A comparative analysis of In vitro and In vivo efficacies of the enantiomers of thioridazine and its racemate

Christensen, Jørn Bolstad; Hendricks, Oliver; Chaki, Shawasti; Mukherjee, Sayanti; Das, Ayan; Pal, Tapan K.; Dastidar, Sujata G.; Kristiansen, Jette E.

Published in:
P L o S One

DOI:
10.1371/journal.pone.0057493

Publication date:
2013

Document Version
Publisher's PDF, also known as Version of record

Citation for published version (APA):
Christensen, J. B., Hendricks, O., Chaki, S., Mukherjee, S., Das, A., Pal, T. K., ... Kristiansen, J. E. (2013). A comparative analysis of In vitro and In vivo efficacies of the enantiomers of thioridazine and its racemate. P L o S One, 8(3), [ e57493]. DOI: 10.1371/journal.pone.0057493
A comparative Analysis of In Vitro and In Vivo Efficacies of the Enantiomers of Thioridazine and Its Racemate

Jørn B. Christensen1, Oliver Hendricks2, Shaswati Chaki3, Sayanti Mukherjee3, Ayan Das4, Tapan K. Pal4, Sujata G. Dastidar3, Jette E. Kristiansen5*

1 Department of Chemistry, University of Copenhagen, Copenhagen, Denmark, 2 King Christian X Hospital for Rheumatic Diseases, University of Southern Denmark, Gråsten, Denmark, 3 Department of Microbiology, Herbicure Healthcare Bio-Herbal Research Foundation, Kolkata, India, 4 Bioequivalence Study Center, Department of Pharmaceutical Technology, Jadavpur University, Kolkata, India, 5 Memphys Center for Biomembrane Physics, Department of Physics and Chemistry Odense, Denmark

Abstract
A long list of chemotherapeutical drugs used in the treatment of the peripheral and the central nervous systems possess anti-microbial activity. Some of these neurotropic compounds are chiral, with the one stereo isomeric form exaggerating reduced neurotropism. This is the case for the levorotatory form of thioridazine. The phenothiazine thioridazine is an interesting compound, characterized by exhibiting a significant growth inhibiting activity on a wide array of microorganisms. Thioridazine is characterized by another challenging feature, because the compound is concentrated in certain human tissue cells. The present study describes a comparative study of the two enantiomers as well as the racemic form of thioridazine. The study exploits the stereochemical aspect and the in vitro and in vivo potential of these compounds, with a focus on the effects on Gram negative organism Salmonella enterica serovar Typhimurium. In summary, the results of this study yielded a significant antibacterial activity of all forms of thioridazine, indicating the levorotatory (−)-form to be superior in terms of both its in vitro and in vivo efficacies.

Introduction
An antibiotic may be defined as a product from a microorganism capable of inhibiting the growth of another microorganism at distinctly low levels. The chemotherapeutics, on the other hand, are primarily synthetic compounds that are able to act on microorganisms in a very similar manner, but at much higher concentrations. It is now known that both antibiotics and antibacterial chemotherapeutics have lost the battle to a large extent in the fight against multidrug resistant (MDR) bacterial pathogens. However, intensive studies by various groups of scientists throughout the world have revealed that there are medicinal compounds used for the therapy of non-infectious pathology possess distinct antimicrobial properties [1–13]. These compounds are termed as non-antibiotics [10]. Non-antibiotics exhibit properties that render them important for the therapy of various MDR infections. Phenothiazines being one of the most important group of non-antibiotics have been studied extensively for their antimicrobial potentiality [1–13,14]. These non-antibiotics possess most of the characteristics of antibiotics and their antibacterial action can be further potentiated by suitable combinations [15–18].

