An Exploratory Descriptive Study of Antimicrobial Resistance Patterns of *Staphylococcus* Spp. Isolated from Horses Presented at a Veterinary Teaching Hospital.

James Wabwire Oguttu[1], Daniel Nenene Qekwana[2], Agricola Odoi[1, 3, *]

Short Title: Antimicrobial resistance of *Staphylococcus* spp. in horses

[1] Department of Agriculture and Animal Health
College of Agriculture and Environmental Sciences
University of South Africa
Florida Science Campus
Johannesburg, South Africa

[2] Section Veterinary Public Health
Department of Paraclinical Sciences
Faculty of Veterinary Sciences,
University of Pretoria
Pretoria, South Africa

[3] Biomedical and Diagnostic Sciences
College of Veterinary Medicine
University of Tennessee,
Knoxville, USA

* Corresponding author

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Abstract

Background: Antimicrobial resistant *Staphylococcus* are becoming increasingly important in horses because of the zoonotic nature of the pathogens and the associated risks to caregivers and owners. Knowledge of the burden and their antimicrobial resistance patterns are important to inform control strategies. This study is an exploratory descriptive investigation of the burden and antimicrobial drug resistance patterns of *Staphylococcus* isolates from horses presented at a veterinary teaching hospital in South Africa.

Methods: Retrospective laboratory clinical records of 1,027 horses presented at the University of Pretoria veterinary teaching hospital between 2007 and 2012 were included in the study. Crude and factor-specific percentages of *Staphylococcus* positive samples, antimicrobial resistant (AMR) and multidrug resistant (MDR) isolates were computed and compared across *Staphylococcus* spp., geographic locations, seasons, years, breed and sex using Chi-square and Fisher's exact tests.

Results: Of the 1,027 processed clinical samples, 12.0% were *Staphylococcus* positive. The majority of the isolates were *S. aureus* (41.5%) followed by *S. pseudintermedius* (14.6%). Fifty-two percent of the *Staphylococcus* positive isolates were AMR while 28.5% were MDR. Significant ($p < 0.05$) differences in the percentage of samples with isolates that were AMR or MDR was observed across seasons, horse breeds and *Staphylococcus* spp. Summer season had the highest (64.3%) and autumn the lowest (29.6%) percentages of AMR isolates. Highest percentage of AMR samples were observed among the Boerperds (85.7%) followed by the American saddler (75%) and the European warm blood (73.9%). Significantly ($p<0.001$) more *S. aureus* isolates

(72.5%) were AMR than *S. pseudintermedius* isolates (38.9%). Similarly, significantly ($p<0.001$) more *S. aureus* (52.9%) exhibited MDR than *S. pseudintermedius* (16.7%). The highest levels of AMR were towards β-lactams (84.5%) followed by trimethoprim/sulfamethoxazole (folate pathway inhibitors) (60.9%) while the lowest levels of resistance were towards amikacin (14.1%)

Conclusions: This exploratory study provides useful information to guide future studies that will be critical for guiding treatment decisions and control efforts. There is a need to implement appropriate infection control, and judicious use of antimicrobials to arrest development of antimicrobial resistance. A better understanding of the status of the problem is a first step towards that goal.

Background

Staphylococcus are Gram-positive cocci that comprise of over 50 species and subspecies, some of which are common commensals of various body sites of different animals [1,2]. Although many *Staphylococcus* spp., are of no clinical significance, some are important opportunistic pathogens [1,3,4]. In equine medicine *S. aureus*, *S. intermedius* and *S. hyicus*, have been associated with clinical infections [2]. From a public health point of view, there are increasing reports of highly virulent staphylococcal infections that can be transmitted between horses and humans [5–7]. For example, a Canadian study reported that 96% of the *Staphylococcus* samples from horses and 93% from humans displayed similar genetic profiles [8]. Another study done in the Netherlands reported that *S. aureus* isolated from a 16 year old girl was genetically similar to that isolated from a horse [5]. Busscher et al. [3] also reported identical Methicillin Resistant *Staphylococcus aureus* (MRSA) from horses and their caregivers.

Infections with antimicrobial drug resistant *Staphylococcus* spp. in both equine and human medicine has been associated with high morbidity, mortality and treatment costs. In animals, infections with antimicrobial drug resistant *Staphylococcus* spp. has also been associated with significant animal welfare implications due to animals staying sick for long periods in event of treatment failures [9–12]. Although coagulase-positive staphylococci (CoPS) are the most important groups associated with severe infections, coagulase-negative staphylococci (CoNS) have emerged as important pathogens as well. Moreover, all *Staphylococcus* spp., regardless of their coagulase activity, have potential to develop resistance to different classes of antimicrobials used for human and animal treatment [13]. Just like CoPS, resistance to antimicrobials such as

gentamycin, macrolides, tetracycline, streptomycin, trimethoprim, sulfamethoxazole and fluoroquinolones is commonly observed among CoNS isolates from pets and horses [13,14].

