**ABSTRACT**

*Staphylococcus aureus* is 1 of the most important causes of bovine mastitis and is responsible for significant economic losses to the dairy industry worldwide. One of the principal approaches used in treating intramammary infections is the administration of antimicrobials. Due to the propensity of *S. aureus* to develop resistance, antimicrobial susceptibility monitoring is necessary to ensure that treatment regimens are effective. As part of this investigation, 90 *S. aureus* strains isolated from mastitis cases submitted to Allerton Provincial Veterinary Laboratory during 2008 and 2009 were evaluated for their susceptibility to a panel of 10 antimicrobials. Only 8 of the 90 *S. aureus* isolates tested (8.9%) were found to be susceptible to all of the antimicrobials evaluated. A very high level of resistance to the beta-lactam antibiotics was noted: 47.8% of the isolates were resistant to penicillin and 65.6% were resistant to ampicillin. Minimal resistance to oxacillin, cephalothin and trimethoprim-sulfamethoxazole (1.1%) was found. Seventeen (18.9%) of the isolates tested were found to be resistant to 3 or more antimicrobials. The need for vigilant monitoring of bacterial resistance trends in the dairy industry is warranted as the potential public health implications are significant.

**Keywords:** antimicrobial susceptibility, bovine mastitis, *Staphylococcus aureus*.


**INTRODUCTION**

*Staphylococcus aureus* is a formidable pathogen and nowhere is this more evident than in the dairy industry, where considerable losses are incurred annually due to intramammary infections caused by this bacterium.

The success of *S. aureus* as a pathogen is due to the variety of strategies the bacterium has evolved which enable it to evade the immune system and counter therapeutic assaults. Once *S. aureus* has breached the physical barriers of the teat canal, the host’s local immune response is challenged by the production of an impressive array of virulence factors that confer protection on the bacteria, enabling them to become established within the udder microenvironment. Enzymes such as hyaluronidase, staphylokinase and proteinases assist tissue invasion, while antiphagocytic factors such as the extracellular polysaccharide capsule compromise the process of phagocytosis, a crucial component of the host’s cellular immune response.

In the event that the invading bacteria are phagocytosed, they are in fact able to survive and even replicate within the phagocyte. The intracellular existence confers protection on the bacteria from the onslaught of the immune response as well as the effects of antimicrobials. With the eventual death of the phagocytic cell the bacteria are released where they are able to induce further damage through the production of multiple haemolysins and other tissue toxins. In severe cases, *S. aureus* is able to induce fibrosis and the formation of microabscesses that further aid the bacteria by limiting the penetration of antibiotics into the site of infection.

Mastitis caused by *S. aureus* ranges in severity from subclinical to a purulent, gangrenous form. Subclinical mastitis is the most common and likely outcome of a *S. aureus* infection and it is also the most problematic, as it usually proceeds undetected, constantly eroding profit margins. The economic losses incurred due to *S. aureus* infections are difficult to quantify and include, but are not limited, to the following: decreased milk production, reduced milk quality, veterinary and treatment costs, premature culling of cows and consequent loss of genetic potential.

The successful implementation of a mastitis control programme is therefore imperative. This requires the prompt identification of *S. aureus*-infected animals before the bacterium has the opportunity to infect other animals in the herd. Management approaches to handling infected animals usually involve segregation, culling or treatment, with the therapeutic approach often being the favoured recourse.

The ability of *S. aureus* to develop or acquire biochemical strategies which confer resistance to different antimicrobials is an additional tactic in this pathogen’s impressive arsenal. One of the diagnostic tools available to practitioners to assist with the selection of an appropriate treatment is the *in vitro* testing of isolates against a representative panel of antimicrobial drugs. The susceptibility pattern of the implicated strain enables decisive action to be taken by the veterinarian in terms of treatment. This avoids the needless application of ineffective antimicrobials and prevents unnecessary costs from being incurred.

The purpose of this investigation was to evaluate the *in vitro* susceptibility of *S. aureus* isolates to different antimicrobial classes that are used in the dairy industry and to use these data to establish the incidence of resistance amongst these pathogens in the KwaZulu-Natal commercial dairy sector.

**MATERIALS AND METHODS**

**Samples**

Allerton Provincial Veterinary Laboratory has a large Mastitis Control and Milk Hygiene Section which, for many years, has provided a diagnostic service to the extensive dairy industry in KwaZulu-Natal. Composite and individual quarter milk samples are submitted routinely or on an ad hoc basis by clients as part of their mastitis management plan. The *S. aureus* strains evaluated during this investigation were isolated during the course of routine diagnostic work carried out at Allerton between January 2008 and December 2009. A total of 90 *S. aureus* isolates from 60 different commercial dairy herds were...
evaluated for their susceptibility to different classes of antimicrobials.