The phenothiazine thioridazine (Tz) is a unique non-antibiotic which is highly bactericidal for Gram positive bacteria and acts as a bacteriostatic agent against Gram negative organisms [19]. Thioridazine is chiral and previous studies have reported that the levorotatory form (−) thioridazine is concentrated in human tissue cells at higher levels than the dextrorotatory form (+) [20]. Furthermore the (−) form of Tz has been reported to have less challenging pharmacodynamics activity, e.g. reduced blocking activity on centrally located dopamine D2-receptors than the (+)-form [21]. Several in vitro studies have shown another feature of racemic Tz.
The compound is concentrated in human macrophages and different tissue types, such as pulmonary epithelial cells. Racemic Tz has a great potentiality for the therapy of MDR-tuberculosis since this compound is concentrated 100-fold in the human macrophages where the tubercle bacilli multiply and remain viable and where antibiotics fail to enter [22]. Furthermore racemic Tz has been shown to possess the capacity to lower the invasion ability of Gram positive and Gram negative bacteria in human epithelial cell lines [23]. Moreover racemic Tz proved to be highly efficient in disintegrating the invading cells of Salmonella enterica serovar Typhimurium in mice at rather low levels [21]. The present study aims to define the specific antibacterial properties of the enantiomeric forms of thioridazine, e.g. the racemic, the (−)- and the (−)- compounds and clarify, whether there is a difference in the efficacy of the drug based on its stereoisomeric profile. In order to achieve this goal, we performed comparative in vitro and in vivo studies with two enantiomers along with the racemic compound available commercially (Sigma Chemicals, Denmark).

Materials and Methods

Bacteria
A total of 55 different bacteria belonging to both Gram positive and Gram negative types were taken for this study (Table 1).
Table 1. Minimum inhibitory concentration (MIC) of three optical forms of Tz with respect to different bacteria.

| BACTERIA | MIC (µg/ml) |
|----------|-------------|
|          | Racemic (+) | (−) |
| S. aureus NCTC 6571, V. cholerae 569B, V. cholerae 1023 | 25 25 25 |
| S. aureus NCTC 8530, S. aureus ATCC 25923, S. dysenteriae 7 NCTC 519, Sh. boydii NCTC 254, V. cholerae ATCC 14033, ATCC 14033, V. cholerae DN7 | 50 100 50 |
| Sh. flexneri 4a NCTC 24, Sh. sonnei NCTC 9774, V.cholerae 713, 820 | 100 100 100 |
| S. aureus ML 16, ML 152, ML 329, ML 358, S. typhi NCTC 59, S. choleraesuis NCTC 36, 37, L. monocytogenes NCTC 7973, NCTC 10351, NCTC 11994 | 200 200 100 |
| B. subtilis ATCC 6633, B. pumilus NCTC 8241, S. aureus ML 266, ML 358, ML 422, E. coli K12 Row, E. coli C600, S. berta NCTC 69, S. abony NCTC 6017 | 200 200 100 |
| B. polymyxa NCTC 4747, B. licheniformis NCTC 10341, S. London NCTC 76, S. enterica serovar Typhimurium NCTC 11, NCTC 74 | 500 500 500 |
| S. aureus ML 277, V. cholerae 137/62 | 1000 1000 1000 |
| K. pneumoniae ATCC 10031, K. oxytoca ATCC 130988 | 2000 2000 2000 |
| L. monocytogenes AMRI 3, A. boumannii KPC 470, 517, P. aeruginosa ATCC 27853, ATCC 25619, C/1/5, K/8/89, BVC 1,2,3,4,5, APC1 | >2000 >2000 >2000 |

**Drugs**

Racemic thioridazine (Sigma Chemicals, Denmark). The two enantiomers of thioridazine were prepared according to the procedure of Bourquin et al [22].

**Media**

Liquid media were nutrient broth (NB; Oxoid), peptone water (PW) containing 1.0% peptone (Oxoid) and 0.5% NaCl and Mueller Hinton broth (MHb, Oxoid). Solid media were nutrient broth and blood agar.
agar (NA, Oxoid), brain heart infusion agar (BHA), Oxoid and Mueller Hinton agar (MHA), Oxoid); pH was always maintained at 7.2 to 7.4.

