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SHORT COMMUNICATION

# Combination of thioridazine and dicloxacillin as a possible treatment strategy of staphylococci

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SUMMARY

We have previously shown that the phenothiazine, thioridazine, acts in synergy with the beta-lactam antibiotic, dicloxacillin, to kill methicillin-resistant *Staphylococcus aureus*. In this study, we investigated whether synergy by combining these two drugs could also be observed in vancomycin intermediate susceptible *S. aureus* (VISA) and methicillin-resistant *Staphylococcus epidermidis* (MRSE). Synergy was observed in three of four tested VISA strains, suggesting that the thickening of cell wall does not interfere with the effects of thioridazine. In *S. epidermidis*, no synergy was observed in all tested strains, suggesting that synergy by combining thioridazine and dicloxacillin is isolated to *S. aureus* species.

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Staphylococci are the causative agents of a wide range of nosocomial and community-acquired infections (Lowy, 1998). The emergence of antibiotic-resistant types, such as methicillin-resistant *S. aureus* (MRSA), methicillin-resistant *S. epidermidis* (MRSE), and vancomycin-intermediate resistant *S. aureus* (VISA) represents an increasing global challenge to health care, due to the limitation of effective drugs for treatment (Boyce *et al*., 2005; Fischbach and Walsh, 2009; Grundmann *et al*., 2006; Howden *et al*., 2010).

We have previously shown that the phenothiazine, thioridazine, acts in synergy with the beta-lactam antibiotic, dicloxacillin, to kill MRSA as well as methicillin-sensitive *S. aureus* (MSSA) (Klitgaard *et al*., 2008; Poulsen *et al.*, 2013). To increase our knowledge on the potential of the drug combination, we examined the synergistic effect of combining thioridazine and dicloxacillin in four VISA strains by performing viability assays as described previously (Poulsen *et al*., 2013). Furthermore, eight clinical MRSE isolates and one MSSE isolate (all from the Odense University Hospital, Denmark) were examined using viability assays or an oCelloScope (Fredborg *et al*., 2013) (Philips BioCell A/S, Denmark). The oCelloScope monitors bacterial growth in a microtiter plate by a light microscope and provides an easy means of performing a large screening assay over a specified time period. The used concentrations are sub-inhibitory in growth experiments performed in liquid culture with normal shaking at 37 degrees Celsius for both the viability assay (Brain Heart Infusion media, Oxoid) and the analyses performed by the oCelloScope (Müeller Hinton media, Merck Chemicals) and thus only moderately affect the growth of the bacteria. Synergy was defined according to the fractional inhibitory concentration index (FICI) where FICI<0.5 showed synergy, 0.5<FICI<4 showed indifference, and FICI>4 showed antagonism (Odds, 2003). FIC index is calculated as FICI = $FIC_A$ + $FIC_B$ = ($C_A$/$MIC_A$) + ($C_B$/$MIC_B$), where $MIC_A$ and $MIC_B$ are the MICs of drugs A and B alone, respectively, and $C_A$ and $C_B$ are the concentrations of the drugs in combination, respectively. All experiments were repeated at least twice with a variability of less than 5%.

A previous study on *S. aureus* showed that the individual isolates responded differently to the combined treatment (1.5-5.5 $\log_{10}$ units difference between dicloxacillin and drug combination) (Poulsen *et al.,* 2013). Importantly, all isolates showed an increased sensitivity to dicloxacillin in the presence of thioridazine compared to either drug alone. In that study 14 out of 20 MRSA isolates and three out of four MSSA isolates showed a synergistic effect accordingly to the definition set for synergy. When testing four VISA strains in the present study, the drug combination displayed synergy in three strains and indifference in a fourth strain, the hVISA (ATCC 700-698 also known as Mu3) (*Table 1*). The finding that the majority of the VISA strains displayed synergy for the combined treatment supports the findings from the study on MSSA and MRSA where 71% (17/24) of the strains displayed synergy as well (Poulsen *et al*., 2013).

