obsolete. The general concept of efflux pump inhibition in bacterial cells depends on the prevention of the energy-dependent extrusion of antimicrobials. Therefore, an efficient efflux pump inhibitor will recover the antimicrobial activity by lowering the minimal inhibitory concentration (MIC), which will lead to the utilization of lower concentrations of the antimicrobial agent [4]. Although targeting efflux pumps in bacteria is one of the main approaches to the restoration of antibiotic activity, most efflux

pump inhibitors are not used clinically, because the effective concentration required to modulate active transport in bacteria is significantly higher than the associated human toxicity levels [3]. The main causes of EPI toxicity are the competitive inhibition of cytochrome P-450 enzymes, increased plasma concentrations of the co-administered drug, and the inhibitory effect on human efflux pumps [5].

This is evident with many natural plant-derived alkaloids, such as Reserpine, Piperine, Capsaicin, and recently Boeravinone B, that have been evaluated and proven to have inhibitory effects on bacterial efflux pumps. Nonetheless, they have also been shown to have various degrees of toxicity, hindering their clinical application [3, 6].

Staphylococcus aureus is a Gram-positive bacterium that is highly abundant as part of the normal human microflora in the respiratory tract, skin, oral cavity, and the gastrointestinal tract. *S. aureus* is also an opportunistic pathogen and is encountered in some serious diseases.

For example, it may cause bacteremia and sepsis when spread directly through the bloodstream as well as pneumonia, endocarditis, and osteomyelitis. Methicillin-resistant *Staphylococcus aureus* (MRSA) is defined as any strain of *S. aureus* that carries an acquired genetic determinant (mecA or mecC), which encodes for lower affinity penicillin-binding protein (PBP-2a) [7]. Over-expressed efflux pumps confer the multidrug-resistant (MDR) phenotype on this bacterium [7].

Valspodar (PSC833) a second-generation mammalian P-glycoprotein efflux pump inhibitor that has less toxicity and higher potency when compared to the first generation P-glycoprotein inhibitors [5]. An earlier study performed by this group showed that an antibiotic dependent concentration of up to 62.5 µg/ml of Valspodar demonstrated a potentiating effect on several antibiotics against an MRSA strain [8]. Valspodar is a substrate for cytochrome p450-3a4 metabolism [9]; lower concentrations are always sought to reduce any expected side effects [10].

This study aims to identify the potentiating effect of lower concentrations of Valspodar when combined with various antibiotics on MRSA and MSSA *in-vitro* and *in-vivo*.

MATERIALS AND METHODS:

Bacterial Strains: Bacterial strains used were *S. aureus* ATCC6538 from the laboratory stocks of the school of Pharmacy, University of Petra), and two randomly selected clinical MRSA isolates from the Jordan University Hospital. All strains were maintained by sub-culturing on freshly prepared Trypticase Soy Agar (TSA; HI Media, Mumbai, India). Backup cultures of the bacterial strains were prepared on TSA slant. All bacterial strains were preserved in 15% glycerol (Gainland Chemical Co., Deeside, UK) and brain heart infusion broth at -20ºC. All strains were cultured on Mannitol Salt Agar (MSA) (Oxoid, Basingstoke, UK) for identification and on OX-MSA to verify resistance [11]. *S. aureus* ATCC6538 showed no growth on OX-MSA agar, and the two strains of MRSA showed variable patterns of growth hence designated MRSA1 and MRSA2.

Antibiotics and Chemicals: Oxacillin sodium salt 95% were purchased from Sigma Pharmaceuticals, LLC. (Melbourne, Australia). Cefoxitin sodium salt of 940 µg/mg was obtained from Fluka Analytical / Sigma Aldrich (Buchs, Switzerland). Valspodar PSC833 vials of 1 ml/mg were purchased from Tocris Bioscience (Bristol, UK).

Determination of Minimal Inhibitory Concentration: The minimal inhibitory concentrations (MIC) of all antibiotics used were determined using the microdilution assay [12]. Briefly, 50µl of sterile Muller Hinton Broth (MHB; HiMedia Mumbai, India) was placed aseptically in all the wells of a sterile 96 well microtiter plate. 50 µl aliquots from stock solutions of antibiotics (16 µg/ml) were added to wells A1 to H1. Serial two-fold dilutions of the antibiotic were performed, followed by the addition of 10µl of 10^5 CFU/ml bacterial suspension to the aforementioned wells. Plates were incubated at 37 ºC for 24 h, and their optical density (OD) was measured at 600 nm using UV spectrophotometer (GloMax®-Multi Detection System (Promega, USA).