Inoculum

Each bacterium was grown in NA/MHA at 37°C, harvested at stationary phase and suspended in 5 ml sterile distilled water. Turbidity of each suspension was matched against 0.5 McFarland standard [14] along with a spectrophotometer at 625 nm corresponding to $2.4 \times 10^5$ colony forming unit (CFU)/ml.

Determination of Minimum Inhibitory Concentration (MIC) of the Enantiomers and the Racemate of Tz

This was carried out according to the guidelines of Clinical and Laboratory Standards Institute (CLSI) [24] by spotting 10^5 CFU contained in a 2 mm loop from diluted 18 h broth cultures on plates containing 0 (control), 50, 100, 200,500, 1000 and 2000 µg/ml of a drug. The plates were incubated at 37°C, observed for appearance of growth after 24 h and again after 72 h.

Spectrophotometry

A stock solution containing 1 mg/ml of thioridazine HCl (Sigma), racemic mixture of thioridazine HCl, dextrorotatory thioridazine HCl (+) and levorotatory thioridazine HCl (−) were prepared by dissolving these separately in methanol. Each stock solution was further diluted to 10µg/ml of methanol. Aliquots of this solution were taken in a quartz cell and scanned for $\lambda_{max}$ in the range of 200–600 nm using methanol as the blank in a double beam UV spectrophotometer (Jasco-V630). The maximum absorbance was determined by using Spectra manager (Version-2.05.03).

HPLC Parameter

- **Name of HPLC:** Jasco.
- **Column:** C8, 250×4.6 mm, 5 µ particle size.
- **Flow Rate:** 1.0 ml/min.
- **Loop size:** 50 µ l.
- **UV Absorption:** 264 nm.
- **Mobile Phase:** Methanol: Water containing 0.1% v/v phosphoric acid.
- **Run Time:** 8 mins.
- **Software used:** Clarity lite (Version: 2.6.4.402)

All the samples were prepared as per the above method and each sample was diluted by methanol (HPLC Grade). Water was used for the analysis was MilliQ water.

Animal Experiments

Swiss albino male mice each weighing 18–20 gm were selected for this work. This study was approved by the Institutional Animal Ethics Committee (IAEC) of Jadavpur University and TAAB Biostudy Services. Animals were maintained under standard conditions of temperature (24±1°C) and relative humidity (50–60%) with a photoperiod of 14:10 h of light:dark. Water and a dry pellet diet were provided ad libitum. The animals were checked regularly for their health and diet according to the rules and guidelines set by the Ethical Committee at definite intervals of time of 12 hr at 8 A.M. in the morning and 8 P.M. in the evening. The animals which showed symptoms of illness were carefully observed, identified and were separated from the healthy ones. These animals were not included in our experiments and were given proper treatment.

The intensity of virulence of infection caused by Salmonella enterica serovar Typhimurium 74 and the median lethal dose (MLD or LD_{50}) of the mouse-passaged strain was as described earlier.

Figure 2. The chromatogram showing that all four different samples had the same retention time (about 4.6 min).

