



**UNIVERSITI PUTRA MALAYSIA**

**THE PREVALENCE OF ANTIBIOTIC RESISTANT  
BACTERIA IN MILK FROM MASTITIC AND NON MASTITIC CASES**

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THE PREVALENCE OF ANTIBIOTIC RESISTANT  
BACTERIA IN MILK FROM MASTITIC AND NON MASTITIC CASES

BY

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ABSTRACT

Sixty cows from three established farms in Malaysia were randomly selected and they were subjected to the California mastitis test to determine the presence of mastitis. The results obtained showed that the prevalence of mastitis in the three farms were very high (81.67%).

Out of 127 organisms isolated, 36 were Staphylococcus pyogenes, 20 Micrococcus spp, 16 Corynebacterium spp, 13 Staphylococcus epidermidis and 10 Streptococcus spp. The predominant organism isolated in each farm was Staphylococcus pyogenes. In Farm A 27.3 percent of the isolates were Staphylococcus pyogenes while in Farms B and C, it was 35 and 23.3 percent respectively. Nearly all of the organisms isolated from the three farms showed resistance to Penicillin. However, all the isolates tested showed complete sensitivity to Gentamycin except for one isolate of Pseudomonas aeruginosa. Fourteen out of 36 Staphylococcus pyogenes isolated showed resistance to Penicillin, while nine and seven isolates showed resistance to Chloramphenicol and Sulfonamides respectively. Streptococcus spp showed a high resistance to Sulfonamides and Tetracycline. In addition to Penicillin, the Staphylococcus pyogenes isolated in Farm A was resistant to Chloramphenicol and Tetracycline whilst in Farms B and C it was also resistant to Sulfonamides.

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## INTRODUCTION

Mastitis is one of the most important economic diseases affecting dairy herds. Losses are incurred through milk discard, cost of treatment, decrease milk yield and long term effects from damage to the cow's udders. Mastitis preventive programs are aimed at reducing the incidence of mastitis. Even with good preventive programs, cases of mastitis do occur and require treatment. Antibiotics play an important role in the treatment of these cases.

Antibiotic sensitivity test is used to determine the most suitable antibiotics for therapy. However, the correlation between antibiotic sensitivity in vitro and clinical response is poor. (Davidson et al, 1982). If the mastitic pathogens are resistant to the antibiotics in vitro, the probability of successful treatment with that antibiotics will be poor.

The emergence of antibiotic-resistant bacteria in bovine milk is a serious problem. Some mastitic pathogens are resistant to one or more antibiotics. This study was conducted in view of the relatively scanty information on the prevalence of antibiotic resistant bacteria from mastitic cases in Malaysia and their antibiotic resistant patterns. The main objectives of this study are to examine the prevalence of bacteria in bovine milk and to determine the antibiotic resistant patterns amongst these bacteria.

## LITERATURE REVIEW

Studies on bovine mastitis have indicated high incidence of infection in many countries. Guzman et al (1982) in Cuba, reported 70 percent (641/916) of cows from four machine-milked herds had

mastitis. In Malaysia, a study of 226 animals in Kluang by Koh and Joseph (1974) showed 54.9 percent of the cows had subclinical mastitis and 2.6 percent had clinical mastitis.

Madariaga-aguilar and Lopez-alvarez (1979) in Mexico isolated several genera of organisms from cases of bovine mastitis. Twenty-seven percent of the organisms were made up of Staphylococcus pyogenes whilst 19 percent were Streptococcus agalactiae. The other organisms isolated were Bacillus spp, Staphylococcus epidermidis and Escherichia coli. In India, Verma and Mishra (1977) found Staphylococcus pyogenes and Staphylococcus epidermidis as the major organisms causing bovine mastitis. Streptococcus agalactiae, Streptococcus uberis, Escherichia coli, Pseudomonas aeruginosa, Klebsiella spp, Corynebacterium spp and Candida spp were also isolated.

The study in Malaysia by Koh and Joseph (1974) showed that Staphylococcus pyogenes and Streptococcus agalactiae were the major organisms causing mastitis. Staphylococcus pyogenes was responsible for 26 percent of mastitis while the latter caused 28 percent. Abas and Mohd Shafit (1984) in MARDI\*, Serdang, also showed that Staphylococcus pyogenes was the predominant organism responsible for 21 percent of the mastitic cases. Staphylococcus epidermidis was next with 11 percent. Sporadic cases of mastitis were found to be due to Streptococcus agalactiae, Pseudomonas aeruginosa, Bacillus subtilis, Corynebacteria pyogenes and Escherichia coli.

