



Letter to the Editor

Effects of tetracycline and zinc on selection of methicillin-resistant *Staphylococcus aureus* (MRSA) sequence type 398 in pigs (Moodley et al., 2011)


A B S T R A C T

Keywords:

Methicillin resistant *Staphylococcus aureus*
MRSA
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Tetracycline

Although this paper was published some time ago, its importance is only recently coming to light as zinc oxide's use as a therapeutic substance for the treatment and control of post-weaning diarrhoea caused primarily by *Escherichia coli*, is being called into question in Europe. One of the main negative aspects of this paper was the conclusion that that feed supplemented with tetracycline or zinc increases the numbers of MRSA ST398 in the nasal cavity of pigs and that they significantly select for such higher counts. It is the purpose of this critique to demonstrate that by using a poor trial method with only a single replicate and small treatment group numbers of only four pigs (two infected with MRSA and two not), by ignoring the MRSA counts on day 0 before treatment started and manipulating the statistics, to increase the low power of the original analysis, has led to the drawing of misleading conclusions regarding the co-selection effect on MRSA by zinc oxide and underestimating the major numerical impact of tetracycline. Pharmacokinetic and pharmacodynamic relationship analysis supports the finding that zinc oxide is unlikely to have any direct co-selector effect on MRSA in the nasal cavity.

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Sir,

Although this paper was published some time ago, its significance is only recently coming to light. The therapeutic use of zinc oxide in pigs for the treatment and control of post-weaning diarrhoea is coming under strong debate in many Member States in Europe and this paper is widely quoted that the use of zinc oxide may co-select for MRSA in pigs.

Having reviewed the paper in depth for the British Pig veterinary Society, as part of a contribution to the Federation of Veterinarians of Europe response to the European Commission, there are a number of inadequacies in the paper that I would like to point out and feel are misleading and therefore make the conclusions misleading.

Under section 2.1: Study design – Firstly, the data was derived from only 16 pigs, 8 were MRSA positive and 8 were MRSA negative at the start of the trial and these were equally divided into the 4 main treatment groups, which were subsequently analysed.

Group 1 was treated with both tetracycline at 25 mg/kg bodyweight for 7 days in feed and zinc oxide at 2500 ppm in feed for the trial period of 21 days. Group 2 received zinc oxide at 2500 ppm (2.5 kg/tonne) for 21 days, Group 3

received tetracycline 25 mg/kg for 7 days and Group 4 received unmedicated feed.

Under section 2.2: MRSA quantification and characterisation – Nasal swabs were taken from the pigs on days 0, 7, 14, and 21 and cultured and the number of MRSA colonies from each pig was counted. According to Fig. 1a there was a large variation (8 fold) in the MRSA counts on day 0 between the treatment groups before the start of medication.

Under section 3: Statistical analysis – It states 'The designed groups (Groups 1–4) were reorganised into treatment groups: tetracycline-treated (Groups 1 and 2) vs. Non-tetracycline treated groups (Groups 3 and 4)' this is in contradiction to the study design section where it states Groups 1 and 3 received tetracycline medication. It goes on to state that 'and zinc-treated (Groups 1 and 3) vs. non-zinc treated (Groups 2 and 4)'. Again, this is a contradiction to the study design section where it states that Groups 1 and 2 received zinc medication. It states 'This was done to increase the statistical power.' Which suggests the original study design was inadequate; there was no replication of the treatment groups, so presumably they used individual animal data in the analysis, which

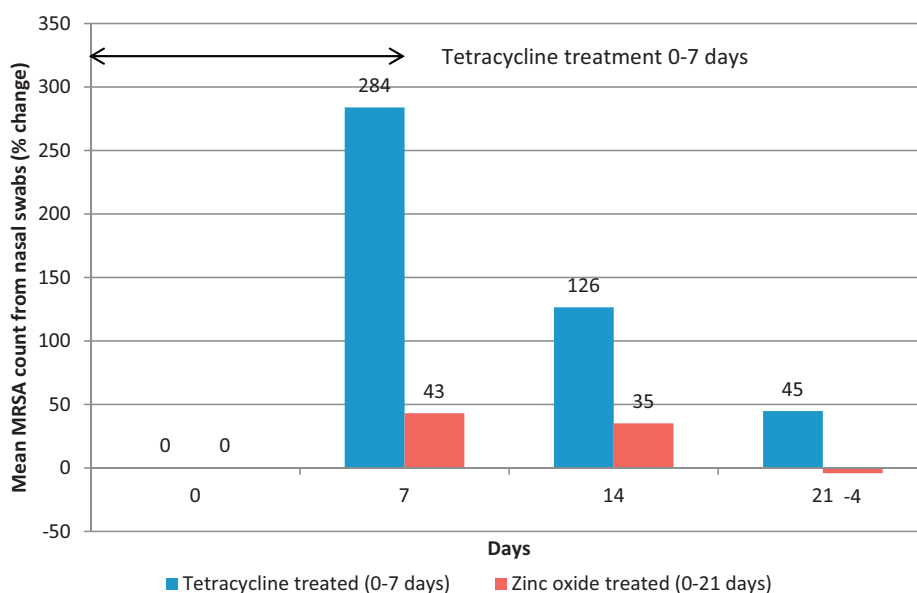


Fig. 1. Comparison of increase in MRSA counts from day 0 (before medication) and days 7, 14 and 21 post-exposure to tetracycline and zinc oxide.

statistically, is poor practice, especially with such small numbers. It is not surprising that there was no significant difference between the zinc and tetracycline groups on day 0, as it involved only 4 MRSA carrying pigs, 2 in each group. In addition, by two groups being used to compare zinc and tetracycline, each negative control also has either a tetracycline-medicated group with it in the case of zinc, or a zinc-medicated group in the case of tetracycline, which may lead to some distortion of the real results. They said that the MRSA colony counts on day 0 were not included as the initial load was not thought to affect MRSA counts on days 7–21. This is surprising, as the day 0 colony count for the zinc medicated groups was high in comparison with all the other groups, especially the untreated controls by up to 8 times. It must be remembered that treatment had not been started at day 0.

