

SENSITIVITY TO COMMONLY USED ANTIBIOTICS OF *STAPHYLOCOCCUS AUREUS* STRAINS ISOLATED FROM MASTITIC COWS.

Machang'u, R.S. and Mlinga, N.J. *Department of Microbiology and Parasitology, Faculty of Veterinary Medicine, Sokoine University of Agriculture, Morogoro, TANZANIA*

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SUMMARY

Twenty one clinically healthy cows were screened for subclinical mastitis. All the cows were in lactation and were maintained at the Sokoine University dairy units. Of 84 udder quarters examined, 37 (44.0 %) were positive for mastitis by the California mastitis test (CMT). *Staphylococcus aureus* was present in 18 quarters. Staphylococci were isolated and tested for sensitivity against antibiotics (cephalosporin, chloramphenicol, erythromycin, gentamicin, kanamycin, neomycin, penicillin, streptomycin, tetracycline). Cephalosporin and gentamicin showed the largest zones of growth retardation, whereas penicillin, tetracycline and chloramphenicol showed the least retardation effect against *S. aureus*. Our findings emphasize the importance of carrying out *in vitro* sensitivity tests before instituting antibiotic treatment.

INTRODUCTION

Treatment of bacterial diseases with antibiotics has become a routine in the veterinary practice. This application of antibiotic therapy has increased proportionately with the emergence of drug resistant organisms, whose numbers have risen considerably in recent years. As early as two decades ago, workers in different countries have observed that the population of antibiotic resistant bacteria found in livestock has become disturbingly high (Cookie *et al.*, 1971; Falkow, 1970; Huber *et al.*, 1971). This situation has necessitated collection of epidemiological information on the prevalence of pathogenic microorganisms resistant to various antibiotic and chemotherapeutic agents.

Antibiotic resistant organisms often maintain atypical conditions (e.g. subclinical mastitis in cows) which may go undetected for a long time. In view of the fact that most mastitic infections in animals are probably treated without the benefit of information derived from antibiotic sensitivity tests, we deemed it

useful to compile data on susceptibility patterns of *S. aureus* organisms isolated from subclinical mastitis at Sokoine University of Agriculture (SUA) dairy farms (1984-1985).

MATERIALS AND METHODS

Isolation of bacterial strains:

Twenty one clinically healthy lactating dairy cows from two units at SUA, Morogoro, were examined for mastitis. The breed and age of these animals were not taken into consideration. Screening was by CMT (Schalm and Noorlander, 1957; Brooks *et al.*, 1982). About 0.5 ml of each CMT positive milk sample was passed onto nutrient agar (NA) plates containing 8% NaCl to facilitate the growth of staphylococci (Collins, 1967). The plates were left for 18-24 h. at 37°C and then examined for bacterial growth and colony morphological characteristics.

Characterization of bacterial isolates:

Colonies from each NA plate were grown on blood agar (BA) plates overnight at 37°C. Smears were then prepared from representative colonies for Gram staining. Cellular morphology was studied microscopically under oil immersion objective (x 1000). Biochemical assays carried out to confirm *S. aureus* were the catalase, coagulase, and mannitol fermentation tests. For the mannitol fermentation test, mannitol agar containing the indicator bromthymol blue (0.5%) was used.

Antibiotic susceptibility of isolated *S. aureus* strains:

Bacterial strains confirmed to be *S. aureus* and which were coagulase positive were tested for antibiotic susceptibility using disc sensitivity test (DST) agar (Difco Laboratories, Detroit). Bacterial colonies were initially suspended in peptone water and 1 ml aliquots used to flood the DST agar surface. Excessive fluid was drained off using sterile Pasteur pipettes and the plates were covered and left to dry at room temperature (20-30 min). Antibiotic discs (Taastrup, Denmark) containing cephalosporin, chloramphenicol, erythromycin, gentamicin, kanamycin, neomycin, penicillin, streptomycin and tetracycline were placed equidistantly on the agar to allow uniform diffusion of the drugs. After overnight incubation (37°C) the diameter of zones of growth inhibition were measured using a millimetre scale. The test was repeated three times and the median value of inhibition for each antibiotic was recorded.