Antimicrobial Activity of PSC833: Valspodar was serially diluted using MHB to yield concentrations ranging from 62 to 0.06 µg/ml in a 96 microtiter plate. An aliquot (10µl) of 10^5 CFU/ml of bacterial strain was added to all wells except the sterility control wells. Plates were

incubated at 37 ºC for 18 to 24 h, and their optical density was measured at 600 nm using a UV spectrophotometer.

Checkerboard Titration Assay: Interaction between antibiotics (OX, FOX) and Valspodar was assessed by checkerboard titration assay. Antibiotics were tested in eight final concentrations ranging from 8 to 0.125 µg/ml, while Valspodar was tested in eleven final concentrations ranging from 62 to 0.06 µg/ml. PSC833 was prepared at a stock concentration equal to four-fold desired final concentration (248 µg/ml). Aliquots (50 µl) of MHB were added to all wells (A1-H11), and 50 µl of PSC833 were dispensed in the 11th wells (A11-H11) and serially two-fold diluted towards the 1st well; Stock solutions of antibiotics (64 µg/ml) were two-fold serially diluted with sterile distilled water in separate sterile test tubes. A 50 µl of each antibiotic concentration was dispensed in the designated well. Finally, 10 µl of the inoculum adjusted bacterial strain was added to all wells. Plates were incubated for 24 h at 37 °C. All plates were read at OD of 600 nm.

Minimum effective concentration (MEC) of PSC833 was taken as the lowest concentration of PSC833 that, in combination with antibiotics, resulted in a clear suspension that gave reading ≤ the reading of the sterility control [1]. Valspodar had now antimicrobial activity; hence, an arbitrary value for Valspodar MEC equal to >1024 (MICb alone) was used to calculate the FICI.

The FIC index was calculated for each drug in each combination using the following formula[13]:

$$FICI = FICa + FICb$$

FICI = (MICa combination / MICa alone) + (MICb combination / MICb alone).

Where, a stands for the antibiotic, b: stands for EPI Valspodar.

Murine Bacteremia Model: Non-pregnant female Balb/c mice, 5–7 weeks of age of average body weight of 20 ± 2 g were kept under controlled conditions of temperature (22-24 ºC), humidity (55-65%) and photoperiod cycles (12 h light/12 h dark).

Bacterial strains (*S. aureus* ATCC6538 (MSSA), MRSA1 and MRSA2) were plated on 5% citrated human blood (discarded blood from blood bank Amman-Jordan)- TSA and incubated for 24 hours at 37 ºC. Three isolated colonies were transferred to 1 ml Tryptic Soy Broth (TSB; HI Media, Mumbai, India) and incubated overnight for 16 to 20 hrs. Subsequently, serial 10-fold dilutions were performed in sterile phosphate buffer saline (PBS) followed by viable plate counting (VPC) on TSA-blood agar. The final concentration of bacterial suspension used was 2×10^7 CFU/ml[14].

In order to validate the bacteremia mouse model, Mice (a group of three) were challenged with a susceptible strain of *S. aureus* ATCC6538 (2×10^7 CFU/ml) by intraperitoneal injection, mice were tail bled at different time intervals (0, 3, 6 and 24 hours), and viable cell count was performed.

Bacterial Challenge: A total of 45 mice were used in each test trial. Mice were divided into three groups according to the bacterial strains, and each group was subdivided into another three subgroups according to the treatment plan as shown in Table 1, each group was composed of three mice. Three trials were performed. Mice were injected intraperitoneally with 500 µl of the chosen bacterial suspension.

TABLE 1: SUMMARY OF BACTERIAL CHALLENGE AND ANTIMICROBIAL TREATMENTS IN FEMALE BALB/c MICE

Mice group	Bacterial Challenge	Treatment
A	*S. aureus* ATCC6538	Saline
B	*S. aureus* ATCC6538	Antibiotic
C	*S. aureus* ATCC6538	Antibiotic-PSC833
D	MRSA1	Saline
E	MRSA1	Antibiotic
F	MRSA1	Antibiotic-PSC833
G	MRSA2	Saline
H	MRSA2	Antibiotic
I	MRSA2	Antibiotic-PSC833

Infections were induced in mice using PBS standardized bacterial broth suspension of 500 µl injected intraperitoneally. Saline injections were used as a negative control (placebo group). All treatments were injected intramuscularly. Each group is represented by three mice. Antibiotic stands for OX or FOX.