doi:10.1371/journal.pone.0057493.g002
Protective efficacies of the forms of Tz in mice infected with virulent *S. enterica* were carried out as described below. Four groups of animals with 60 mice in the control group and 20 mice each in the 6 experimental groups were taken. The control group received 0.1 ml of sterile saline while for each form of Tz there were 40 animals, in which 20 mice received 100 µg and the other 20 received 200 µg of the drug. After 3 h all the animals were infected with 50 MLD of virulent *S. enterica* 74 as described [25]. Protective capacity of all 3 forms of Tz was determined by recording the mortality of mice in the different groups up to 100 h after the challenge. The animals which survived after 100 h of infection were euthanased with the help of cervical dislocation as suggested in the Ethical Committee. The end point for performing euthanasia was observation up to 100 h with regular monitoring at 12 h intervals. The animals which were euthanased prior to 100 h were based on the condition of their health. Generally within 72 h if they showed severe signs of illness, for example loss of appetite, weight loss, lack of movement, breathlessness, shivering etc. they were euthanased as advised by the veterinary doctor in the Ethical Committee. The number of animals euthanased prior to 100 h were based on the condition of their health. Generally within 72 h if they showed severe signs of illness, for example loss of appetite, weight loss, lack of movement, breathlessness, shivering etc. they were euthanased as advised by the veterinary doctor in the Ethical Committee. The number of animals euthanased prior to 100 h varied from 2–7 in each group. Assessment of the animals euthanased prior to 100 h was monitored by the veterinary doctor after every 12 h as mentioned 8 AM in the morning and 8 PM in the evening.

### Determination of the Effect of Treatment by 3 Forms of Tz on CFU of *S. enterica* 74 from the Liver, Spleen and Heart Blood of Infected Mice

In a separate experiment 4 groups of 5 mice each were given the following treatment: Group 1 was administered 0.1 ml saline while the other 3 groups received 200 µg each of racemic or (+) or (−) forms of Tz. After 3 h all the animals were challenged with 50 MLD of the same organism. After 18 h mice in each of the 4 groups were sacrificed by cervical dislocation (as recommended by the Ethical Committee) and their spleens and livers were aseptically removed; heart blood was drawn directly from the heart with the help of a micro-syringe for determination of CFU. The spleens and livers were homogenized separately in a tissue homogenizer maintained at 4°C and each specimen was processed for CFU counts.

### Statistical Analysis

The results were statistically evaluated by students’ *t* test and *χ²* test wherever applicable, Using freely available statistical software GraphPad (www.graphpad.com).

### Results

#### Bacterial Inhibitory Spectra of 3 Different Forms of Tz

A total of 55 different Gram positive and Gram negative bacteria when tested against the 3 forms of Tz, racemic, (+) and (−), it was found that *S. aureus* NCTC 6571, *V. cholerae* 569B, 1023 could be inhibited at 25 µg/ml of each agent (Table 1). Among others it was found that strains of *S. aureus*, *V. cholerae* and shigellae were also sensitive to these agents MIC of racemic, (+) and (−) forms of Tz produced almost identical type of inhibition in such organisms. However, (+) variety was less inhibitory than the other two. Strains of *S. enterica* serovar Typhimurium were inhibited at 500 µg/ml of all the compounds. *L. monocytogenes* NCTC 7973, NCTC 10351, NCTC 11994 were inhibited at 200 µg/ml of racemic and (+) forms and at 100 µg/ml of (−) form. The strains of

### Table 3. In vitro activity of sera obtained from blood and homogenates of liver and spleen of mice treated for *Salmonella enterica* 74.

| Group | Treatment | CFU/ml¹ | Sera | Homogenate |
|-------|-----------|---------|------|-----------|
|       |           | Heart blood | Liver | Spleen    |
| Control | 0.1 ml of a sterile saline | 1.8×10⁶ to 5.9×10⁶ | 6.5×10⁶ to 9.2×10⁶ | 1.6×10⁶ to 8.7×10⁶ |
| I      | 200 µg of Racemic form | 9.0×10⁵ to 6.6×10⁵ | 2.8×10⁶ to 3.8×10⁶ | 2.5×10⁶ to 4.8×10⁶ |
| II     | 200 µg of (+) form | 3.6×10⁵ to 9.6×10⁵ | 2.2×10⁶ to 5.0×10⁵ | 7.8×10⁵ to 2.8×10⁵ |
| III    | 200 µg of (−) form | 1.0×10⁶ to 8.0×10⁵ | 2.0×10⁵ to 4.7×10⁵ | 1.8×10⁵ to 5.7×10⁵ |

¹Mice received a challenge of 0.95×10⁸ colony-forming units of *S. enterica* NCTC 74 in 0.5 ml of brain-heart infusion medium.