Excessive use of broad spectrum antimicrobials has been hypothesized as the main driver of antimicrobial drug resistance in *Staphylococcus* spp. [15]. For example, Bagcigil et al. [16] reported high levels of resistance to multiple antimicrobial agents including β-lactams in horses with previous history of treatment with β-lactams. Failure to complete the course of antimicrobial treatment has also been identified as a risk factor for development of resistance among staphylococcal isolates from horses [17]. Recent studies show that colonization with *Staphylococcus* spp. carrying antimicrobial resistance genes increases the risk of infection with resistant *Staphylococcus* [2,17]. Transfer of resistance genes between pathogenic organisms and commensal flora has also been hypothesized as a risk factor for infection with resistant isolates [18]. Morton et al. [19] were able to demonstrate horizontal transfer of one conjugate mupirocin plasmid by finding the same plasmid in different staphylococcal isolates from patients in different areas of the hospital, which suggested that isolates had acquired new genes. Moreover, conjugative plasmids can move between CoPS and CONS [19]. Furthermore, molecular epidemiological analyses by pulsed-field gel electrophoresis has shown that horizontal transfer of plasmid borne genes within and between different equine staphylococcal species is possible [20].

Despite the existence of evidence suggesting that horses play a significant role as sources of staphylococcal infections for humans [5,6], it is surprising that studies of staphylococcal infections in Africa in general, and South Africa in particular, have mainly

111 focussed on humans [12,21,22]. Available studies in human medicine suggest a high
112 prevalence of resistant staphylococcal infections, especially MRSA, associated with
113 both hospital and community acquired infections [9]. Therefore, studies of horses are
114 critically needed to fill this knowledge gap. Given the paucity of data on both the burden
115 of staphylococcal infections and the antibiotic resistance profiles among *Staphylococcus*
116 isolates from horses in Africa, the present study is an exploratory descriptive
117 investigation of the burden of staphylococcal infections and their antimicrobial drug
118 resistance patterns among horses presented at the University of Pretoria veterinary
119 teaching hospital from 2007 to 2012. This exploratory investigation is expected to
120 provide useful information to guide future hypothesis driven studies in this region of the
121 world.

Materials and Methods

Study area

This study was conducted using retrospective laboratory data collected from Gauteng province in South Africa. Gauteng province is approximately 18,178 km^2 in size, and has an estimated population of 13.2 million people (24% of the South African population). During the time period covered by this study, the province had a total of five metropolitan municipalities: Ekurhuleni, Sedibeng, Johannesburg, Tshwane and West Rand. The province has a subtropical climate, and is cooler in Johannesburg and slightly warmer in Pretoria. Gauteng is located in the Highveld region of South Africa, and has an annual summer rainfall of approximately 700 mm. It has four seasons: summer (November-March), autumn (April-May), winter (June-August) and spring (September-October). Winter is the driest season, while December and January are the wettest months. The province experiences annual maximum temperatures of about 22°C in the south and 25°C in the north [23,24].

Data source

Laboratory records of all 1,027 clinical samples from horses, from Gauteng Province, presented at the University of Pretoria bacteriology diagnostic laboratory for isolation and susceptibility testing of *Staphylococcus* spp. from January 2007 to December 2012 were included in the study. The data were received as paper records, reviewed and entered into an electronic database. The following fields were extracted for each record: horse breed, sex, age (in months), date sample was submitted as well as culture and antimicrobial susceptibility test results. The data were assessed for

duplicate entries and if any horses had been sampled multiple times during the study period. No duplicates were identified. The dataset did not contain multiple tests from the same horse, neither were there mixed infections in the samples analysed. The breeds of horses were re-classified to identify the top 10 breeds. All other breeds that had small numbers were grouped into one category and called other breeds.

Isolation of *Staphylococcus* spp. and testing for antimicrobial susceptibility

The Bacteriology Laboratory at the University of Pretoria veterinary teaching hospital, from where the study data were obtained, follows standardized protocols for isolation of *Staphylococcus* spp. based on the methods described in Quinn et al [25]. Susceptibility testing of samples was conducted using the Kirby Bauer disc diffusion technique following the guidelines described in the Clinical and Laboratory Standards Institute (CLSI) document [26]. The isolates were subjected to antimicrobial susceptibility testing against a panel of 9 drugs using the disc diffusion method. Antimicrobial resistance (AMR) was defined as resistance to at least one antimicrobial while multidrug resistance (MDR) was defined as resistance to 3 or more classes of antimicrobials [27]. Thus, some of the isolates classified as AMR were also included in the MDR group if they exhibited resistance to 3 of more classes of antimicrobials.

Statistical analysis

Shapiro-Wilks test [28] was used to test normality of continuous variables. Non-normally distributed continuous variables were summarized using medians and interquartile ranges. Age was categorized into four categories: foals (<1 year old), yearlings (1-2 years old), fillies and colts (2-4 years old) and adults (>4 years old). Crude and factor-specific percentages of *Staphylococcus* positive samples, AMR and MDR isolates as well as their 95% confidence intervals were computed. The factors (categorical variables) considered were: species of *Staphylococcus*, geographic location, season, year, breed, age group and sex. Associations between these categorical variables and percentages of *Staphylococcus* positive samples, AMR and MDR isolates were assessed using Chi-square and, in cases of small expected cell sizes, Fishers Exact test. Significance was assessed at $p \leq 0.05$. Due to the small sample sizes involved in this exploratory descriptive investigation, adjusted associations using multiple regression models could not be assessed. All statistical analyses were performed in STATA [29].