**Bacteriological culture and isolation**

Milk samples were cultured onto Columbia blood agar (Oxoid) supplemented with 5% sheep blood. Inoculated plates were incubated at 35–37 °C for approximately 36 h before being examined. All staphylococcal colonies showing yellow pigmentation were tested for coagulase production using diluted rabbit plasma (Bio-Rad) according to the method described by Quinn et al. Isolates which tested positive for coagulase production using the overnight tube coagulation test were identified as *S. aureus*. In the event that multiple *S. aureus* isolates were cultured and identified in a batch of samples from the same herd, visual inspection of colonies was used to select representative isolates for antimicrobial susceptibility testing.

**Antimicrobial susceptibility testing**

Antimicrobial susceptibility testing was carried out in accordance with the guidelines published by the Clinical and Laboratory Standards Institute (formerly the National Committee for Clinical Laboratory Standards). Briefly, a suspension of each test isolate was prepared in 0.9% physiological saline to a turbidity equivalent to a 0.5 McFarland standard. Each suspension was streaked onto Mueller Hinton Agar (Oxoid) following which antimicrobial discs (Oxoid) were positioned onto the plates. The panel of antimicrobials tested was selected in such a way as to ensure that each of the classes of antimicrobials available as mastitis remedies was represented. Selection was also to a certain extent restricted by the availability of published interpretive data. The panel of antimicrobials tested is summarised in Table 1.

**RESULTS**

The susceptibility data of the 90 *S. aureus* isolates evaluated during the assessment period are summarised in Table 2, while the percentage of isolates showing resistance to the different antimicrobials tested is graphically depicted in Fig. 1.

Only 8 of the 90 *S. aureus* isolates tested (8.9%) were found to be susceptible to all of the antimicrobials evaluated. Overall the greatest degree of resistance was observed to the beta-lactam antibiotics penicillin (47.8%) and ampicillin (65.6%). Minimal resistance (1.1%) to oxacillin, cephalothin and trimethoprim/sulfamethoxazole was observed.

Seventeen (18.9%) of the isolates tested were found to be resistant to 3 or more antimicrobials. The susceptibility patterns of these multi-drug-resistant isolates are summarised in Table 3.

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**Table 1: Antimicrobials evaluated during this investigation**

<table>
<thead>
<tr>
<th>Antimicrobial class</th>
<th>Class representative</th>
<th>Disk concentration</th>
<th>Disk concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Natural penicillin</td>
<td>Penicillin</td>
<td>10 IU</td>
<td></td>
</tr>
<tr>
<td>Aminopenicillin</td>
<td>Ampicillin</td>
<td>10 µg</td>
<td></td>
</tr>
<tr>
<td>Penicillinase-resistant penicillins³</td>
<td>Oxacillin</td>
<td>1 µg</td>
<td></td>
</tr>
<tr>
<td>First-generation cephalosporin</td>
<td>Cephalothin</td>
<td>30 µg</td>
<td></td>
</tr>
<tr>
<td>Aminoglycoside</td>
<td>Streptomycin</td>
<td>10 µg</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Neomycin</td>
<td>30 µg</td>
<td></td>
</tr>
<tr>
<td>Tetracycline</td>
<td>Tetracycline</td>
<td>30 µg</td>
<td></td>
</tr>
<tr>
<td>Potentiaded sulphonamide</td>
<td>Trimethoprim/sulfamethoxazole</td>
<td>1.25 µg/23.75 µg</td>
<td></td>
</tr>
<tr>
<td>Fluoroquinolone</td>
<td>Enrofloxacin</td>
<td>5 µg</td>
<td></td>
</tr>
<tr>
<td>Macrolide</td>
<td>Tylosin</td>
<td>15 µg</td>
<td></td>
</tr>
</tbody>
</table>

†It is standard practice to select and test a representative antimicrobial from each class. Test results for the chosen antimicrobial are therefore representative of the entire class.

‡The disc concentration has no practical significance; it is used for in vitro testing purposes.

³Methicillin resistance is evaluated in the laboratory by testing the susceptibility of isolates to oxacillin. The use of oxacillin is favoured as the antimicrobial has better storage stability and is more reliable in the detection of MRSA strains.