²*p<0.001 vs. controls (*t* test).

doi:10.1371/journal.pone.0057493.t003
Klebsiella, *P. aeruginosa* and *L. monocyctogenes* AMRI 3 were highly resistant to all the compounds.

**UV Spectrophotometer analysis.** Maximum absorbance ($\lambda_{\text{max}}$) of the 3 forms of Tz and the reference thioridazine (Sigma) were found to be 264 nm. All the four analytes had the same absorbance as well as identical absorption spectra (Fig. 1).

**HPLC analysis.** The chromatogram of the three forms of Tz and the reference Tz from Sigma had the same retention time (4.6 min). All the chromatograms were identical showing peaks at the same concentration (Fig. 2).

**In vivo Experiments**

Virulence of the infection produced by *S. enterica* NCTC 74 is being presented in Table 2. In a control group of 60 mice that received only the challenge the mortality was 86.7%. As the number of CFU of *S. enterica* 74 injected intraperitoneally into mice increased, the % mortality increased, becoming 100% with a dose of 0.95 x $10^6$ CFU (Table 2). The protective capacity offered by the 3 different forms of Tz showed that there was 100% survival with 200 mg/mouse with the (+) form. With the (+) variety the survival was 70% with 200 µg/mouse dose. However, racemic proved to be better than (+) as there was 95% survival with 200 mg/mouse dose (Table 2). It may be mentioned here that the animals that received (+) form went to sleep within a few minutes after intraperitoneal injections. Animals of the other two forms went to sleep after 30–40 minutes of intraperitoneal injection of the compounds.

The tests on the 3 forms of Tz in mice infected with *S. enterica* 74 revealed that 5 animals which received only saline and challenge had $>10^5$ live cells in liver, spleen and blood after 18 hr infection. However, the number of CFUs in all the organs were between $10^3$ and $10^4$, being much less in the test batches of mice that received one of the 3 drugs along with challenge. The data were statistically significant (Table 3).

**Discussion**

Results obtained in the present study show that racemic, (+) and (-) forms of Tz did not have much difference in their *in vitro* action, only except that (+) had shown slightly better inhibitory activity than the other two. Standard strains of *L. monocytogenes*, eg. NCTC 7973, 10551, 11994 could be inhibited at 500 µg/ml of the 3 forms of Tz, while *L. monocytogenes* AMRI 3 that was isolated from an acute systemic infection in Kolkata was highly resistant to all the drugs. Both the spectrophotometric and HPLC studies carried out with the 3 forms along with standard thioridazine from Sigma Chemicals, Denmark showed that there was no difference in the $\lambda_{\text{max}}$ and that absorption spectra were identical.

This study further revealed that administration of any form of Tz successfully protected the mice infected with virulent *S. enterica* from lethality. The protection offered by the drugs were also statistically significant as evidenced by the reduction in the viable cell count in the organs of infected mice compared to the animals that were not administered any drug.

Intraperitoneal infection by *S. enterica* in mice is likely to cause phagocytosis by neutrophils [24]. According to Gunn [26], salmonellae can efficiently resist the action of hydrolases due to the action of PmrA/B regulon responsible for inactivation of hydrolases. The MIC of all the compounds with respect to *S. enterica* 74 was 500 µg/ml and is equivalent to weight of water. The amount of Tz forms in a mouse receiving 200 µg dose each would be equivalent to 10 µg/ml, which is one-twentieth of the actual MIC value. Such a distinct protection by Tz forms in mice may be explained by the studies of Ordway et al [27]. Since these authors could demonstrate that phenothiazines get concentrated 100–1000 fold inside macrophages maintained in a suitable medium, it may be possible that the concentration takes place inside the lysozome leading to rupture of bacterial cell wall. Furthermore the phenothiazines are known to promote loss of 55 kD protein [28], there may have been a significant reduction of virulence of bacterial cells in the phagolysosome and hence the lethality might have diminished distinctly. In absence of a direct proof regarding the actual mechanism of action of the different forms of Tz, the protection offered by these compounds remains an assumption. Although it may not be possible to recommend Tz alone against bacterial infections on the basis of our observation in the present study, it may be suggested that structural modifications of the original Tz molecule may open up an avenue on the possibilities of producing highly potent protective antibacterial agents in course of time.