RESULTS

The CMT results for the 84 udder quarters examined were as shown in Table 1. Table 2 shows the morphological and biochemical characteristics of isolated *S. aureus* strains. Table 3 shows the variability of sensitivity to

antibiotics of the *S. aureus* strains and Figure 1 shows the relative (%) numbers of isolates resistant to the antibiotics tested.

A wide variation in the effectiveness of the antibiotics tested was demonstrated against *S. aureus*. The best inhibitory effect was shown by cephalosporin and gentamicin. The majority (77.8%) of the tested strains (n = 18) were highly sensitive to cephalosporin. The remaining strains were moderately sensitive. All strains were moderately sensitive to gentamicin. The widest range (from highly sensitive to resistant) was recorded against penicillin (27.8% highly sensitive and 38.9% resistant) and ampicillin (22.2% highly sensitive and 44.4% resistant). The highest resistance (77.7% strains) was recorded against tetracycline and chloramphenicol. The other drugs (erythromycin, kanamycin, neomycin, streptomycin) were either moderately effective or not effective against the isolates, (Table 3), with neomycin and kanamycin showing relatively fewer resistant *S. aureus* isolates than streptomycin or erythromycin.

DISCUSSION

Antibiotic sensitivity tests are simple laboratory models, which do not necessarily simulate the real body conditions of the host animal. However, they are the best *in vitro* indicators for determining the antibiotic(s) of choice before treatment of bacterial disease may begin. Sensitivity of a microorganism may vary considerably depending on the host species from where it has been isolated (Hareharan *et al.*, 1974; Biberstein *et al.*, 1974).

Bacterial organisms of the same species, isolated from one and the same animal host but from different bouts of infection may vary in their sensitivity to antibiotics. Also, factors related to seasons and geographical zones may

Table 1. California mastitis test (CMT) of milk samples collected at the Sokoine University of Agriculture dairy farms

Cow Number	RF	RH	LF	LH
1	+	-	+	+
2	-	-	+	-
3	+	+	+	-
4	-	+	+	-
5	-	-	-	-
6	+	-	+	-
7	-	+	-	-
8	-	-	-	-
9	+	+	+	+
10	+	-	-	-
11	+	+	-	+
12	+	-	-	-
13	+	+	+	-
14	+	-	+	-
15	+	-	+	+
16	+	+	+	+
17	-	+	-	+
18	-	-	-	+
19	-	-	+	-
20	-	-	-	-
21	-	-	-	-

RF = right fore quarter,
 LF = Left fore quarter,
 + = CMT positive,

RH = right hind quarter
 LH = left hind quarter
 - = CMT negative.

Table 2. Biochemical reactions of bacterial isolates (*) from California mastitis test positive milk samples

Sample number	Coagulase test		Mannitol fermentation
	slide test	tube test	
1	+	++	+
2	+	+	+
3	+	++	+
4	++	++	+
5	++	++	+
6	++	++	+
7	-	-	-
8	++	++	+
9	-	-	-
10	-	-	-
11	-	-	-
12	-	-	-
13	++	++	+
14	-	+	+
15	-	-	-
16	-	-	-
17	-	-	-
18	-	-	-
19	++	++	+
20	+	+	+
21	-	-	-
22	-	++	+
23	+	++	+
24	+	++	+
25	-	-	-
26	+	+	+
27	+	+	+
28	-	-	-
29	-	-	-
30	-	-	-
31	-	++	+
32	-	++	+
33-37	ND	ND	ND

(*) All isolates were Gram +ve and tested +ve to catalase test. ND = not determined.