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**Table 2: Susceptibility data for *Staphylococcus aureus* isolates (n = 90).**

<table>
<thead>
<tr>
<th>Antimicrobial</th>
<th>Percentage</th>
<th>Sensitive</th>
<th>Intermediate</th>
<th>Resistant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Penicillin</td>
<td>52.2 0 47.8</td>
<td>52.2</td>
<td>0</td>
<td>47.8</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>34.4 0 65.6</td>
<td>34.4</td>
<td>0</td>
<td>65.6</td>
</tr>
<tr>
<td>Oxacillin</td>
<td>95.6 3.3 1.1</td>
<td>95.6</td>
<td>3.3</td>
<td>1.1</td>
</tr>
<tr>
<td>Cephalothin</td>
<td>98.9 0 1.1</td>
<td>98.9</td>
<td>0</td>
<td>1.1</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>61.1 22.2 16.7</td>
<td>61.1</td>
<td>22.2</td>
<td>16.7</td>
</tr>
<tr>
<td>Neomycin</td>
<td>77.8 16.7 5.6</td>
<td>77.8</td>
<td>16.7</td>
<td>5.6</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>60 28.9 11.1</td>
<td>60</td>
<td>28.9</td>
<td>11.1</td>
</tr>
<tr>
<td>Trimethoprim/sulfamethoxazole</td>
<td>98.9 0 1.1</td>
<td>98.9</td>
<td>0</td>
<td>1.1</td>
</tr>
<tr>
<td>Enrofloxacin</td>
<td>91.1 7.8 1.1</td>
<td>91.1</td>
<td>7.8</td>
<td>1.1</td>
</tr>
<tr>
<td>Tylosin</td>
<td>91.1 6.7 2.2</td>
<td>91.1</td>
<td>6.7</td>
<td>2.2</td>
</tr>
</tbody>
</table>

---

**Fig. 1: Resistance of *Staphylococcus aureus* (n = 90) isolates to the different antimicrobial drugs tested.**
ent provinces were screened for anti-
Aureus University of Pretoria, a number of
by the Faculty of Veterinary Science of the
and Monitoring Programme coordinated
lates tested were resistant to penicil-
mammary preparations available are
97 % of the intramammary preparations
antimicrobials used in the dairy industry;
The extensive resistance to this class of
strains was to the beta-lactams.
As part of a recent National Surveillance
Monte Carlo, while studies in Denmark,
Brazil and Argentina reported figures of
obtained in similar studies carried out
elsewhere. A study in China reported
obtained are in agreement with those
countries, all studies indicated that the
greatest resistance observed amongst
S. aureus strains was to the beta-lactams.
The extensive resistance to this class of
antimicrobials is not altogether surprising
considering the fact that the penicillins
are 1 of the oldest groups of antimi-
crobials and have been available for many
years. They are among the most common
antimicrobials used in the dairy industry;
97 % of the intramammary preparations
available in South Africa are penicillins or
penicillin-dihydrostreptomycin combi-
nations. The remaining 3 % of intra-
mammary preparations available are
tetracyclines and cephalosporins.

Table 3: Resistance patterns of Staphylococcus aureus strains showing multi-drug resistance.

<table>
<thead>
<tr>
<th>Antimicrobials tested</th>
<th>Number of isolates</th>
<th>Number of antimicrobials resistant to</th>
<th>Number of isolates with the same resistant pattern</th>
</tr>
</thead>
<tbody>
<tr>
<td>Penicillin</td>
<td></td>
<td>3</td>
<td>R S S S/I</td>
</tr>
<tr>
<td>Oxacillin</td>
<td></td>
<td>2</td>
<td>R S S S/I</td>
</tr>
<tr>
<td>Cephalothin</td>
<td></td>
<td>1</td>
<td>R S S S R S S S R S R S</td>
</tr>
<tr>
<td>Streptomycin</td>
<td></td>
<td>1</td>
<td>S R S S I</td>
</tr>
<tr>
<td>Neomycin</td>
<td></td>
<td>1</td>
<td>S R S S</td>
</tr>
<tr>
<td>Tetracycline</td>
<td></td>
<td>1</td>
<td>S R S S</td>
</tr>
<tr>
<td>Trimethoprim/</td>
<td></td>
<td>1</td>
<td>S R S S</td>
</tr>
<tr>
<td>Sulphonamide</td>
<td></td>
<td>1</td>
<td>S R S S</td>
</tr>
<tr>
<td>Enrofloxacin</td>
<td></td>
<td>1</td>
<td>S R S S</td>
</tr>
<tr>
<td>Tylosin</td>
<td></td>
<td>1</td>
<td>S R S S</td>
</tr>
</tbody>
</table>

3According to CLSI guidelines, MRSA strains should be reported as being resistant to all beta-lactam antimicrobials. Although the zone size obtained indicated that this isolate was susceptible to cephalothin, the result was reported as “resistant”.
S = sensitive; I = intermediate; R = resistant.

