East African Medical Journal Vol. 79 No. 1 January 2002
ANTIMICROBIAL SUSCEPTIBILITY OF STAPHYLOCOCCI SPECIES FROM COW FOREMILK ORIGINATING FROM DAIRY FARMS AROUND GABORONE, BOTSWANA
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C. GUTA, T.K. SEBUNYA and B.A. GASHE

ABSTRACT

Objective: To determine the prevalence of antibiotic susceptibility of _Staphylococcus_ species isolated from foremilk samples.
Setting: Milk was collected from five farms within a 70 km radius of Gaborone, Botswana.
Subjects: Two hundred and twenty five staphylococci isolates from foremilk samples.
Main outcome measures: Antibiotic susceptibility tests to penicillin G, ampicillin, tetracycline, erythromycin, cephalothin, chloramphenicol, methicillin, gentamicin and vancomycin.
Results: The susceptibility patterns of the staphylococcal strains to the antibiotics were as follows: penicillin G (47.1%), ampicillin (58.7%), tetracycline (62.7%), erythromycin (72%), cephalothin (72.9%), chloramphenicol (79.1%), methicillin (86.2%), gentamicin (88.9%) and vancomycin (100%). Lower susceptibility to chloramphenicol, methicillin and gentamicin was displayed by _Staphylococcus epidermidis, S. haemolyticus_ and _S. saprophyticus_. Only 19 (8.5%) of the isolates were susceptible to all the antibiotics tested. The most common multiple resistance patterns encountered were penicillin-ampicillin (9.3%), penicillin-erythromycin-ampicillin (6.1%) and erythromycin-tetracycline - ampicillin (3.6%).
Conclusion: Most of the _Staphylococcus_ isolates were resistant to one or more of the antimicrobial agents, with none being resistant to vancomycin. Inappropriate use of antibiotics is suspected to be a major contributory factor in the relatively high level of resistance to antimicrobial agents observed in this study. Therefore, milk can act as a very good source of antibiotic resistant _Staphylococcus_ species posing a threat to consumers.

INTRODUCTION

Antibiotics, which became widely available in the 1940s, have been labelled "magic bullets" able to eliminate bacteria, without doing much harm to individuals. They have found widespread use, at sub-therapeutic levels, in animal production(1-3). They are used to increase growth rates, improve yields and prevent the spread of diseases among herds(2,4). However, several researchers have reported on an increase in antimicrobial resistance of pathogens due to the practice of dosing animals with sub-therapeutic concentrations of antibiotics(1-4).

The multiplication of the resistant pathogens, together with the potential for lateral transfer of resistant elements(5), leads to the development of bacterial communities that are largely resistant to a wide spectrum of antibiotics. Cattle can be a source of antibiotic resistant strains for humans with a possible interchange of strains between humans and animals(6,7). It has also been reported that misuse of antibiotics in the treatment of bovine mastitis has resulted in the development of resistance among organisms responsible for the condition(8-10).

Resistance of *Staphylococcus* species to antibiotics has been extensively studied(8,14-17). In Rather *et al's*(16) study, overall all staphylococci isolates were susceptible to chloramphenicol, amikacin and gentamicin. *Staphylococcus saprophyticus* was generally more resistant to penicillin (100%), methicillin (66.7%), tetracycline (66.7%), erythromycin (66.7%) and novobiocin (100%). In another study, by Adesiyun *et al*(8), 49% of the *Staphylococcus aureus* strains tested were resistant to one or more of eight antimicrobial agents with resistance being high to penicillin (48%), ampicillin (45%), methicillin (21%) and cephalothin (17%), but none were resistant to gentamicin, chloramphenicol and neomycin. However, Abbar *et al*(14) reported a 5.66% resistance to chloramphenicol, among staphylococcal species. Myllys *et al*(17) reported an overall increase in resistance to antimicrobial agents from 36.9% in 1988, to 63.6% in 1995 for *S. aureus* and with coagulase negative staphylococci (CNS) from 26.6% to 49.7%.

This study was therefore carried out to determine the susceptibility patterns of staphylococci isolated from foremilk originating from cows at selected farms around Gaborone, Botswana.

MATERIALS AND METHODS

Sample collection: Foremilk was collected from five dairy farms within a 70 km radius of Gaborone. Each herd was visited on one, two or three occasions, depending on the number of milking cows at each farm. After washing, disinfecting and drying the teats, quarter foremilk samples were hand drawn into sterile tubes. The samples were transported to the laboratory in cooler boxes where they were kept at 4°C for use within 24 hours.