Table 3. Susceptibility to antibiotics of *Staphylococcus aureus* isolates

Sample No.	Am.	Ch.	Cp.	Er.	Ge.	Ka.	Ne.	Pe.	St.	Te.
1	R	R	S+	R	S	S	S	S	R	R
2	R	R	S	R	S	S	S	R	R	R
3	S	S	S+	S	S	S	S	S	S	R
4	S	R	S+	R	S	S	S	S	S	R
5	S	R	S+	S	S	S	S	S	R	R
6	R	R	S+	S	S	S	R	S+	R	R
7	S+	R	S+	S	S	S	S	S+	S	R
8	S+	S	S+	S	S	S	S	R	S	S
9	R	R	S	R	S	S	S	R	S	S
10	S	R	S+	R	S	R	S	R	S	R
11	R	S	S+	R	S	S	S	R	S	R
12	R	R	S	R	S	S	S	S	S	R
13	S	R	S+	R	S	R	S	R	R	R
14	R	S	S+	S	S	S	S	S+	S	S
15	S+	R	S+	S	S	S	S	S+	S	S
16	S+	R	S+	S	S	S	R	S+	S	R
17	R	R	S	S	S	S	S	R	S	R
18	S	R	S+	S	S	S	S	S	S	R

R = resistant (< 20 mm), S = sensitive 20-30 mm,
S+ = Strongly sensitive >30 mm

Am = Ampicillin,
Er = Erythromycin,
Ne = Neomycin,
Te = Tetracycline.

Ch = Chloramphenicol,
Ge = Gentamicin,
Pe = Penicillin,

Cp = Cephalosporin,
Ka = Kanamycin,
St = Streptomycin,

affect the sensitivity of microorganisms to antibiotics (Gay, 1977).

Huber *et al.* (1977) found that strains of *S. aureus* resistant to cephalosporin, were also resistant to other antibiotics of the penicillin group. Our findings have shown that of the four isolates which were moderately sensitive to cephalosporin three were resistant to penicillin and ampicillin. Resistance of *S. aureus* strains to penicillin was shown by

seven strains (38.8 %).

Elsewhere resistance of *S. aureus* to penicillin was reported to be as high as 75% (Finland, 1955).

The relatively high degree of resistance of the *S. aureus* isolates to tetracycline and chloramphenicol was probably due to frequent exposure of the strains to suboptimal dosage of these antibiotics in previous treatments. These

two antibiotics are the most frequently used broad spectrum antimicrobials for the treatment of mastitis. The resistance observed among *S. aureus* isolates was probably genotypic due to enzymic adaptation, or could be R-factor mediated (Biville, 1977).

Lack of a wide variety of antibiotics often compels the clinician to use available broad spectrum antibiotics erroneously. The dairy farms under study have predominantly used intramammary infusions with tetracycline in the treatment of mastitis not because tetracycline was the most effective but because it was readily available. Although such usage of broad spectrum antibiotics may appear time, material, and even cost effective, it may in the long run lead to big losses due to emergence of resistant strains and the development of

persistent subclinical mastitis.

The use of tetracycline and chloramphenicol for treatment of mastitis is widely practised in Tanzania and hence our findings may be regarded a reflection of the general situation in farms other than those of SUA. These findings further recommend the use of cephalosporin, gentamicin, penicillin and ampicillin, in that order for the treatment of mastitis.

However, treatment should always be guided by the results of antibiograms except for emergency cases, or where an immediate possibility exists for carrying out this antimicrobial test. In the latter cases, however, sensitivity tests should later be sought from Veterinary Investigation Centres.