Treatments: Treatments were initiated three hours' post-infection on day one. Antibiotic doses were calculated according to mouse body weight and to the values obtained from the in vitro experiments. OX dose was 50 mg $kg^{-1,}$ and the FOX dose was 14.3 mg kg^{-1}. The safe dose of PSC833 was determined according to the American Society of Clinical Oncology, which equals 6.6 mg

$kg^{-1}day^{-1,}$ and the steady-state concentration (Cpss) achieved is 1 mg L^{-1} [15]. The maximum non-toxic concentration of PSC833 that was used in vivo for all treatments was 1mg L^{-1}. This concentration also represents an intermediate concentration between the highest and lowest MEC values of combined PSC833 obtained from in vitro studies. 100 µl of the saline, antibiotics, or antibiotics - PSC833 mixture was administered intramuscularly three times daily with eight hours' interval between doses for three days.

Day of the bacterial challenge was designated as day one of the experiment. Using heparinized capillaries, 10 µl of blood samples were collected after 24, 48, and 72 h of infection, and dispensed into sterile 1 ml Eppendorf tubes. Blood tubes were vortexed and serially diluted (1:10) in sterile PBS, and 30 µl were inoculated on 5% blood TSA plates in triplicates. CFUs were quantified following overnight incubation at 37 °C. The results presented are the mean of three readings.

All experiments were given ethical approval in accordance with the University of Petra Institutional Guidelines on Animal Use, which complies with the guidelines of the Federation of European Laboratory Animal Science Association (FELASA).

The study protocol was revised and approved by the Council of Research of the Faculty of Pharmacy and Medical Sciences (University of Petra, Amman, Jordan).

Statistical Analysis: One-way ANOVA test followed by Tukey's post-hoc test was used to compare more than two variables using SPSS statistical software (IBM, USP, version 21). Data of the animal model challenge are represented as mean ± standard error of the mean (SEM). P-value <0.05 was considered statistically significant.

RESULTS:

Effect of Antibiotic / Valspodar Combination *in-vitro*: The MIC for each antibiotic is presented in **Table 1**. Bacterial strains were classified as Susceptible (S), Intermediate (I), and Resistant (R) according to CLSI breakpoints [12]. Antibiotics/ PSC833 combination showed synergism that manifested in reductions in MIC of antibiotics and Minimal effective concentrations (MEC) values for PSC833. Strain dependent variation in synergism was also observed in **Table 2**. PSC833 alone had no inhibitory effect on the growth of all strains and in all concentrations used. Minimal effective concentration (MEC) of PSC833 in combination with antibiotics varied between strains, as seen in **Table 2**.

TABLE 2: MICs OF ANTIBIOTICS TOWARDS *S. AUREUS* STRAINS

Bacterial Strain/ Antibiotics	MIC µg/ml	
	FOX*	OX
CLSI breakpoints	≤ 4 and ≥ 8 µg/ml	≤ 2 and ≥ 4 µg/ml
S. aureus ATCC6538	1 (s)♠	0.125 (s)
MRSA1	>8 (r)	8 (r)
MRSA2	>8 (r)	>8 (r)

*OX – Oxacillin, FOX – Cefoxitin.
♠ (s) Susceptible, (r) Resistant.

TABLE 3: EFFECT OF PSC338 ON MIC OF DIFFERENT ANTIBIOTICS *IN-VITRO*

Antibiotics	Bacterial Strains	MIC (µg/ml)		MEC (µg/ml)	% Reduction of Antibiotic MIC	% Reduction of PSC833 MEC	FICI ∗
		Antibiotic	*Antibiotic*∞	*PSC833*Ʊ			
Oxacillin (OX)	*S.aureus*ATCC6538	0.125 (s)	0.006	0. 06	52	99.99	0.048
	MRSA 1	8 (r)	1	0.125	87.5	99.97	0.125
	MRSA 2	> 8(r)	NK	-	-	-	-♠
Cefoxitin (FOX)	*S.aureus*ATCC6538	1 (s)	0.5	15.5	50	75	0.5
	MRSA 1	> 8 (r)	NK	-	-	-	-
	MRSA 2	> 8 (r)	NK	-	-	-	-