Results

A total of 1,027 clinical samples from horses in Gauteng Province were submitted to the University of Pretoria bacteriology diagnostic laboratory from 2007 to 2012 and were included in this study. Of the 1,027 processed samples, 12.0% (123/1,027) were positive for staphylococci, the majority of which were *S. aureus* (41.5%) followed by *S. pseudintermedius* (14.6%), *S. epidermidis* (4.9%), *S. equinus* (0.8%), and *S. chromogens* (0.8%). The remaining 37.4% of the samples did not have species information. Of the *Staphylococcus* positive samples, a total of 52.0% (64/123) had isolates that exhibited antimicrobial resistance to at least one antimicrobial (AMR), while 28.5% (35/123) had isolates that exhibited multidrug resistance (MDR). Significantly (p=0.002) more samples had isolates that exhibited AMR (52.0%; 95% CI: 42.8-61.1) than those that had MDR (28.5%; 95% CI: 20.7-37.3%).

Distribution by animal characteristics

The samples that were submitted and processed, came from 29 different breeds of horses. The five most common breeds contributing samples were: Thoroughbreds (26.8%), European warm blood (12.6%), Arab (11.6%), South African warm blood (6.4%) and Friesian (6.0%) (Table 1). A significant (p=0.001) association was observed between breed and the percentage of *Staphylococcus* positive samples, with the highest percentage of *Staphylococcus* positive samples observed among cross-breeds (20.5%), followed by Boerperds (18.9%) and European breeds (17.8%). There was also a significant (p=0.015) association between breed of the horse and the percentage of

200 samples carrying resistant isolates, with the highest percentage of samples with
201 resistant isolates being observed among the Boerperds (85.7%) followed closely by the
202 American saddler (75%) and the European warm blood (73.9%). Similarly, there was a
203 significant (p=0.006) association between the percentage of samples carrying MDR
204 isolates and horse breed, with the American Saddler having the highest percentage
205 (75%) of MDR followed by European warm blood (56.5%), Thoroughbreds (33.3%) and
206 the Boerperds (28.6%).

207 The median age of the horses was 4.3 years, but ranged from 0 to 25.7 years
208 (interquartile range: 0.7-9 years). The majority of the samples were from adults (50.6%),
209 followed by foals (27.1%). Yearlings contributed the lowest percentage of samples
210 (8.1%) (Table 1). A significant (p=0.001) association was observed between age group
211 and percentage of *Staphylococcus* positive samples. Foals had the highest percentage
212 (20.9%; 95% CI: 16.2, 26.1%) of *Staphylococcus* positive samples followed by yearlings
213 (10.8%; 95% CI: 5.1, 19.6), fillies/colts (8.9%; 95% CI: 4.8, 14.7%) and adults (8.3%;
214 95% CI: 6.0, 11.0%). However, there was no significant difference (p=0.360) in the
215 levels of antimicrobial resistance across age groups. On the contrary, a significant
216 (p=0.012) association in the levels of samples that had MDR isolates was observed
217 across age groups, with fillies/colts having the highest levels (46.2%; 95% CI: 19.2,
218 74.9%) followed by adults (42.0%; 95% CI: 27.0, 57.9%), foals (17.2%; 95% CI: 8.6,
219 29.4%) and yearlings had the lowest (11.1%; 95% CI: 0.2, 48.2%).

220 Significantly (p = 0.001) more samples from males (48.6%) than from females
221 (42.2%) were processed. However, there were no significant (p=0.438) differences in
222 the percentages of *Staphylococcus* positive samples obtained from males (9.6%) and

223 females (10.1%) (Table 1). Similarly, no significant (p=0.231) differences in the
224 percentage of samples with resistant isolates were observed between males (64.6%);
225 95% CI: 49.5, 77.8%) and females (52.3%; 95% CI: 36.7, 67.5%). Interestingly, there
226 was a significantly (p=0.024) higher percentage of MDR isolates among samples from
227 male (41.7%; 95% CI: 27.6, 56.8) than female horses (20.5%; 95% CI: 9.8, 35.3%).

228

229 *Temporal Patterns*

230 The largest percentage of samples were submitted during summer (40.8%) while
231 the lowest (15.6 %) were submitted during the spring (Table 2). Although spring
232 (14.4%) and autumn (14.1%) tended to have higher percentages of *Staphylococcus*
233 positive samples than the other two seasons, there was no significant (p=0.357)
234 association between season and percentage of *Staphylococcus* positive samples (Table
235 2). However, a significant (p=0.002) association between season and percentage of
236 isolates resistant to at least one antimicrobial was observed. Summer had the highest
237 (64.3%; 95% CI: 48.6, 77.4%) and autumn the lowest (29.6%; 95% CI: 15.2, 49.6%)
238 percentage of antimicrobial resistant isolates (Table 2). On the contrary, there was no
239 significant (p=0.137) association between the percentage of MDR isolates and season.

240 A comparison of the percentage of samples submitted and processed each year
241 revealed that significantly more samples were processed in 2007; and the lowest
242 percentage of samples were received and processed in 2011. Moreover, there was a
243 significant (p<0.001) association between year and the percentage of *Staphylococcus*
244 positive samples, with the largest percentage of positive samples occurring in 2012
245 (24.2%) (Table 2). Thus, except for the anomaly observed in 2011, there was an

246 increasing trend in the percentage of *Staphylococcus* positive samples during the study
247 period (Table 2). By contrast, there were no significant (p = 0.707) differences in the
248 percentage of AMR isolates across the years, neither were there significant differences
249 (p=0.304) in the percentage of MDR isolates across the years.