VISA and hVISA strains are characterized by common biochemical and morphological changes, with cell wall thickening being a consistent feature of both clinical and laboratory-induced isolates. The thickened cell wall of VISA and hVISA results in an increased vancomycin binding capacity of these strains, which is thought to delay the access of vancomycin to its active site in the cytoplasmic membrane of the bacterial division septum (Cui *et al*., 2000). Recent studies on the resistance mechanism of VISA strains suggest a predominance of

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**Table 1 -** *Strains used in the study and overview of MIC (mg/L) and FIC index.*

| *Strain* | $MIC_{DCX}$ | $MIC_{TDZ}$ | $MIC_{DCX\ combi}$ | $MIC_{TDZ\ combi}$ | $FIC_{index}$ | *Synergy*[a)] |
|---|---|---|---|---|---|---|
| *VISA* | | | | | | |
| 1458 | 2048 | 64 | 32 | 16 | 0.27 | S |
| 3727 | >2048 | 32 | 2048 | 20 | <1.31 | S[b)] |
| 40638 | 2048 | 64 | 32 | 20 | 0.33 | S |
| ATCC700-698 | 2048 | 64 | 1536 | 8 | 0.88 | I |
| *MRSE* | | | | | | |
| 7272 | 256 | 32 | 1.5 | 16 | 0.51 | I |
| 324308 | 256 | 32 | 4 | 16 | 0.52 | I |
| 334410 | 256 | 32 | 24 | 16 | 0.59 | I |
| MRSE A | 600 | 8 | 400 | 4 | 1.17 | I |
| MRSE B | 400 | 8 | 256 | 6 | 1.39 | I |
| MRSE C | 256 | 8 | 100 | 6 | 1.14 | I |
| MRSE D | 128 | 8 | 64 | 6 | 1.25 | I |
| MRSE E | 128 | 8 | 64 | 8 | 1.50 | I |
| *MSSE* | | | | | | |
| MSSE F | 2 | 8 | 1.5 | 4 | 1.25 | I |

DCX: dicloxacillin; TDZ: thioridazine; FIC: fractional inhibitory concentration.
[a)] FIC index shows synergy (S) at values <0.5, indifference (I) at values >0.5 <4, and antagonism at values >4.
[b)] The $MIC_{DCX}$ of VISA strain 3727 was not technically feasible due to extreme high MIC. FIC index is calculated with a $MIC_{DCX}$ of 2048 mg/L. The strain does however show synergy when determining CFU/mL in viability assay.

amino acid substitutions within regulatory loci, such as the cell wall related two-component system VraSR (Kato *et al*., 2010). It has been shown that thioridazine induces *vraSR* and target genes of VraSR indicating a cell wall damaging effect of thioridazine, supporting that thioridazine treatment can be efficient against VISA (Bonde *et al*., 2011). Our study demonstrates that thioridazine increases the susceptibility to dicloxacillin in VISA strains, despite the fact that VISA strains generally have thickening of the cell wall. In case of MSSE and MRSE, no synergy was observed in this study. According to the applied criteria, all strains showed indifference when treated with dicloxacillin and thioridazine in combination (*Table 1*). *S. aureus* and *S. epidermidis* differ slightly in the teichoic acid content of the cell wall. *S. aureus* normally contains a ribitol teichoic acid with *N*-acetylglucosamine residues, whereas *S. epidermidis* contains a glycerol teichoic acid with glucosyl residues (Parisi, 1985). The ionic charge imparted by teichoic acids through modulation of divalent cation binding to teichoic acids influences the stability and folding of proteins in the cell wall (Neuhaus and Baddiley, 2003). In addition, *S. epidermidis* has the capability to incorporate a large proportion of serine residues in the cross-linking of the peptidoglycan layer whereas *S. aureus* mainly incorporates glycine (Navarre and Schneewind, 1999; Schleifer and Kandler, 1972). In *S. aureus*, it is known that glycine is involved in the synergistic mechanism as glycine is depleted from the peptidoglycan in the presence of thioridazine (Thorsing *et al*., 2013).

In conclusion, a synergistic effect by combining thioridazine and dicloxacillin was observed in the majority of VISA strains indicating that the thickened cell wall of the VISA strains does not interfere with the effects of thioridazine. The combination of thioridazine and dicloxacillin did not affect the viability of *S. epidermidis* and the effect of the combined treatment seems isolated to the *S. aureus* species.