DISCUSSION
A high percentage of the isolates tested
were resistant to the beta-lactam antibiot-
ics penicillin and ampicillin. The figures
obtained are in agreement with those
obtained in similar studies carried out
elsewhere. A study in China reported
that 77.3 % of the S. aureus mastitis iso-
lates tested were resistant to penicil-
lin/ampicillin, while studies in Denmark,
Brazil and Argentina reported figures of
75 %, 55.1 % and 40 %, respectively.
Although the percentage resistance to
this class of drugs has varied between
countries, all studies indicated that the
greatest resistance observed amongst
S. aureus strains was to the beta-lactams.
Unfortunately certain groups of bacte-
ria, including the staphylococci, have
evolved new strategies that led to the
emergence of methicillin-resistant strains.
This has had the greatest impact in
human medicine, where methicillin-
resistant S. aureus (MRSA) has emerged
as a major nosocomial pathogen. Until
recently the problem was limited to hos-
pitals, but the MRSA strains have started
to spread in the human community at
large. The presence of MRSA strains has
been reported in animals but accounts of
isolations from dairy cattle have been
rare. One of the isolates evaluated during
this investigation was found to be resis-
tant to oxacillin, and by virtue of group
representation, methicillin resistant. Two
further isolates were found to be moder-
ately susceptible to oxacillin. The presence
of MRSA strains is hereby confirmed in
KZN dairy herds but to date this particular
resistance pattern appears to have a
limited distribution. Owing to the public
health significance of MRSA, ongoing
monitoring for methicillin-resistant
strains in the dairy industry is warranted.
Seventeen of the S. aureus isolates tested
were found to be multi-drug-resistant, i.e.
resistant to 3 or more of the antimicrobials
tested. One of these isolates was in fact
found to be resistant to 5 of the anti-
microbials, namely penicillin, ampicillin,
oxacillin, tetracycline and the potentiated
sulphonamide (trimethoprim/sulphameth-
oxazole). The isolate was found to be sen-
tive to cefotaxin but according to
CLSI guidelines methicillin-resistant
staphylococci should be reported as resis-
tant to all beta-lactams, including cephal-
osporins, despite any apparent in vitro sus-
cceptibility. The occurrence of multi-drug-
resistant strains is a cause of great concern
as these strains may be readily transmit-
ted to other dairy cows or even workers in
the dairy. Treatment options are limited
and culling of animals becomes necessary
to remove these strains from circulation.
The disc diffusion method is 1 of the
standardised protocols recommended by

<table>
<thead>
<tr>
<th>Number of antimicrobials resistant to</th>
<th>Number of isolates</th>
<th>Number of isolates with the same resistant pattern</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>12</td>
<td>R S S S/I</td>
</tr>
<tr>
<td>6</td>
<td>R S R S S/I R S S/I</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>R S S S/I</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>R S S S R S S S R S S</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>S R S S I</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>S R S S</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>S R S S</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>S R S S</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>S R S S</td>
<td></td>
</tr>
</tbody>
</table>
the CLSI for the in vitro antimicrobial susceptibility testing of bacterial isolates. The method has found application in many diagnostic laboratories due to ease of use, flexibility and economic feasibility. It needs to be borne in mind that all in vitro tests were initially developed and used in the human diagnostic field. Therefore, most of the interpretive data available for use are derived from studies carried out in human medicine. To date very little research has been carried out using veterinary isolates and evaluating the clinical efficacy of antimicrobials against these isolates in different animal hosts. Diagnostic results should therefore be used as a guideline only. A further consequence of the lack of experimental data in animal hosts is that there are limited interpretative data available that permit the synergistic effect of different drug combinations to be assessed in vitro.

Ultimately the in vivo success of antimicrobial therapy is reliant upon multiple factors. In addition to the virulence of the causative agent, the physicochemical characteristics of the drug, the prevailing udder microenvironment and the in vivo interaction of all components significantly influence the success of treatment. Where S. aureus is implicated, the successful treatment of infected animals is significantly compromised by the strategies of a cunningly resourceful pathogen.

CONCLUSION

Staphylococcus aureus has gained notoriety in the dairy industry due to its success as a pathogen and its consequent impact on animal health and profit margins. One of the principal approaches adopted in combating S. aureus is the administration of antimicrobials. Few accounts exist that document the susceptibility of S. aureus strains implicated in bovine mastitis to the different classes of antimicrobials used in the local dairy industry. Although treatment guidelines will ultimately be based on the susceptibility pattern of the specific S. aureus strain implicated in a particular herd, the data obtained from this study may provide practitioners with an insight into existing resistance patterns and assist when immediate treatment action needs to be taken. The data obtained from this investigation indicate that resistance to the beta-lactam antibiotics, penicillin and ampicillin, is common. Resistance to the other classes of antimicrobials tested varies between 1.1% and 16.7%. The detection of strains exhibiting intermediate susceptibility and, in 1 case, resistance to methicillin is a cause for concern, as is the occurrence of multi-resistant strains which are present within some KwaZulu-Natal dairy herds.

The data obtained in this study support the need for continued rigorous monitoring of drug resistance patterns. This will ensure that appropriate treatment regimens are implemented that will reduce the indiscriminate use of antimicrobials that has often been implicated in the emergence of resistant bacterial strains.

ACKNOWLEDGEMENTS

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