250

251 *Geographic Patterns*

252 The geographic distribution of sample submissions was a reflection of the
253 distribution of horse populations across the different municipalities with Tshwane
254 submitting most (61.2%) of the samples followed by Johannesburg (20%) (Table 2).
255 Although the percentage of *Staphylococcus* positive samples varied from 10% in
256 Ekurhuleni municipality to 20% in Sedibeng municipality (Table 2), these differences
257 were not statistically significant (p=0.563). The percentage of isolates that were
258 resistant to at least one antimicrobial varied from 40% in Sedibeng to 63.6% in
259 Ekurhuleni (Table 2). Again, there were no significant (p=0.857) differences in the
260 percentage of isolates that were resistant to at least one antimicrobial. Similarly, the
261 percentage of MDR isolates varied from 20% in Sedibeng to 36.4% in Ekurhuleni
262 municipality (Table 2). As was the case with AMR isolates, there were no significant
263 (p=0.891) differences in the percentage of MDR isolates across the municipalities.

264

265 *Antimicrobial susceptibility profiles*

266 There was a significant (p<0.001) difference in the percentages of samples with
267 resistant isolates across the *Staphylococcus* spp., with *S. aureus* (72.5%) showing

much higher levels of resistance than *S. pseudintermedius* (38.9%) (Table 3). Similarly, there was a significant (p=0.0001) difference in the percentages of samples with MDR isolates across species, and again *S. aureus* (52.9%) exhibited higher levels of MDR compared to *S. pseudintermedius* (16.7%) (Table 3).

Significant ($p<0.05$) differences in the levels of resistance to each antimicrobial was also observed, with resistance to β-lactams being the highest (ampicillin: 84.5%; penicillin: 74.1%) followed by Trimethoprim/sulfamethoxazole (folate pathway inhibitors) (60.9%), while the lowest level of resistance was towards amikacin (14.1%) (Table 3). Most MDR isolates tended to exhibit resistance to trimethoprim/sulfamethoxazole (53.1%), followed by gentamicin (48.9%) (Table 3). Only 9.1% of these isolates exhibited resistance towards amikacin and only 18.8% displayed resistance towards enrofloxacin. The distribution of the number of classes of antimicrobials to which isolates were resistant is shown in Table 4.

The majority of MDR combinations observed involved *S. aureus* (Table 5). Multidrug resistance involving combinations of four antimicrobials tended to occur more frequently in young foals as compared to combinations that involved more antimicrobials. Most samples with MDR isolates came from Tshwane municipality (Table 5) and involved mainly European warm blood (31.1%) and Thoroughbreds (20.0%). It is interesting to note that a high percentage (35.6%) of MDR isolates were from horses less than 1 month old (Table 5).

Discussion

The current study is an exploratory descriptive analysis of antimicrobial resistance patterns of *Staphylococcus* spp. isolated from horses presented at a veterinary teaching hospital in South Africa. Since very little is known regarding the epidemiology of antimicrobial resistance in horses in Africa and many other developing economies, this study is intended to provide preliminary information to guide future more detailed hypothesis driven epidemiological studies of antimicrobial drug resistance among *Staphylococcus* spp. not only in horses, but other domestic species as well.

Contrary to findings by Leekha et al. [30] the present study did not find significant seasonal differences in the percentage of *Staphylococcus* positive samples. This could be due to aggregation of cases by pre-defined seasons, as was done in the present study. Other authors have argued that this aggregation leads to loss of information if infection occurrences are not seasonal but follow other cyclical patterns such as biannual patterns [30]. Some authors have suggested use of time-series analytical approaches in such situations [31]. Unfortunately, due to the exploratory nature and the small samples sizes of the current study, time-series analysis could not be performed. The significant association observed between season and percentage of antimicrobial resistance could be attributed to weather conditions such as temperature and humidity. However, it may also be due to seasonal differences in staffing levels (i.e., fewer regular staff during vacation months) and seasonal differences in antimicrobial drug use due to differences in infection rates from other bacteria resulting in differences in selection pressure [30].

Although the percentages of *Staphylococcus* positive samples increased over time, there were no similar increases in the number of samples submitted for processing. Therefore, the observed increases in percentage of *Staphylococcus* positive samples may be a reflection of a true increase in the number of staphylococcal infections over the years. However, more detailed primary base studies will need to be performed to further investigate this trend. The higher levels of Staphylococcal infections in some breeds of horses seem to suggest potential breed predisposition. However, the reason for this is unclear and no previous studies have reported this. Therefore, this warrants further investigations to establish the potential existence of breed predisposition. Although sex dimorphism of staphylococcal infections, especially methicillin resistant infections, have been observed in humans [32], no similar associations were observed in this study and to our knowledge, no studies have reported differences in staphylococcal infections in horses based on sex.

Coagulase positive (CoPs) *Staphylococcus* spp., especially *S. aureus* are responsible for a significantly large percentage of infections in horses as opposed to coagulase negative strains [1]. Therefore, the higher levels of resistance observed among *S. aureus* in this study is of concern, from a therapeutic point of view, due to the resulting potential treatment failures associated with the observed high levels of resistance. The relatively high levels of AMR and MDR observed in this study may suggest high selection pressure among *Staphylococcus* spp. isolates from horses treated at the veterinary teaching hospital. This may be due to the fact that most of the cases seen in the teaching hospital tend to be referral cases that may not have responded well to initial antimicrobial treatments by the primary care veterinarians.