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## References

Bonde M., Hojland D.H., Kolmos H.J., Kallipolitis B.H., Klitgaard J.K. (2011). Thioridazine affects transcription of genes involved in cell wall biosynthesis in methicillin-resistant Staphylococcus aureus. *FEMS Microbiol Lett.* **318**, 168-176.

Boyce J.M., Cookson B., Christiansen K., Hori S., Vuopio-Varkila J., Kocagöz S., Öztop A.Y., Vandenbroucke-Grauls CMJE, Harbarth S, Pittet D. (2005). Meticillin-resistant Staphylococcus aureus. *The Lancet Infectious Diseases.* **5**, 653-663.

Cui L., Murakami H., Kuwahara-Arai K., Hanaki H., Hiramatsu K. (2000). Contribution of a Thickened Cell Wall and Its Glutamine Nonamidated Component to the Vancomycin Resistance Expressed by Staphylococcus aureus Mu50. *Antimicrob Agents Chemother.* **44**, 2276-2285.

Fischbach M.A., Walsh C.T. (2009). Antibiotics for emerging pathogens. *Science.* **325**, 1089-1093.

Fredborg M., Andersen K.R., Jorgensen E., Droce A., Olesen T., Jensen B.B., Rosenvinge F.S., Sondergaard T.E. (2013). Real-time optical antimicrobial susceptibility testing. *J Clin Microbiol.* **51**, 2047-2053.

Grundmann H., Aires-de-Sousa M., Boyce J., Tiemersma E. (2006). Emergence and resurgence of meticillin-resistant Staphylococcus aureus as a public-health threat. *The Lancet.* **368**, 874-885.

Howden B.P., Davies JK, Johnson PD, Stinear TP, Grayson ML. (2010). Reduced vancomycin susceptibility in Staphylococcus aureus, including vancomycin-intermediate and heterogeneous vancomycin-intermediate strains: resistance mechanisms, laboratory detection, and clinical implications. *Clin Microbiol Rev.* **23**, 99-139.

Kato Y., Suzuki T., Ida T., Maebashi K. (2010). Genetic changes associated with glycopeptide resistance in Staphylococcus aureus: predominance of amino acid substitutions in YvqF/VraSR. *J Antimicrob Chemother.* **65**, 37-45.

Klitgaard J.K., Skov M.N., Kallipolitis B.H., Kolmos H.J. (2008). Reversal of methicillin resistance in Staphylococcus aureus by thioridazine. *J Antimicrob Chemother.* **62**, 1215-1221.

Lowy F.D. (1998). Staphylococcus aureus infections. *The New England Journal of Medicine.* 520-532.

Navarre W.W., Schneewind O. (1999). Surface proteins of gram-positive bacteria and mechanisms of their targeting to the cell wall envelope. *Microbiol Mol Biol Rev.* **63**, 174-229.

Neuhaus F.C., Baddiley J. (2003). A continuum of anionic charge: structures and functions of D-alanyl-teichoic acids in gram-positive bacteria. *Microbiol Mol Biol Rev.* **6**, 686-723.

Odds FC. (2003). Synergy, antagonism, and what the chequerboard puts between them. *Journal of Antimicrobial Chemotherapy.* **52**, 1.

Parisi J.T. (1985). Coagulase-negative staphylococci and the epidemiological typing of Staphylococcus epidermidis. *Microbiol Rev.* **49**, 126-139.

Poulsen M.O., Jacobsen K., Thorsing M., Kristensen N.R., Clasen J., Lillebaek E.M., Skov M.N., Kallipolitis B.H., Kolmos H.J., Klitgaard J.K. (2013). Thioridazine potentiates the effect of a beta-lactam antibiotic against Staphylococcus aureus independently of mecA expression. *Res Microbiol.* **164**, 181-188.

Schleifer K.H., Kandler O. (1972). Peptidoglycan types of bacterial cell walls and their taxonomic implications. *Bacteriol Rev.* **36**, 407-477.

Thorsing M., Klitgaard J.K., Atilano M.L., Skov M.N., Kolmos H.J., Filipe S.R., Kallipolitis B.H. (2013). Thioridazine induces major changes in global gene expression and cell wall composition in methicillin-resistant Staphylococcus aureus USA 300. *PLoS One.* 8, e64518.