**Author Contributions**

Conceived and designed the experiments: JBC OH SGD JEk. Performed the experiments: SC SM AD TKP. Analyzed the data: SC SM AD TKP SGD. Contributed reagents/materials/analysis tools: JBC SG SM AD TKP SGD. Wrote the paper: JBC OH SGD JEk.

**References**

1. Dastidar SG, Saha PK, Sanyamat B, Chakraborty AN (1976) Antibacterial activity of ambodril and benadryl. J Appl Bacteriol 41: 209–214.

2. Dastidar SG, Chaudhury A, Amadouras S, Roy S, Moorkeyee M et al. (1995) In vivo and in vitro antimicrobial action of phlumazine. J. Chemotherapy 7: 201–206.

3. Dastidar SG, Jairaj J, Moorkeyee M, Chakraborty AN (1997) Studies on antimicrobial effect of the antihistaminic phenothiazine tramprazine tartarate, Acta Microbiol. Immun. Hung. 44: 241–247.

4. Dastidar SG, Ganguly K, Chaudhury K, Chakraborty AN (2000) The anti-bacterial action of dicrofenac shown by inhibition of DNA synthesis, International J. Antimicrobial Agents 14: 249–251.

5. Mazumdar R, Ganguly K, Dastidar SG, Chakraborty AN (2001) Trifluoperazine: A broad-spectrum bactericide specially active on staphylococci and vibrios. International J. Antimicrob. Agents 18: 403–406.

6. Kumar KA, Ganguly K, Mazumdar K, Dutta NK, Dastidar SG et al. (2003) Amlodipine: a cardiovascular drug with powerful antimicrobial property, Acta Microbiol. Pharmacol. Immun. Hung. 51: 75–83.

7. Dastidar SG, Debnath S, Mazumdar K, Ganguly K, Chakraborty AN (2004) Trifluoperazine: a microbicide: non-antibiotic compound, Acta Microbiol. Pharmacol. Immun. Hung. 52: 285–292.

8. Basu LR, Mazumdar K, Dutta NK, Karak P, Dastidar SG (2005) Antibacterial activity of the antipsychotic agent prochlorperazine, and its synergism with methidathion, Microbiol. Res. 160: 95–100.

9. Jeyaseeli L, DasGupta A, Kumar KA, Mazumdar K, Dutta NK et al. (2006) Antimicrobrial potentiality of thioxanthene fluphenthixol through extensive in vitro and in vivo experiment, Int. J. Antimicrob. Agents 27: 58–62.

10. Kristiansen JE (1992) The antimicrobial activity of non-antibiotics. Acta Path. Micro. Immun. Scand. 100: 7–14.

11. Kristiansen JE, Amaral L (1997) The potential management of resistant infections with non-antibiotics. J. Antimicrobial Chemother. 40: 319–327.

12. Molnar J, Kiraly J (1976) Antibacterial effect of some phenothiazine compounds and the R-factor elimination by chlorpromazine. Acta Microbiol. Acad. Sci. Hung. 23: 45–54.

13. Molnar J, Fischer J, Foldeak S, Guttmann F, Nakamura MJ (1992) Thiazines and structurally related compounds, ed. By Keyzer H, Eckert GM, Forrest IS, Gupta RR, Guttmann F et al. Krieger Publishing Company, Malabar, U.S.A. 197–202.