Thus, it is possible that most of these horses would have been on antibiotic treatment for prolonged periods before being transferred to the referral veterinary teaching hospital. Another plausible explanation for the high levels of antimicrobial resistance observed in the study could be the result of failure of horse owners to ensure that the animals complete the full course of prescribed antimicrobial therapy. This issue has been highlighted by some authors who reported that caretakers have a tendency to stop drug administration as soon as disease symptoms abate, a practice that has been incriminated in the development of antimicrobial drug resistance [17]. Transportation stress has also been incriminated in the high carriage of resistant *Staphylococcus* in equine patients admitted to clinics as a result of direct contact with referring veterinarians who might be carrying resistant *Staphylococcus* [4].

The high levels of antimicrobial resistance observed in this study is consistent with the observations in humans in South Africa by Essa et al [12], who observed that up to 95.1% of the samples were MDR, and only 3.7% of the samples were susceptible to all antibiotics tested in the study. The very high levels of resistance to β-lactams observed in the current study is consistent with reports by Weese [1] who reported high levels of resistance to β-lactams among staphylococci and especially *S. aureus*. Some studies have reported that routine use of β-lactam antibiotics in prevention of surgical infections, predisposes horses to acquisition of methicillin resistant *S. aureus* [33,34]. Therefore, the high levels of resistance to β-lactam antibiotics in the present study could signal the existence of methicillin resistance in staphylococcal infections in horses presented at the hospital under study. Unfortunately, methicillin was not routinely included in the susceptibility test panels used by the veterinary teaching hospital that supplied the data

for the current study, and hence the levels of resistance to methicillin could not be assessed in the current study. However, it is encouraging to note that resistance to fluoroquinolones was relatively low implying that these antimicrobials are still relatively effective and therefore their use could more likely be associated with successful treatment outcomes compared to β-lactams. It is worth noting that in South Africa, some of the older antibiotics are readily available to farmers over the counter while the newer ones require prescription [35]. This may have impacted selection pressure for antimicrobial resistance especially influencing antimicrobials readily available over the counter.

The observed low levels of resistance towards aminoglycosides was not unusual. For instance, Abrahamsen [17] in a study conducted in Maine (USA), also observed that *Staphylococcus* spp tended to be susceptible to both gentamicin and amikacin, both of which are aminoglycosides. Furthermore, a study by Schnellmann et al. [33], investigating emergence of resistance after hospitalization in Switzerland, found much lower levels of resistance to amikacin compared to other antimicrobials. However, it is worth noting that the level of resistance observed here may not be a true reflection of the level of resistance in the larger horse population. For example, Van den Eede et al [4] observed a much lower level of resistance among horses on farms that were situated in a region surrounding an equine clinic in Belgium whereas much higher carriage rates of resistant *Staphylococcus* had been detected in horses presented at the clinic [4].

The significant differences in the percentage of MDR isolates across species, with *S. aureus* exhibiting higher levels of MDR compared to other species, was anticipated given that it is the most common species in horses and therefore more likely to be

exposed to higher selection pressure during antibiotic treatments compared to the other less common species [30]. Worth noting was that MDR was frequently observed among isolates from horses less than 1 month old. This could be due to the fact that foals frequently require antimicrobial treatment to combat a variety of conditions. Moreover, the basic principle of antimicrobial treatment in neonatal foals is the use of broad-spectrum antimicrobials [36]. This practice potentially increases the selection pressure for antimicrobial resistance among foals. However, it is interesting to note that MDR isolates resistant to six or more drugs tended to occur in older horses, which suggests that the overall selection pressure for antimicrobial drug resistance could be higher in older horses. Some authors have indicated that after 3 days of hospitalization and treatment with penicillin, the percentage of *Staphylococcus* isolates showing antibiotic resistance dramatically increases in horses [33].

Although some authors have reported geographic differences in levels of AMR staphylococcal isolates [37], the current study did not identify significant geographic differences. This could most likely be due to the small samples sizes involved in some of the municipalities included in the study. Therefore, more detailed and larger studies need to be performed to more fully investigate geographic and other determinants of variations in AMR and MDR staphylococcal infections. Thus, our next set of studies will focus on these issues as well as investigating practices in the use of antimicrobials by both veterinary practitioners and animal owners.

A limitation of this retrospective laboratory-based study is that the source of samples (e.g. nasal, skin, etc) could not be assessed due to quality of these data. A positive outcome of this finding is that we will work with the laboratory to improve

laboratory data capture. This will have a positive impact of improved data quality for similar studies in the future. Additionally, information on past antimicrobial use was not available and therefore we could not assess its associations with levels of AMR or MDR. Moreover, detailed analysis of the β-lactam resistant isolates were not performed and hence we could not assess their susceptibility to methicillin. Finally, it is possible that some of the isolates reported as *Staphylococcus* spp. may have belonged to *S. aureus*, *S. pseudintermedius*, *S. epidermidis* or *S. equinus*. Unfortunately, information was not available to elucidate this.

