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Antimicrobial Resistance Trends of *Staphylococcus Aureus* Isolated from Milk in South Africa Differs between Seasons and Regions

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Introduction

Little was known about the seasonal and regional effects on antibiotic resistance of *Staphylococcus aureus (S. aureus)* to 8 commonly used antibiotics (Ampicillin, Cloxacillin, penicillin G, Cefuroxime, Cephalexin, Clindamycin, Oxy-tetracycline and Tylosin) in South African dairy herds. The discovery and use of antimicrobial agents in the last century has

been one of medicine's greatest achievements. However, bacteria are becoming increasingly resistant to these agents (Roberts, 2002). Bacterial antimicrobial resistance in humans is inter-linked with antimicrobial resistance in other populations, especially farm animals, which are exposed to enormous quantities of antibiotics (despite attempts at reduction) and act as another reservoir of resistance genes (Woolhouse et al. 2015).
In South Africa little information is available on the variation of the prevalence of antibiotic resistance of *S. aureus* (in different seasons and regions). Such knowledge will have an important impact on the treatment of animals with antibiotics, especially in South Africa with a unique climatic environment between the different provinces of the country.
The aims of this study were to identify the relationship, if any, of seasonal weather effects in the nine provinces to the prevalence of *S. aureus* resistance to eight antibiotics that had been tested in South African dairy herds over a ten year study period.

Materials and Methods

A study was done where on a total of 3410 susceptibility tests were performed on *S. aureus* isolates collected from milk samples with high Somatic Cell Count (SCC), of more than 400 000 cells/ml (NMC, 2004), from South African dairy herds over all seasons from 2000 to 2010. The samples represented approximately 830 commercial dairy herds, out of the approximate 2000 dairy herds in eight of nine provinces of South Africa namely, Gauteng, KwaZulu Natal (subtropical climate), Free State, Eastern Cape, Western Cape (Mediterranean climate, winter rainfall), North West, Limpopo(North) and Mpumalanga (subtropical climate).

All samples were analysed at the Milk Laboratory, Faculty of Veterinary Science, University of Pretoria. Microbiological and cytological examinations were performed on all milk samples (NMC, 2004). All these isolates were tested using the Kirby Bauer method (Bauer et al. 1966) with published clinical breakpoints. The results were based on the diameter of the inhibition zones and were classified as sensitive, intermediate or resistant in accordance with the Clinical and Laboratory Standard Institute (Rodloff et al. 2008). Eight antibiotics used in intramammary treatment (dry and lactating remedies) that are available in South Africa were tested. These were beta-lactams (ampicillin 10 μg, cloxacillin 5 μg, penicillin G 10 IU), cephalosporins (cephalexin 30 μg, cefuroxime 30 μg), lincosamides (clindamycin 10 μg), tetracyclines (oxy-tetracycline 30 μg) and macrolides (tylosin 30 μg).

Statistical Analysis

Data from Northern Cape (NC) province were removed from analysis as they were not sufficient compared to the information available from other provinces. The results were grouped into two categories: as resistant or susceptible. Results that were originally listed as being intermediate were grouped together with the resistant results in this analysis.
Further apparent relationships between season and province on the prevalence of antibiotic resistance of all *S. aureus* isolates were tested with a general linear mixed model ('glmer' within 'lme4' package with R software © version 3.3.3) using a logit link-function. The data analysis accounted for all seasons: spring (n= 482) (1 September to 30 November); summer (n= 404) (1 December to 28 February); autumn (n= 530) (1 March to 31 May); winter (n= 607) (1 June to 31 August). This is a broad classification used by the weather services, and definitions of seasons and rainfall patterns can be variable.

Results & Discussion

In this study, the overall comparison of proportions of resistance of *S. aureus* (Gram positive) between antibiotics investigated, was significantly different for all pairs indicated, except for the pairs of penicillin G and ampicillin, clindamycin and ampicillin and for oxy-tetracycline and ampicillin. Of the eight antibiotics tested tylosin (0.671) and penicillin G (0.5031) showed the highest prevalence of resistance overall and cefuroxime (0.1439) and cephalexin (0.2305) the lowest. Concerning the GLMM, the repetition of the data collected did not have an effect whereas there was a clear effect of farm, for all the antibiotics tested. The GLMM applied to the data for *S. aureus* resistance to ampicillin, penicillin G, clindamycin and tylosin showed that there was no interaction between season and province. In addition no significant differences were shown between the prevalence of antibiotic resistance according to the season for tylosin. For the prevalence of antibiotic resistance for *S. aureus* to cefuroxime, cephalexin, oxy-tetracycline and cloxacillin, the GLMM analysis showed significant interactions between season and province. In addition for cloxacillin an effect of season and an effect of province were found. The prevalence of *S aureus* resistance to cloxacillin in winter was shown to be significantly higher than in spring and the prevalence of antibiotic resistance in North West and Free State provinces was also significantly higher than the prevalence of antibiotic resistance in KwaZulu Natal. It is difficult to account for the reasons causing these differences. In KwaZulu Natal during spring the *S. aureus* isolates had the lowest prevalence of antibiotic resistance for clindamycin (lincosamide) and for the beta lactam group (ampicillin, cloxacillin and penicillin G) when compared to all other provinces and seasons. Findings generated in KwaZulu Natal during spring were therefore used as baseline against which the other provinces and seasons were compared. Conversely, cefuroxime and cephalexin showed a different trend to the rest of the antibiotics, by having the lowest prevalence of *S. aureus* antibiotic resistance in Gauteng and in winter. Autumn in KwaZulu Natal was the season that showed the lowest prevalence of *S. aureus* antibiotic resistance for oxy-tetracycline. In addition the prevalence of resistance of *S. aureus* was found to have a direct relationship to season and provinces as well as the interaction of season and provinces.

The hypothesis is that the variation in prevalence of antibiotic resistance of bacteria amongst seasons presenting extreme weather conditions may be due to the variation in the population of bacteria producing biofilm in the different seasons (da Silva-Meira et al. 2012; Melo et al. 2014), which agrees with the findings of this study. There were seasonal and regional apparent effects on the prevalence of antibiotic resistance to *S. aureus* in South Africa. A study by Fox et al. (1995) also found that location, herd and season significantly influenced prevalence of intramammary infections.

Reasons for these associations may be attributed to the different weather, ecological and difference in rainfall per region and different management such as milking intervals during the different seasons and in different provinces as well as the feeding methods (TMR versus pasture based herds) used in different provinces. Resistance patterns were also found to differ between bacteriostatic and bactericidal antibiotics depending on spectrum (narrow versus broad). Management and hygiene challenges are increased under warmer and wetter conditions in the various South African provinces and at different times of the year. It is of concern that all the antibiotics tested, except for cephalosporins showed a predicted prevalence of resistance of above 50% in most provinces and was the lowest in KwaZulu-Natal in spring. However, cephalosporins had the lowest levels of prevalence of bacterial antibiotic resistance in Gauteng during winter.

Conclusion

Resistance of *S. aureus* to the eight antibiotics varied in the different seasons and provinces, possibly due to different weather conditions and biofilm formation. The lowest prevalence of resistance to the majority of the categories of antibiotics tested were present in KwaZulu Natal during spring, although the predicted prevalence of antibiotic resistance for tylosin in KwaZulu Natal was still 56%. The cephalosporins had the lowest levels of prevalence of bacterial resistance in Gauteng during winter.

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