Bacteriological analyses isolation of staphylococci: Within 24 hours of sample collection, 0.1ml of an appropriate dilution of the samples in 0.01% peptone water (Oxoid) was surface plated on mannitol salt agar (Oxoid). Colonies whose cultural characteristics resembled that of staphylococci species were selected. Those found to be Gram-positive, catalase-positive cocci were stored on nutrient agar slants at 4°C for further tests.

Oxidative-fermentative (OF) test: Duplicate tubes of OF medium were inoculated with bacteria by stabbing with a straight wire. To one of the tubes of each pair, sufficient sterile liquid paraffin was added to form a layer about 10mm in depth. Tubes were incubated at 37°C for five to seven days, after which they were observed for acid production (colour change from purple to yellow).

Coagulase test: The tube coagulase test was performed using the test procedure described by Guitierrez *et al*(11). Colonies were transferred into small tubes containing 0.3 ml Brain Heart Infusion (BHI) broth (Oxoid) and incubated for 24 hours at 37°C. A mixture of the BHI culture suspension and reconstituted rabbit plasma was then incubated at 37°C and was examined after 30 minutes, 2, 4, and 24 hours for clot formation. Only firm and complete clots that stayed in place when tubes were tilted were considered positive for coagulase activity.

APISTAPH kits: The isolates were identified to species level using the APISTAPH kits (bioMerieux, France). The tests were carried out according to the manufacturer's instructions. Twenty four-hour cultures of staphylococci isolates on sheep blood agar (Oxoid) were used to inoculate the APISTAPH medium. Identification was facilitated by the use of the APISTAPH Analytical Profile Index provided by the manufacturer of the strips.

Resistance of isolates to antimicrobial agents: The Mast diagnostics antibiotics used were as follows: chloramphenicol (25 µg), erythromycin (5 µg), methicillin (10 µg), penicillin G (1 unit), tetracycline (25 µg), ampicillin (10 µg), cephalothin (5 µg), gentamicin (10 µg) and vancomycin (30 µg). Isolates were inoculated into Meuller-Hinton broth and incubated at 37°C until turbidity matched that of a 0.5 MacFarland standard. Antibiotic susceptibility testing was carried out as described by Umoh *et al*(12). After surface spreading of the standardised Broth culture (0.1 ml) on Mueller- Hinton agar plates, excess moisture was allowed to evaporate from the agar surface before placing the antibiotic discs firmly on the surface of the agar. The plates were incubated at 37°C for 24 hours, upon which the zones of inhibition were measured. The breakpoints for classifying strains as resistant or susceptible were obtained from the Performance Standards for Antimicrobial Disc Susceptibility Tests(13).

RESULTS

Of the 225 staphylococci identified, 92 (40.9%) were coagulase-positive and the remaining 133 (59.1%) were coagulase-negative. The results of the species identified and their antibiotic susceptibilities are given in Table 1. The results displayed in Table 1 show higher resistance to chloramphenicol, methicillin and gentamicin by *S. epidermidis, S. haemolyticus* and *S. saprophyticus*. For example, about forty eight per cent (47.8%), (50%) and (66.7%) of the *S saprophyticus, S. epidermidis* and *S. haemolyticus* respectively, were resistant to chloramphenicol, only 11.1%, 12.5% and 14.7% of *S. xylosus, S. hyicus* and *S. aureus* respectively, were resistant to this drug.