Conclusions

The above limitations notwithstanding, the study has shown evidence of seasonal patterns of staphylococcal infections and occurrence of AMR and MDR in horses. There is also evidence of differences in the occurrence of AMR and MDR by species of *Staphylococcus* and breed of horse. Resistance among *Staphylococcus* isolates was highest towards β-lactam antibiotics, but lowest towards amikacin. It should be pointed out that this was an exploratory descriptive study that did not lend itself to more detailed analyses such as use of multivariable models due to the small sample sizes involved. However, this being the first study of its kind in Africa, it provides useful descriptive information to guide future more detailed studies intended to address the problems of equine staphylococcal infections and antimicrobial resistance. Routine surveillance, prudent antimicrobial use and efficient infection control should be advocated as strategies to contain development of antimicrobial resistance in horse infections. Future studies will need to be primary-base involving large sample sizes to help understand

425 specific local factors contributing to development of antimicrobial resistance so as to
426 better guide treatment and control efforts.

List of Abbreviation

AMR: Antimicrobial Resistant

β-lactam: Beta-lactam

95% CI: 95% Confidence Interval

CoNS: coagulase-negative staphylococci

CoPS: coagulase-positive staphylococci

MDR: Multidrug Resistant

MRSA: Methicillin Resistant *Staphylococcus aureus*

p: p-value

S. aureus: *Staphylococcus aureus*

S. epidermidis: *Staphylococcus epidermidis*

S. equinus: *Staphylococcus equinus*

S. hyicus: *Staphylococcus hyicus*

S. intermedius: *Staphylococcus intermedius*

S. pseudintermedius: *Staphylococcus pseudintermedius*

spp: Species

USA: United States of America

Declarations

Ethics approval and Consent to participate

This study was approved by the Animal Ethics Committees of both the University of Pretoria and the University of South Africa (reference number V051-14 and Ref. NR.: 2014/CAES/077 respectively). Consent for animal samples to be used in research and for the findings to be published was obtained from animal owners at the time they consented for their animals to receive care at the veterinary teaching hospital.

Consent for Publication

Not applicable

Availability of data and materials

The data that support the findings of this study are available from the bacteriology laboratory of the University of Pretoria that has legal ownership of the data. The data are not publicly available and can be requested for and obtained from the above listed legal owner.

Competing Interests

The authors declare that they have no competing interests.

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Authors' contributions

JWO was involved in study design, results interpretation and editing of the manuscript. DNQ was involved in study design, data entry and manuscript writing. AO conceived the study idea, was involved in study design, analysed the data, interpreted the results and was involved in extensive editing of the manuscript. All authors read and approved the final manuscript

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Table 1: Host factor distribution of equine samples from Gauteng Province (South Africa) tested for *Staphylococcus*, 2007-2012

	All Samples Processed (n=1,027)			Staphylococcus Positive Samples (n=123)		
Breed	**n**[a]	**%**[b]	**95% CI**[c]	**n**[a]	**%**[b]	**95% CI**[c]
American Saddler	39	3.8	2.8, 5.2	4	10.3	3.8, 24.6
Arab	119	11.6	9.8, 13.7	8	6.7	3.4, 12.9
Boerperd	37	3.6	2.6, 4.9	7	18.9	9.2, 35.0
Crossbreed	39	3.8	2.8, 5.2	8	20.5	10.5, 36.2
European warm blood	129	12.6	10.7, 14.7	23	17.8	12.1, 25.4
Friesian	62	6.0	4.7, 7.7	2	3.2	0.8, 12.1
Nooitgedachtpony	43	4.2	3.1, 5.6	5	11.6	4.9, 25.3
South African warm blood	66	6.4	5.1, 8.1	1	1.5	0.2, 10.1
Thoroughbred	275	26.8	24.2, 29.6	21	7.6	5.0, 11.4
Welshpony	25	2.4	1.6, 3.6	3	12.0	3.8, 31.9
Percheron	13	1.3	0.7, 2.2	0	0	-
All other breeds	73	7.1	5.6, 8.9	6	8.2	3.1, 17.1
Unspecified or missing	107	10.4	8.7, 12.4	35	32.7	24.0, 42.5
Age Group						
Foal (< 1 year)	278	27.1	24.4, 29.9	58	20.9	16.2, 26.1
Yearling (1-2 years)	83	8.1	6.5, 9.9	9	10.8	5.1, 19.6
Filly or Colt (2-4 years)	146	14.2	12.1, 16.5	13	8.9	4.8, 14.7
Adult (> 4 years)	520	50.6	47.5, 53.7	43	8.3	6.0, 11.0
Sex						
Males	499	48.6	45.5, 51.7	48	9.6	7.2, 12.6
Females	434	42.2	39.2, 45.3	44	10.1	7.6, 13.4
Unspecified or missing	94	9.2	7.5, 11.1	31	33.0	23.6, 43.4

[a]number of samples
[b]percentage of samples
[c]95% confidence interval

Table 2: Temporal and Geographic distribution of equine samples from Gauteng Province assessed for antimicrobial susceptibility, 2007-2012