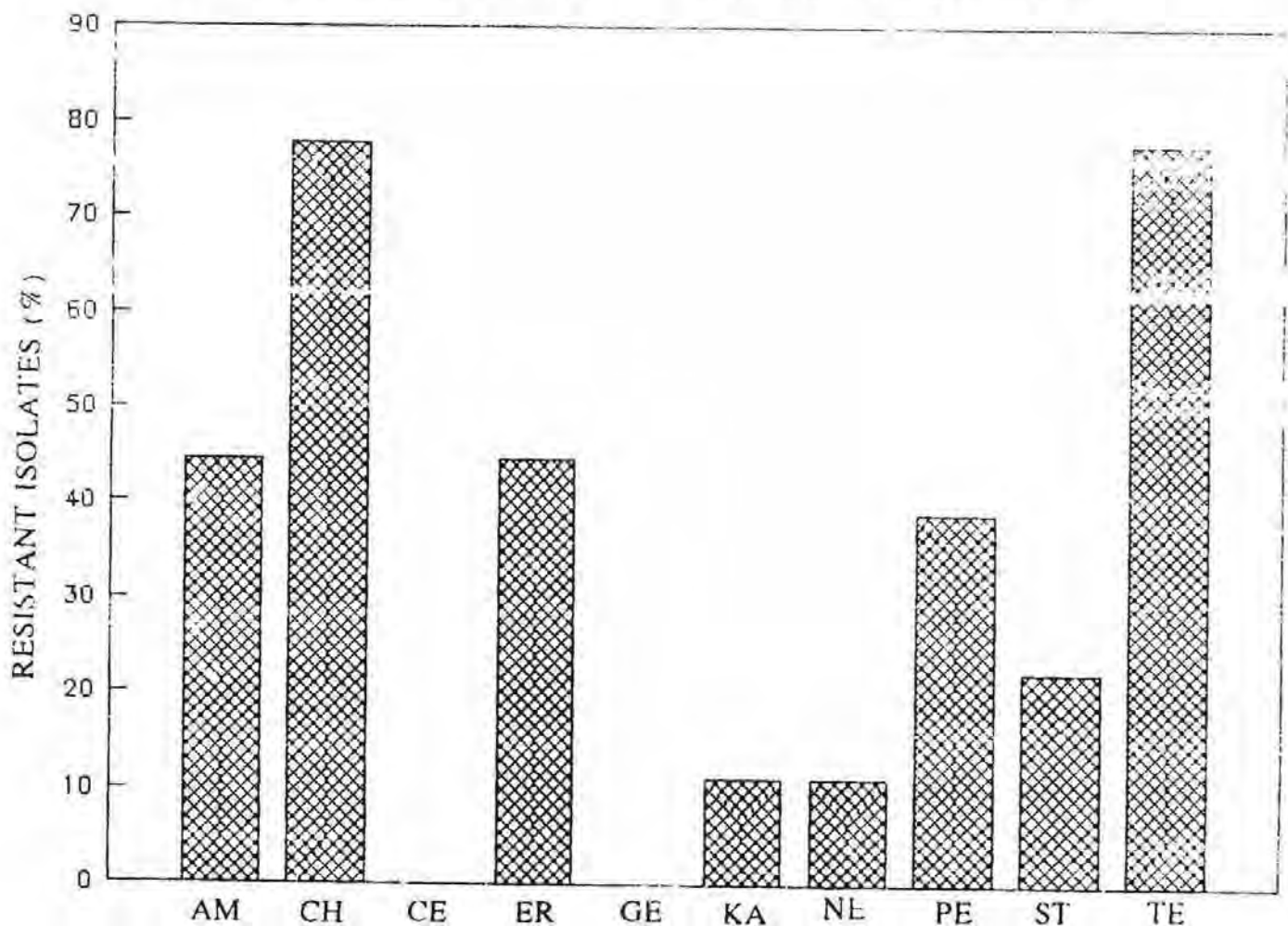


Figure 1: Relative number (%) of *Staphylococcus aureus* isolates resistant to antibiotics. Am = Ampicillin, Ch = Chloramphenicol, Cp = Cephalosporin, Er = Erythromycin, Ge = Gentamicin, Ka = Kanamycin, Ne = Neomycin, Pe = Penicillin, St = Streptomycin, Te = Tetracycline.

If resistance to one antibiotic emerges, a replacement with another effective antimicrobial should be done, taking care not to substitute between closely related antibiotics. Through such a regime, bacterial mastitis could be successfully controlled.

The following additional points, if observed should assist in the prevention of the emergence of resistant bacterial strains:

- i) treatment should be initiated as early as possible in the course of infection.
- ii) the use of antibiotics without a well documented effective dosage should be avoided. Never economize on drugs by reducing dosage or by changing treatment regime.
- iii) periodic (annual or biannual) survey of all dairy herds should be done to detect subclinical disease. Initiation of mass treatment during the dry period of the herds is useless without the prior survey of the ever changing bacterial populations.
- iv) tetracycline, chloramphenicol, or other related broad spectrum antimicrobials should not be used for treatment of mastitis unless if strictly recommended by antibiograms; for after all broad spectrum antibiotics are meant to be used only after other target antibiotics have proved ineffective.

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