Table 1

Resistance of identified staphylococcal species to nine antimicrobial agents

Species	No. of isolates	% Resistance PG (1 unit)	AP (10 µg)	T (25 µg)	C (25 µg)	GM (10 µg)	MT (10 µg)	E (5 µg)	KF (5 µg)
S. aureus	68	34 (50.0)	24 (35.3)	25 (36.8)	10 (14.7)	3 (4.4)	11 (16.2)	15 (22.1)	14 (20.6)
S. xylosus	27	12 (44.4)	11 (40.7)	9 (33.3)	3 (11.1)	2 (7.4)	3 (11.1)	5 (18.5)	8 (29.6)
S hyicus	24	11 (45.8)	9 (37.5)	6 (25.0)	3 (12.5)	1 (4.2)	2 (8.3)	6 (25.0)	4 (16.7)
S saprophyticus	23	12 (52.2)	10 (43.5)	13 (56.5)	11 (47.8)	6 (26.1)	7 (30.4)	11 (47.8)	7 (30.4)
S. sciuri	17	9 (52.9)	7 (41.2)	5 (29.4)	2 (11.8)	1 (5.9)	3 (17.6)	4 (23.5)	7 (41.2)
S epidermidis	12	9 (75.0)	7 (58.3)	8 (66.7)	6 (50.0)	5 (41.7)	1 (8.3)	8 (66.7)	5 (41.7)
S. lugdunensis	11	5 (45.5)	4 (36.4)	3 (27.3)	2 (18.2)	2 (18.2)	-	2 (18.2)	2 (18.2)
S. lentus	11	6 (54.5)	4 (36.4)	3 (27.3)	1 (9.1)	1 (9.1)	1 (9.1)	3 (27.3)	2 (18.2)
S. hominis	8	4 (50.0)	3 (37.5)	2 (25.0)	1 (12.5)	1 (12.5)	–	2 (25.0)	3 (37.5)
S. cohnii	7	4 (57.1)	3 (42.9)	3 (42.9)	1 (14.3)	–	–	–	1 (14.3)
S haemolyticus	6	5 (83.3)	4 (66.7)	4 (66.7)	4 (66.7)	3 (50.0)	2 (33.3)	4 (66.7)	4 (66.7)
S. chromogenes	4	2 (50.0)	2 (50.0)	1 (25.0)	1 (25.0)	–	–	1 (25.0)	2 (50.0)
S capitis	3	3 (100)	2 (66.7)	2 (66.7)	2 (66.7)	–	–	1 (33.3)	2 (66.7)
S auricularis	2	1 (50.0)	2 (100)	–	–	–	1 (50.0)	–	–
S. simulans	2	2 (100)	1 (50.0)	–	–	–	–	1 (50.0)	–
Total	225	119 (52.9)	93 (41.3)	84 (37.3)	47 (20.9)	25 (11.1)	31 (13.8)	63 (28)	61 (27.1)

PG= Penicillin G, AP= Ampicillin, T= Tetracycline, C= Chloramphenicol, GM= Gentamicin, MT= Methicillin, E= Erythromycin, KF= Cephalothin
None of the isolates were resistant to vancomycin (30 µg)

Figure 1

Overall percentage antibiotic susceptibility of the 225 staphylococcal strains

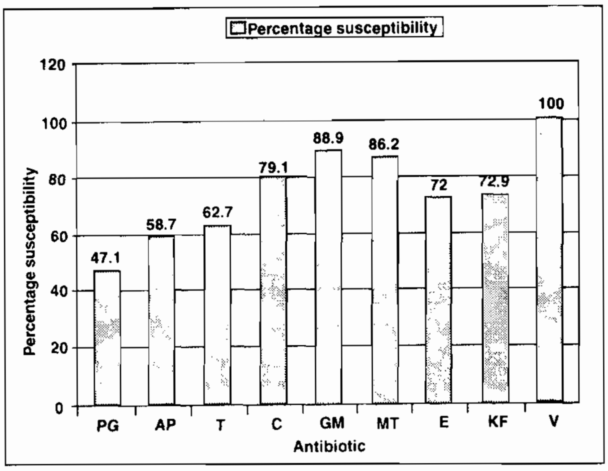

Figure 1 shows the overall susceptibility patterns of the staphylococcal strains to nine antimicrobial agents. Resistance to penicillin G (PG), ampicillin (AP), tetracycline (T), erythromycin (E) and cephalothin (KF) was relatively high among the 225 staphylococcal strains. Penicillin G, to which only 47.1% of the isolates were susceptible, was the least effective of the nine drugs tested.

Table 2

Resistance antibiograms patterns of staphylococci isolated from foremilk

Combination of drugs	No. resistant	% resistance
PG, AP	21	9.3
PG alone	16	7.1
PG, E, T	14	6.2
AP alone	10	4.4
E, T, AP	8	3.6
T alone	8	3.6
E alone	6	2.7
PG, E, T, AP	5	2.2
PG, E, AP	5	2.2
PG, T, AP	5	2.2
KF alone	5	2.2
PG, T, AP, KF	4	1.8
E,.AP	4	1.8
PG, T, KF	3	13
PG, AP, KF, C, GM	2	0.8
PG, E, KF	2	0.8
PG, E, KF, C	2	0.8
T, AP, KF	2	0.8
PG, KF	2	0.8
PG, AP, C, GM	2	0.8
MT alone	2	0.8
PG, E, T, KF, GM	2	0.8
PG, T, E, AP, KF, C	2	0.8
PG, E, T, AP, KF, C, GM	1	0.4
C alone	1	0.4
PG,E,T,AP,C	1	0.4
PG, E, T, MT, GM	1	0.4
E, AP, KF, C	1	0.4
PG, E, T, KF	1	0.4

PG= penicillin G, AP = ampicillin, T= tetracycline, C= chloramphenicol, GM = gentamicin, MT= methicillin, E= erythromycin, KF= cephalothin.
-19 (8.4%) of staphylococci were sensitive to all drugs
- All were sensitive to vancomycin

Susceptibility to the other three remaining antimicrobial agents, chloramphenicol (C), gentamicin (GM) and methicillin (MT) was generally high. All the isolates were susceptible to vancomycin (V). Of the commonly used drugs, gentamicin was the most effective drug.