	All Samples Processed (n=1,027)		*Staphylococcus* positive samples (n=123)		AMR[c] Isolates (n=64)		MDR[d] *Isolates* (n=35)	
	%[b]	95% CI[a]	%	95% CI[a]	%	95% CI[a]	%	95% CI[a]
Season								
Summer	40.8	37.8, 43.8	10.0	7.4, 13.3	64.3	48.6, 77.4	38.1	24.6, 53.8
Autumn	18.6	16.3, 21.1	14.1	9.9, 19.9	29.6	15.2, 49.6	14.8	5.5, 34.2
Winter	25.0	22.5, 27.8	12.1	8.6, 16.7	58.1	40.0, 74.2	41.9	25.8, 60.0
Spring	15.6	13.5, 17.9	14.4	9.7, 20.7	47.8	28.3, 68.1	8.7	2.1, 29.9
Year								
2007	34.1	31.2, 37.0	7.7	5.3, 11.0	55.6	36.4, 73.2	14.8	5.5, 34.2
2008	15.5	13.4, 17.8	8.2	4.8, 13.6	53.8	27.0, 78.6	15.4	3.6, 47.0
2009	16.1	13.9, 18.4	12.1	7.9, 18.1	55.0	32.9, 75.3	35.0	17.2, 58.3
2010	8.8	7.2, 10.7	13.3	7.7., 22.1	33.3	12.4, 63.9	8.3	1.0, 44.1
2011	7.1	5.7, 8.9	6.8	2.9, 15.5	60.0	16.6, 91.9	20.0	2.1, 74.8
2012	18.5	16.2, 21.0	24.2	18.6, 30.8	52.2	37.7, 66.3	43.5	29.8, 58.2
Municipality								
Johannesburg	20.0	17.6, 22.5	11.7	7.6, 16.9	54.2	34.0, 73.0	33.3	17.2, 54.5
Tshwane	61.2	58.2, 64.2	11.6	9.2, 14.4	49.3	37.9, 60.8	26.0	16.5, 37.6
Ekurhuleni	10.1	8.4, 12.1	10.6	5.4, 18.1	63.6	32.3, 86.5	36.4	13.5, 67.7
Sedibeng	2.4	1.6, 3.6	20.0	6.8, 40.7	40.0	5.3, 85.3	20.0	0.5, 71.6
West Rand	6.2	4.9, 7.9	10.0	7.8, 26.9	60.0	26.3,87.8	30.0	6.7, 65.2

[a] 95% confidence interval

[b] Percent

[c] AMR: Antimicrobial resistant (defined as resistance to at least one antimicrobial)

[d] MDR: Multidrug resistant (defined as resistance to at least three classes of antimicrobials; includes a subset of AMR isolates)

Table 3: Distribution of antimicrobial resistance by species of *Staphylococcus* and antimicrobial agent

	Resistant Isolates		MDR Isolates	
	%	95% CI	%	95% CI
Species		n = 123		n=35
S. aureus	72.5	58.3, 83.3[a]	52.9	38.5, 67.1[a]
S. pseudintermedius	38.9	19.2, 63.0[b]	16.7	3.6, 41.4[b]
S. spp[1]	30.4	18.7, 45.4[b]	2.2	0.1, 11.5[c]
All other species[2]	75.0	34.9, 96.8[a]	50.0	15.7, 84.3[a]
Antimicrobial & Antimicrobial Class		n=64		n=35
Aminoglycosides[3]				
Gentamycin	54.6	42.1, 66.7[a]	48.9	38.1, 59.8
Amikacin	14.1	7.3, 25.2[e]	9.1	4.0, 17.1
B-lactams[4]				
Ampicillin	84.5	72.3, 91.9[b]	37.5	27.4, 48.5
Penicillin	74.1	61.0, 84.0[b]	37.5	27.4, 48.5
Ceftriaxone	44.8	32.3, 58.1[c]	30.0	20.3, 41.3
Fluoroquinolones[5]				
Enrofloxacin	23.4	14.5, 35.7[c]	18.8	10.1, 30.5[b]
Folate Pathway Inhibitors[6]				
Trimotherim-sulfamethoxazole	60.9	48.2, 72.3[a]	53.1	40.2, 65.7[a]
Tetracyclines[7]				
Doxycyline	46.9	34.8, 59.4[a,d]	35.9	24.3, 48.9[c]
Phenicols				
Chloramphenicol	44.1	31.7, 57.2[d]	31.3	20.2, 44.1[c]

[1]Samples that were not identified to species level and were thus reported as *Staphylococcus spp.*
[2]Other species included *S. equinus*, *S. chromogens* and *S. epidermidis*
[3]Gentamycin and amikacin
[4]Penicillin, ampicillin, ceftriaxone
[5]Enrofloxacin
[6]Sulphamethoxazole
[7]Chloramphenicol
[a-d]Estimates with different superscripts are significantly different at 5% significance level

Table 4: Distribution of number of antimicrobial classes to which equine samples exhibited resistance (n=64)

Number of antimicrobial classes to which isolates exhibited resistance	Number of isolates	Percent	95% CI[a]
1	17	26.6	16.3, 39.1
2	12	18.8	10.1, 30.4
3	7	10.9	4.5, 21.2
4	12	18.8	10.1, 30.4
5	8	12.5	5.6, 23.2
6	8	12.5	5.6, 23.2

[a] 95% confidence interval

Table 5: Antimicrobial resistance patterns of staphylococcal isolates from equine samples from Gauteng Province (South Africa), 2007-2012.