∗ FICI ≤ 0.5 µg/ml synergistic, FIC > 0.5 µg/ml additive, FIC >1 to ≤4 no interaction, FIC >4antagonistic. PSC833 MEC was considered >1024. ♠- Could not be obtained due to the inability to achieve complete killingat the concentration used of the antibiotic. ∞- MIC of antibiotics when combined with PSC833. Ʊ- MEC of PSC833 when combined with an antibiotic. NK: Reduction in growth (only reduction in optical density readings compared to controls).

Effect of Antibiotic/Valspodar Combination *in-vivo*: Mice infected with *S. aureus* ATCC6538 (MSSA) failed to clear the systemic infection without antibiotic treatment (control group). When mice were treated with OX alone, complete clearance of the bacteria from the blood was detected on the fourth day of **Fig. 1**. However, complete clearance of bacteria from the blood was observed on the second day of the challenge after using the combination therapy of OX and PSC833. When treated with OX alone, the bacterial counts were reduced by 46 % (p = 0.023) on the third day and by 95.4 % on the fourth day in comparison to control **Fig. 1**. However, when treated with OX-PSC833 combination, the systemic bacterial counts of mice infected with MRSA1 on the third and fourth day significantly (p = 0.002) decreased by 79.6% and 99.4% respectively in comparison to control. Although the percentage reduction in bacterial counts in mice treated with OX- PSC833 was more than that seen in mice treated with OX alone, the combination therapy failed to achieve complete killing, as seen in **Fig. 1**. The use of OX-PSC833 combination towards an MRSA2 strain infection similarly showed 86.7 % and 97.7 % (P= 0.001) reduction in bacterial counts on days three and four respectively when compared to placebo as opposed to 75.5 % and 90.3 % (p =0.02) reduction in MRSA2 CFU/ml counts on days three and four of treatment with OX alone in comparison to placebo **Fig. 1**.