Only 19 (8.4%) of the 225 staphylococcal isolates were susceptible to all nine antibiotics, 48 (21.3%) were resistant to only one of the nine agents, 58 (25.8%) to two, 59 (26.2%) to three, 28 (12.4%) to four, eight (3.6%) to five, four (1.8%) to six and only one (0.4%) was resistant to seven of the antimicrobial agents. The strain that was resistant to seven of the nine antimicrobial agents belonged to the species *S. haemolyticus*. This strain was only susceptible to methicillin and vancomycin. None of the strains belonging to the species, *S. saprophyticus, S. epidermidis, S. haemolyticus, S. chromogenes, S. capitis, S. auricularis* and *S. simulans* were susceptible to all the nine antibiotics.

Table 2 displays the multiple resistance patterns of some of the isolates to the antimicrobial agents. In situations where there was multiple resistance the most encountered drugs were penicillin, erythromycin, tetracycline, ampicillin and cephalothin. The most common multiple resistance patterns encountered were penicillin-ampicillin, penicillin-erythromycin-tetracycline and penicillin-erythromycin-ampicillin.

DISCUSSION

The susceptibilities of the characterised staphylococcal isolates to nine antimicrobial agents obtained in this study are not very different from those reported by other workers. Resistance to penicillin G, ampicillin, tetracycline, erythromycin and cephalothin was noted in 52.9%, 41.3%, 37.3%, 28.0% and 27.1% of the 225 isolates investigated, with a smaller percentage showing resistance to chloramphenicol (20.8%), gentamicin (11.1%), methicillin (13.8%). All the Staphylococcus species were sensitive to vancomycin. The results for penicillin, cephalothin, gentamicin, vancomycin, ampicillin and tetracycline resistance tend to agree with findings by other workers(8,14-16).

In contrast, Garcia *et al*(15) reported a 3.3% resistance rate among bovine strains to erythromycin, Abbar *et al*(14) reported 9.43%, with the current investigation reporting a 28.0% resistance rate to this drug. Given the fact that these findings by other workers were reported 15 - 20 years back, significant changes might have occurred in the effectiveness of this drug against bovine staphylococci strains. This assumption is supported by Myllys *et al*(17) who reported an increase in resistance to antimicrobial agents from 36.9% in 1988, to 63.6% in 1995 for *S. aureus* and with coagulase negative staphylococci species from 26.6% to 49.7%. Resistance to chloramphenicol observed in this study is higher (20.8%) than previously reported by other workers(8,16). The higher resistance in this study is, however, supported by Abbar *et al*(14), who despite observing a lower resistance

(5.7%) to this drug predicted higher resistance in future. The prediction was because chloramphenicol was the drug of choice for treatment of bovine staphylococci mastitis hence the projected higher incidences of abuse resulting in development of resistance to the drug. The differences can also be attributed to variations in concentration of the antibiotics used, the possibility of resistance transfer among strains and the frequency of use and abuse of a particular antibiotic in a given location(12).

The susceptibility tests also showed that *S. epidermidis, S. haemolyticus,* and *S. saprophyticus* were generally the least susceptible isolates to most of the antimicrobial agents used. This is in agreement with findings of Rather *et al*(16) and Adesiyun *et al*(8). The most active microbial agent (besides vancomycin), according to this study, was gentamicin, which was effective against 88.9% of the isolates tested for antibiotic susceptibility. Even though there are worrying reports of the emergence of human *S. aureus* strains resistant to the wonder drug, vancomycin(18,19), no resistance to this antibiotic was encountered among bovine strains investigated in this study.

The resistance patterns to antibiotics observed in this study are worrying, given reports that milk can act as a very good source for the transfer of antibiotic-resistant staphylococci(20). It is, therefore, important to conduct antibiotic susceptibility tests on mastitis staphylococci so that the most effective antibiotic(s) can be selected for the treatment of affected cows. Inappropriate use of antibiotics by farm workers is suspected to be a major contributory factor in the appearance of resistant strains. A comprehensive educational programme to both farmers and farm workers on the use of antibiotics may, therefore, result in a drastic reduction in prevalence of resistance to antimicrobial agents among staphylococci species isolated from milk in Botswana.

ACKNOWLEDGEMENTS

Mr. C. Gupta was supported by the Belgian Government SADC Scholarship fund. Technical support was provided by Mr. Nkosi Ndabambi at the Department of Biological Sciences, University of Botswana, Gaborone.

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