14. Daugupta A, Dastidar SG, Shirakiti Y, Motohashi N (2004) Antibacterial activity of artificial phenothiazines and isoflavones from plants, In: Bioactive Heterocycles VI, Vol. 15, 67–132, Springer, Berlin/Heidelberg.

15. Chatopadhyay D, Dastidar SG, Chakraborty AN (1988) Antimicrobial property of methidathion and its synergism with antibiotics and some chemotherapeutic agents, Arzneimitte Forsch (FrG) 38: 869–872.

16. Arok KK, Mazumdar K, Dutta NK, Karak P, Dastidar SG et al. (2004) Evaluation of synergism between the aminoglycoside antibiotic streptomycin and the cardiovascular agent amiodpine, Biol. Pharm. Bull. 27: 1116–1120.
17. Dasgupta A, Chaki S, Mukherjee S, Jeyaseeli L, Mazumdar K et al. (2010) Experimental analyses of synergistic combinations of antibiotics with a recently recognized antibacterial agent, lacidipine. Eur. J. Clin. Microb. Infect. Dis. 29: 239–243.

18. Jeyaseeli L, Dasgupta A, Dastidar SG, Molnar J, Amaral L (2012) Evidences of Significant Synergism between Antibiotics and the Antipsychotic Antimicrobial Drug Flupenthixol. Eur. J. Clin. Microb. Infect. Dis. 31: 1243–1250.

19. Radhakrishnan V, Ganguly K, Ganguli M, Dastidar SG, Chakrabarty AN (1999) Potentiality of tricyclic compound thioridazine as an effective antibacterial and antimplasmid agent. Indian J. Exp. Biol. 671–675.

20. Jortani SA, Valentour JC, Poklis A (1994) Thioridazine enantiomers in human tissues. Forensic Sci. Int. 64: 163–170.

21. Svendsen CN, Froimowitz M, Hrbek C, Campbell A, Kula N et al. (1988) Receptor affinity, neurochemistry and behavioral characteristics of the enantiomers of thioridazine: evidence for different stereoselectivities at D1 and D2 receptors in rat brain. Neuropharmacology 27: 1117–1124.

22. Ordway D, Viveiros M, Leandro C, Bettenocourt R, Almeida J et al. (2003) Clinical concentration of thioridazine kill intracellular multidrug-resistant Mycobacterium tuberculosis. Antimicrob Agent Chemother. 47: 917–922.

23. Hendricks O (2007) Antimicrobial effects of selected Non-antibiotics on sensitivity and invasion of Gram positive bacteria; PhD Thesis, University of Southern Denmark.

24. Clinical and Laboratory Standards Institute. 2009. Methods for dilution antimicrobial susceptibility testing of bacteria that grow aerobically. 7th ed., approved standard M7-A7. 2009. Clinical and Laboratory Standards Institute, Wayne, PA.

25. Dasgupta A, Mukherjee S, Chaki S, Dastidar SG, Hendricks O, et al, 2010, Thioridazine protects the mouse from a virulent infection by Salmonella enterica serovar Typhimurium 74. Int. J. Antimicrob. Agents 35: 174–176.

26. Gunn JS, 2008, The Salmonella PmrAB regulon: Lipopolysaccharide modifications, antimicrobial peptide resistance and more. Trends Microbiol. 14: 225–226.

27. Ordway D, Viveiros M, Leandro C, Arroz MJ, Amaral L, 2002, Intracellular activity of clinical concentrations of phenothiazines including thioridazine against phagocytosed Staphylococcus aureus. Int J Antimicrob Agents 20: 34–43.

28. Amaral L, Kristiansen JE, Frohland Thomsen V, Markovich B, 2000, The effects of chlorpromazine on the outer cell wall of Salmonella typhimurium in ensuring resistance to drug. Int J Antimicrob. Agents 14: 225–229.