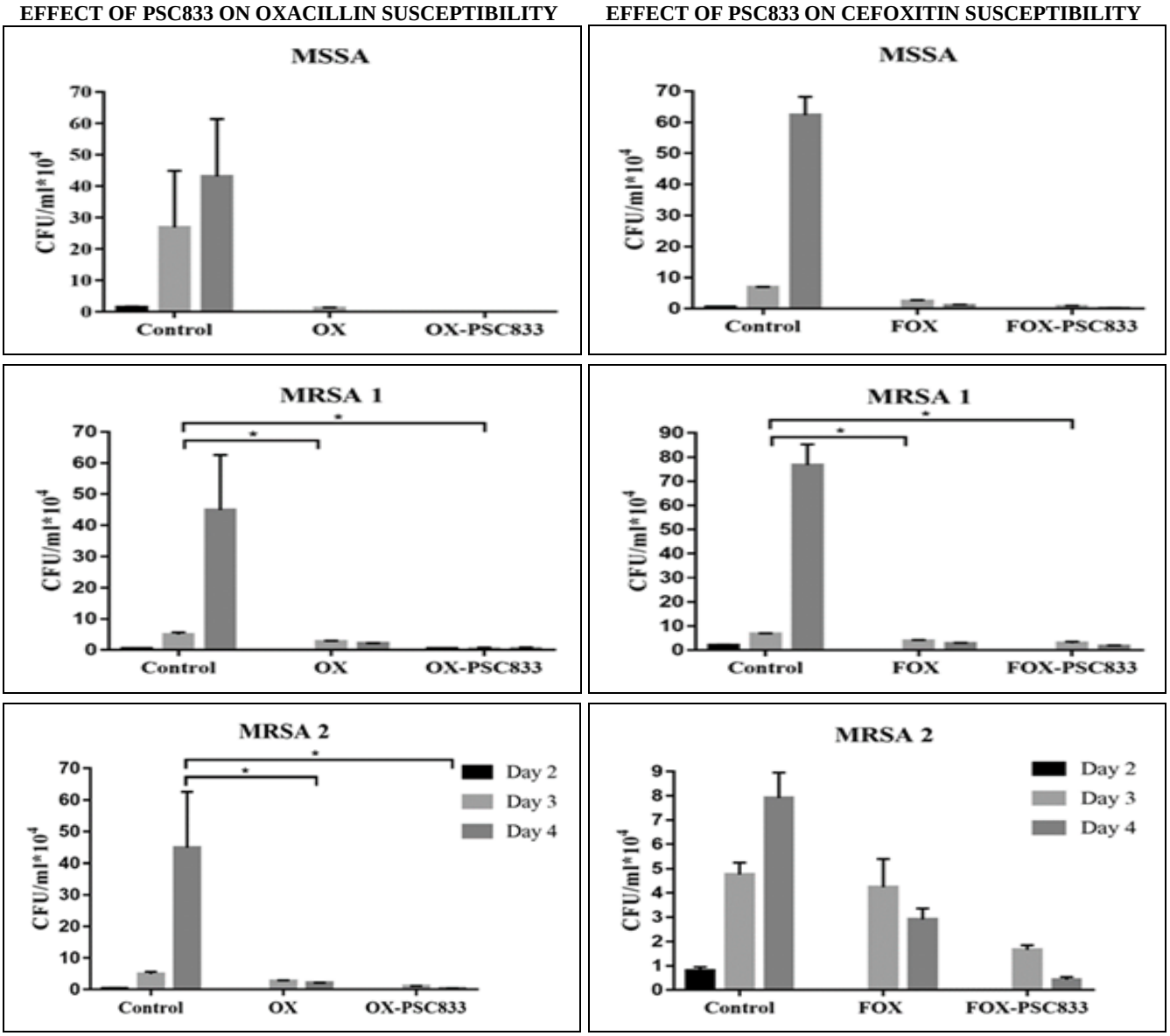

FIG. 1: BACTERIAL COUNTS IN BALB/C MICE TREATED WITH COMBINATION THERAPY. *P-value < 0.05.

Mice infected with *S. aureus* ATCC6538 and treated with FOX-PSC833 showed 97.4% and 99.8% reduction in bacterial counts CFU/ml on days three and four of a bacterial challenge compared to controls. However, mice treated with FOX alone achieved 91.4% and 98% reduction in bacterial counts when compared to controls; thus no complete clearance was achieved neither in combination therapy nor in FOX treatment alone **Fig. 1**.

Mice infected with MRSA1 and treated with FOX-PSC833 showed 65.2% and 98.5% reduction in bacterial $CFUml^{-1}$ on days three and four, respectively, when compared to placebo. However, FOX alone achieved a percentage reduction of 11% and 90% on days three and four of a challenge when compared to placebo **Fig. 1**. On the other hand, MRSA2-infected mice and treated with FOX-PSC833 combination showed 57.07% ($p = 0.002$) reduction of bacterial counts on the third day of treatment and 96.6% reduction in CFU/ml on the fourth day of challenge compared to controls. However, mice treated with FOX alone achieved 42.4% reduction in CFU/ml counts on the third day ($p = 0.01$), and 94.5% reduction in bacterial counts on the fourth day in comparison to placebo **Fig. 1**.

DISCUSSION: This study was conducted to detect an effective concentration of PSC833 that would synergistically improve the effectiveness of antibiotics on multi-drug resistant strains of *S. aureus*. Previously this group demonstrated the MEC for PSC833 to be $62.5\mu gml^{-1}$, or less, depending upon the type of antibiotic used. A clinical strain of MRSA obtained from KHCC in Amman Jordan was used in that study, and the MEC for Valspodar that resulted in 99% reduction in Oxacillin MIC was 62.5 μgml^{-18}. In fact, in the previous study, Valspodar at a concentration of 3.12 μgml^{-1} resulted in an increase in the zone of inhibition of Oxacillin from 6mm to 20 mm on the MRSA strain used and for cefoxitine from 10 to 29mm. In the current study, another two MRSA samples that were randomly chosen from another health facility (Jordan University Hospital) and 3 years after the performance of the original study, have exhibited an increased susceptibility to Oxacillin when exposed to Valspodar at lower concentrations. This shows the consistent effect of Valspodar in retrieving the antibiotic sensitivity of MRSA towards important antibiotics such as Oxacillin and cefoxitin. The mean MEC (calculated from the values obtained on the different strains used) of PSC833 was found to be 5.2 µg/ml ± 9. Reduction in MEC drives the combination of antibiotic/PSC833 away from the toxicity zone of PSC833. This is especially important as higher doses of PSC833 are known to be competitive inhibitors of cytochrome P450-3A4, which may result in unpredictable pharmacokinetic interactions that could put patients at risk due to the accumulation of the combined cytotoxic agents [10]. Both PSC833 MEC and antibiotic MIC values were variable between strains. MRSA1 had a MIC of 8 µg/ml towards oxacillin, while MRSA2 had a MIC of >8 µg/ml. Both values exceeded the previously reported MIC breakpoints towards this antibiotic, which is 4 µg/ml. This variation in MIC between *S. aureus* ATCC6538 and resistant strains likely contributed to the difference in MEC of PSC833 used in combination with antibiotics.