If the day 0 count is included and then compared with days 7, 14 and 21 results, a very different picture becomes apparent in percentage increase terms (see Fig. 1).

It is interesting that the tetracycline-treated group actually shows a more marked increase in MRSA count than the zinc-treated group, contrary to what is reported in Section 4: Results of 4–6 fold increases in MRSA counts for zinc and tetracycline. However, neither group is just zinc or just tetracycline each one has one group included. On this basis, it can be seen that a nearly 7 fold increase in the tetracycline group during treatment is likely to have had an influence on the zinc-treated group results, especially, as the zinc group count is declining by day 21 below the day 0 count, in spite of constant zinc exposure. This can hardly be considered co-selective pressure.

Similarly, if the untreated control groups are compared the main increase is seen by day 7 from day 0 is in the non-zinc medicated group but containing 1 tetracycline medicated group (see Fig. 2.)

There is a rise in the non-tetracycline group, containing one zinc medicated group but only 1/7th of the non-zinc

medicated group containing one tetracycline group. The subsequent variation after the removal of the single tetracycline can be seen by days 14 and 21 in the no zinc group and the variation in the zinc may be due to normal trial and swabbing variation.

Overall, the way the results have been analysed by ignoring day 0 initial MRSA counts and confusing the picture by re-mixing the groups appears to have distorted the possible outcomes and conclusions of the trial. Tetracycline at 25 mg/kg bodyweight given for 7 days has a marked effect in increasing MRSA counts during medication, a possible real co-selection, whereas the use of zinc oxide at 2500 ppm in feed for 21 days appears to have a minimal effect, 1/7th of that of tetracycline, and the variation may be due to sampling variations only, as the colony counts are extremely variable on an individual basis, as reported and also includes a tetracycline-treated group.

A pharmacokinetic and pharmacodynamic relationship analysis of zinc oxide supports the findings that zinc is unlikely to have a direct co-selector effect on MRSA in the nose. Poulsen (1995) demonstrated that the zinc concentration in serum after feeding zinc oxide to give 2500 ppm of zinc over 21 days reached 2.3 µg/ml (0.23 mg/100 ml). Sawai (2003) evaluated the antimicrobial activity of zinc oxide in a slurry-like suspension due to its lack of solubility and showed that concentrations of 990 µg/ml (0.99 mg/ml) were required to completely inhibit the growth of *Staphylococcus aureus*, using a conductimetric assay. Aarestrup and Hasman (2004) demonstrated the MIC 90 for 26 susceptible *S. aureus* using zinc chloride dissolved at pH 5.5 in agar to be 2 micromoles (equivalent to 272.63 µg/ml of ZnCl₂ or 130.77 µg/ml Zn) and Aarestrup et al. (2010) confirmed these figures. On this basis, serum concentrations of zinc are 1/430 of the MIC for zinc oxide and 1/119 for zinc chloride and 1/57 for zinc itself. The likelihood of zinc oxide having a direct impact on co-selection of MRSA in the nose can be considered remote.

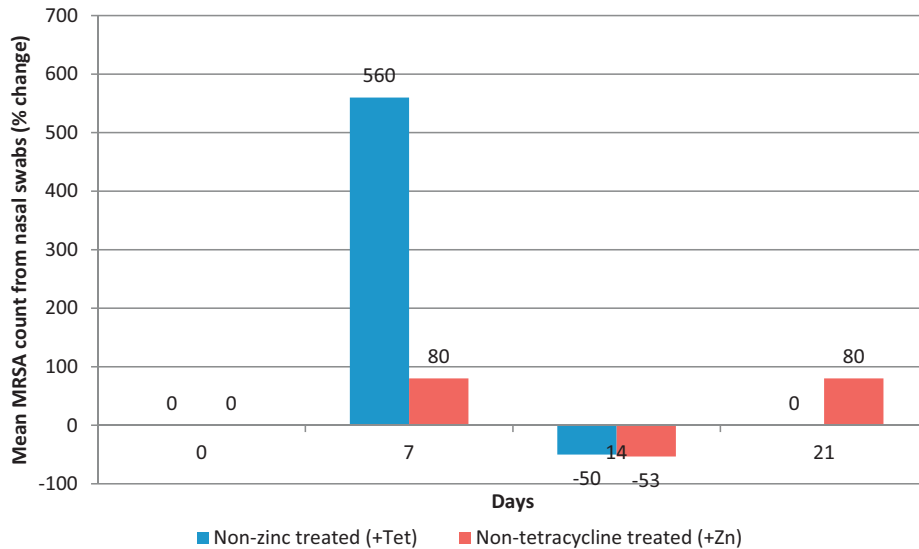


Fig. 2. Comparison of increase in MRSA counts from day 0 (before medication) and days 7, 14 and 21 post-exposure to non-tetracycline and non-zinc oxide medicated groups.

I hope you will consider publishing this letter so that a poor paper can be discounted from reviews of such a valuable pig medicine as zinc oxide.

Yours sincerely,

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