Horse Age (months)	Breed of Horse	Municipality	Year	Staphylococcus Species	Antimicrobial Resistance Patterns [a]
0	Thoroughbred	Tshwane	2008	*S. aureus*	AMP-DOX-PEN
0	Europeanwarmblood	Mogale City	2011	*Staph. spp.*	AMP-PEN-SUL
132	Crossbreed	Tshwane	2007	*S. aureus*	AMI-AMP-PEN
0	Boerperd	Tshwane	2007	*S.pseudintermedius*	AMP-DOX-PEN
0	Boerperd	Tshwane	2007	*S.aureus*	AMP-DOX-PEN
0	Unpecified or Missing	Tshwane	2007	*Staph. spp.*	AMP-CEF-PEN
131	Europeanwarmblood	Johannesburg	2008	*S. aureus*	AMP-PEN-SUL
48	Europeanwarmblood	Tshwane	2012	*S.aureus*	AMP-GEN-PEN-SUL
182	Boerperd	Tshwane	2012	*S.aureus*	AMP-GEN-PEN-SUL
1	Arab	Tshwane	2007	*S. equinus*	AMP-CEF-PEN-SUL
96	All other breeds	Tshwane	2012	*S. epidermidis*	DOX-ENR-GEN-SUL
40	All other breeds	Tshwane	2007	*S. aureus*	AMP-CEF-GEN-PEN-SUL
96	Europeanwarmblood	Tshwane	2012	*S. epidermidis*	DOX-ENR-GEN-PEN-SUL
0	Unpecified or Missing	Tshwane	2012	*S. aureus*	AMP-CEF-CHL-PEN-SUL
83	Europeanwarmblood	Johannesburg	2012	*S. aureus*	AMP-CHL-GEN-PEN-SUL
48	Europeanwarmblood	Tshwane	2012	*S. aureus*	AMP-DOX-GEN-PEN-SUL
84	Thoroughbred	Tshwane	2012	*S. aureus*	AMP-CEF-GEN-PEN-SUL
0	Europeanwarmblood	Johannesburg	2008	*S. aureus*	AMP-CEF-GEN-PEN-SUL
0	Unpecified or Missing	Tshwane	2012	*S. aureus*	AMP-CEF-CHL-GEN-PEN-SUL
0	Unpecified or Missing	Emfuleni	2012	*S. aureus*	AMP-CEF-CHL-GEN-PEN-SUL
0	Unpecified or Missing	Tshwane	2012	*S. aureus*	AMP-CEF-DOX-GEN-PEN-SUL
84	Thoroughbred	Ekurhuleni	2007	*S. aureus*	AMP-CEF-DOX-GEN-PEN-SUL
168	Europeanwarmblood	Ekurhuleni	2012	*S. aureus*	AMP-CEF-CHL-GEN-PEN-SUL

0	American Saddler	Ekurhuleni	2007	*S. aureus*	AMP-CEF-DOX-GEN-PEN-SUL
81	Thoroughbred	Tshwane	2009	*S. aureus*	AMP-CEF-DOX-GEN-PEN-SUL
0	American Saddler	Ekurhuleni	2007	*Staph. spp.*	AMP-CEF-DOX-GEN-PEN-SUL
48	Europeanwarmblood	Westonaria	2012	*S. aureus*	AMP-CHL-DOX-GEN-PEN-SUL
19	Europeanwarmblood	Westonaria	2012	*S. aureus*	AMP-CHL-DOX-ENR-GEN-PEN-SUL
108	Thoroughbred	Tshwane	2009	*S. aureus*	AMP-CEF-CHL-DOX-GEN-PEN-SUL
0	Arab	Johannesburg	2012	*S. aureus*	AMI-AMP-CHL-DOX-GEN-PEN-SUL
168	Europeanwarmblood	Johannesburg	2012	*S. aureus*	AMP-CEF-CHL-DOX-GEN-PEN-SUL
36	Europeanwarmblood	Westonaria	2010	*S.pseudintermedius*	AMP-CEF-CHL-DOX-ENR-GEN-SUL
111	All other breeds	Tshwane	2009	*S.pseudintermedius*	AMP-CEF-CHL-DOX-GEN-PEN-SUL
96	American Saddler	Tshwane	2012	*S.aureus*	AMI-AMP-CEF-CHL-GEN-PEN-SUL
0	Nooitgedachtpony	Tshwane	2009	*S. aureus*	AMI-AMP-CEF-CHL-DOX-ENR-GEN-PEN
120	Europeanwarmblood	Johannesburg	2009	*S.epidermidis*	AMI-AMP-CEF-DOX-ENR-GEN-PEN-SUL
182	Boerperd	Tshwane	2012	*S.aureus*	AMP-CEF-CHL-DOX-ENR-GEN-PEN-SUL
48	Europeanwarmblood	Tshwane	2012	*S.epidermidis*	AMP-CEF-CHL-DOX-ENR-GEN-PEN-SUL
155	Thoroughbred	Johannesburg	2012	*S.aureus*	AMI-AMP-CHL-DOX-ENR-GEN-PEN-SUL
83	Thoroughbred	Johannesburg	2011	*S.pseudintermedius*	AMI-AMP-CEF-CHL-DOX-ENR-GEN-PEN-SUL
0	Unpecified or Missing	Tshwane	2009	*S.aureus*	AMI-AMP-CEF-CHL-DOX-ENR-GEN-PEN-SUL
72	Thoroughbred	Tshwane	2009	*S.aureus*	AMI-AMP-CEF-CHL-DOX-ENR-GEN-PEN-SUL

[a]AMI=Amikacin; AMP= Ampicillin; CEF=Ceftriaxone; CHL=Chloramphenicol; DOX=Doxycycline; ENR= Enrofloxacin; GEN=Gentamicin; PEN=Penicillin; SUL= Sulphametho