Although antibiotics / PSC833 combination had an evident effect on the reference strain and on MRSA1, an intermediate effect was observed on MRSA2. *S. aureus* has the ability to rapidly develop resistance to new antibiotics through various mechanisms [10]. Resistance mechanisms include hydrolysis of the β-lactam ring by β-lactamases and modification of the PBP target, which decreases its affinity for antibiotics [7]. The selection of antibiotics used in this study was based on their β-lactamase resistance: OX and FOX are both resistant to β-lactamases hydrolysis [16]. Since ABC efflux pump inhibition by PSC833 was not able to restore the full activity of the antibiotics used on the MRSA strains, residual B-lactam resistance could be attributed to the alternative penicillin-binding protein (PBP 2a) which has low affinity for Oxacillin and most other β –lactams [7]. Combination therapy surpassed the effect of each antibiotic alone. This was evident in the significant reduction in MRSA2 bacterial counts as a result of combination therapy and in the retrieval of MRSA1 sensitivity to Oxacillin. However, retrieval of susceptibility was not achievable for MRSA2 *in-vivo*.

This study confirmed that PSC833 synergistically improved the action of β-lactamase resistant

antibiotics on antibiotic sensitive strains and recovered the activity of OX on MRSA as indicated by a synergistic FIC value. Additionally, PSC833 use improved the susceptibility of MRSA for FOX, although FIC values could not be calculated. The synergy between PSC833 and antibiotics in this study was seen at a low MEC of PSC833. The MEC of PSC833 was directly proportional to MIC levels in resistant bacterial strains. That is, the more resistant the strain is, the more EPI concentration is required. It is important to note that the variability in efflux pump relative expression levels between strains likely contributed to a difference in MEC of the efflux inhibitor [17].

Despite the fact that no complete killing was achieved *in-vitro*, *in-vivo* trials against resistant strains of bacteria in Balb/c murine model indicated high levels of antibiotic-EPI synergy on all bacterial strains. Reduction in MRSA strain counts suggests a relative improvement in susceptibility; however, no complete killing was achieved throughout the three days of treatment, proposing the possibility of better outcomes upon extending the duration of treatment. MRSA2 has changed from resistant (R), where "there is a high likelihood of therapeutic failure at that certain concentration of used antibiotic," to intermediate resistance (I) "inhibited in vitro by a concentration of the antibiotics that are associated with an uncertain therapeutic outcome" [18], through the four-days exposure to the antibiotics and that was evident from the reduction of microbial counts in vivo.

The *in-vitro* and *in-vivo* data are consistent with the results of another study that confirmed the role of PSC833 in augmenting and improving antibiotic action and proved that it could be used at very low, clinically safe concentrations without reducing its effectiveness [8].

CONCLUSION: In conclusion, a combination of antibiotics with a resistance mechanism inhibitor continues to be a viable approach for improving therapeutic options against MDR bacteria. The involvement of variable mechanisms of resistance in bacterial strains could necessitate the use of multiple resistance mechanism inhibitors. We have shown here that PSC833, an efflux pump inhibitor, augments antibiotic action against MRSA *in-vitro* and *in-vivo*. Further work needs to be done to determine the efficacy of this approach in other bacterial strains and different infection models *in-vivo*.

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REFERENCES:

1. Mullin S, Mani N and Grossman TH: Inhibition of antibiotic efflux in bacteria by the novel multidrug resistance inhibitors biricodar (VX-710) and timcodar (VX-853). Antimicrob Agents and Chemother 2004; 48: 4171-76.
2. Douafer H, Andrieu V, Phanstiel IVO and Brunel JM: Antibiotic adjuvants: make antibiotics great again!. Journal of Medicinal Chemistry 2019; 62: 8665-81.
3. Ahmad I, Qais FA, Maheshwari M and Rumbaugh KP: Efflux Pump Inhibitors and Their Role in the Reversal of Drug Resistance. In Antibacterial Drug Discovery to Combat MDR. Springer, Singapore 2019; 251-75.
4. Abdel-Halim H, Abdelhalim A and Abdelmalek S: The search of potential inhibitors of the AcrAB–TolC system of multidrug-resistant Escherichia coli: an in silico approach. Applied Microbiology and Biotechnology 2019; 103: 6309-18.
5. Ughachukwu PO and Unekwe PC: Efflux Pump Mediated Resistance in Chemotherapy. Annals of Medical and Health Sciences Research 2012; 2: 191-98.
6. Singh S, Kalia NP, Joshi P, Kumar A, Sharma PR, Kumar A, Bharate SB and Khan IA: Boeravinone B:Boeravinone B, A novel dual inhibitor of NorA bacterial efflux pump of *Staphylococcus aureus* and human P-glycoprotein, reduces the biofilm formation and intracellular invasion of bacteria. Frontiers in Microbiology 2017; 8: 1868.
7. Vestergaard M, Frees D and Ingmer H: Antibiotic resistance and the MRSA problem. Gram-Positive Pathogens 2019: 747-65.
8. Dheyab HH, Muhi-eldeen Z and Abdelmalek SM: The mammalian efflux pump inhibitor Valspodar (PSC833) improves susceptibility of MRSA to antibiotics. The International Arabic Journal of Antimicrobial Agents 2016; 6: 1-10.
9. Advani R, Lum BL, Fisher GA, Halsey J, Chin DL, Jacobs CD and Sikic BI: A phase I trial of liposomal doxorubicin, paclitaxel and Valspodar (PSC-833), an inhibitor of multidrug resistance. Annals of Oncology 2005; 16: 1968-73.
10. Wandel C, Kim RB, Kajiji S, Guengerich FP, Wilkinson GR and Wood AJ: P-glycoprotein and cytochrome P-450 3A inhibition: dissociation of inhibitory potencies. Cancer Research 1999; 59: 3944-48.
11. Kumurya AS: Use of Mannitol Salt Agar (MSA) and cefoxitin as a selective culture medium for growing MRSA strains 2017; 1-5.
12. Patel JB, editor. Performance standards for antimicrobial susceptibility testing. Clinical and Laboratory Standards Institute 2017.
13. Sopirala MM, Mangino JE, Gebreyes WA, Biller B, Bannerman T, Balada-Llasat JM and Pancholi P: Synergy

testing by Etest, microdilution checkerboard, and time-kill methods for pan-drug-resistant *Acinetobacter baumannii*. Antimicrobial Agents and Chemotheraby 2010; 54: 4678-83.

14. Schuch R, Lee HM, Schneider BC, Sauve KL, Law C, Khan BK and Fischetti VA: Combination therapy with lysin CF-301 and antibiotic is superior to antibiotic alone for treating methicillin-resistant *Staphylococcus aureus*–induced murine bacteremia. The Journal of Infectious Diseases 2013; 209: 1469-78.
15. Boote DJ, Dennis IF, Twentyman PR, Osborne RJ, Laburte C, Hensel S, Smyth JF, Brampton MH and Bleehen NM: Phase I study of etoposide with SDZ PSC833 as a modulator of multidrug resistance in patients with cancer. Journal Clinical Oncology 1996; 14: 610-18.
16. Katzung BG and Trevor AJ: Basic & clinical pharmacology, McGraw Hill Inc., 14th Edition 2015.
17. Jo A and Ahn J: Phenotypic and genotypic characterization of multiple antibiotic resistant Staphylococcus aureus exposed to subinhibitory levels of oxacillin and levofloxacin. BMC Microbiology 2016; 16: 170.
18. MacGowan AP and Wise R: Establishing MIC breakpoints and the interpretation of *in-vitro* susceptibility tests. Journal of Antimicrobial Chemotherapy 2001; 48: 17-28.

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