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## Antibiotic Resistant Bacteria

From a study of antibiotic resistant bacteria in mastitis, Mueller and Thaller (1979) reported that 50 percent of the Staphylococcus spp isolated were resistant to Benzylpenicillin and 51 percent to Ampicillin. The Staphylococcus spp were also resistant to Tetracycline and Streptomycin. Among the Escherichia coli isolates, 26 percent were resistant to Ampicillin and 35 percent to Streptomycin and Tetracycline. The Escherichia coli was also found to be resistant to Neomycin and Chloramphenicol. The Staphylococcus spp examined by Frost and O'Boyle (1981) showed resistance to Streptomycin and Penicillin. Some of the isolates showed resistance to Erythromycin, Tetracycline and Chloramphenicol. Multiple resistance were uncommon. Davidson et al (1982) in New York reported that a high percentage of Staphylococcus pyogenes was resistance to Penicillin, Ampicillin and Dihydrostreptomycin. A lower percentage of the organism is resistant to Tetracycline. A large number of the Escherichia coli isolated was resistant to Penicillin. The organism was also resistant to Dihydrostreptomycin, Tetracycline and Ampicillin. Klebsiella spp were also resistant to Ampicillin and Tetracycline.

The study by Koh and Joseph (1974) showed that 29 percent of Staphylococcus pyogenes isolated from bovine milk in Kluang were resistant to Tetracycline and 22 percent to Penicillin. The organism was also resistant to Erythromycin, Ampicillin and Chloramphenicol. It is interesting to note that the Pseudomonas aeruginosa isolated were resistant to all antibiotics tested except

Neomycin. In a recent study, Mohd Shafit and Abas (1984) showed all Staphylococcus pyogenes isolates were resistant to Carbenicillin, Colistin and Polymyxin-B. A smaller percentage of the isolates was resistant to Ampicillin, Tetracycline and Streptomycin. The Pseudomonas spp isolated were resistant to Penicillin, Tetracycline and Chloramphenicol. They were found to be sensitive to Streptomycin and Polymyxin-B.

#### MATERIALS AND METHODS

The study was carried out on 60 lactating cows from three established farms in Malaysia. Twenty lactating cows were randomly selected from each farm. The animals were mainly Sahiwal-Friesian crosses while a small percentage were made up of Jersey, Friesian and Local Indian Dairy crosses. All of the animals were machine-milked. They were fed with grass and concentrates and were milked twice daily. This study was carried out in the month of May 1984.

##### Collection of samples

The udders of the animals were washed with water and dried. During this procedure, the mammary gland was examined for clinical mastitis. Milk was withdrawn from the four quarters into the four test wells. Gross abnormalities of the milk were examined before subjecting the milk to the California mastitis test (Appendix-A) to detect the presence of subclinical mastitis.

Collection of the milk samples for bacteriological study was done immediately after performing the California mastitis test. The teats were cleaned with 70 percent alcohol and left to dry. Milk

from all the four quarters were collected aseptically into sterile Bijou bottles. The milk samples were immediately transported to the Bacteriology Laboratory for examination. Samples from long distance were packed in ice before being taken to the laboratory.

#### Bacteriological examination

Bacteriological examination consists of isolation and identification of the bacterial organisms and testing the organisms for antibiotic sensitiveness.

The milk samples were incubated at 37°C for an hour. The contents of each bottles were thoroughly mixed before being streaked onto Blood Agar plates (5% cattle blood in Oxoid blood agar base #2, and MacConkey Agar plates (Oxoid). The Blood Agar plates were incubated under microaerophilic condition whilst the MacConkey Agar plates were incubated aerobically at 37°C for 24 hours before examination. If no growth was observed, the plates were further incubated for another 24 hours. Plates were discarded if no growth was observed after 48 hours.

After incubation, the plates were examined and representative colonies were Gram-stained and subcultured onto another Blood Agar plates before any attempt was made to identify them. Colonies on MacConkey Agar plates were classified as either lactose fermenters or non lactose fermenters. Identification of the bacteria were based on Cowan and Steel's Manual for the identification of medical bacteria (Cowan, 1974). Isolates of Staphylococcus pyogenes, Streptococcus spp and Pseudomonas aeruginosa were kept on Nutrient Agar slant (Oxoid) for antibiotic sensitivity test. These isolates were incu-

bated aerobically at 37°C for 24 hours and stored at room temperature.

#### Antibiotic sensitivity test

A modified Kirby-Bauer disk technique (Appendix-B) was used for antibiotic sensitivity testing for the selected isolates. The antibiotics tested were Ampicillin (10 mcg), Chloramphenicol (30 mcg), Erythromycin (15 mcg), Gentamycin (10 mcg), Penicillin G (10 mcg), Polymyxin-B (300 U), Streptomycin (10 mcg) and Sulfonamide compound (300 mcg). The choice of antibiotics was in accordance to the current antibiotics used for therapy.

Ampicillin was used for Gram negative isolates, Penicillin C for Gram positive isolates and Polymyxin-B for isolates of Pseudomonas aeruginosa only.

#### RESULTS

Table 1 : The prevalence of mastitis in the three study farms

| FARMS | CALIFORNIA <u>MASTITIS TEST</u> |             |
|-------|---------------------------------|-------------|
|       | NOS OF ANIMALS EXAMINED         | POSITIVES   |
| A     | 20                              | 15 (75%)    |
| B     | 20                              | 15 (75%)    |
| C     | 20                              | 19 (75%)    |
| TOTAL | 60                              | 49 (81.67%) |

Based on examination of the milk samples by the California mastitis test, the overall prevalence of mastitis in the three farms were found to be very high (81.67%). The prevalence of mastitis in Farms A and B was 75 percent while in Farm C the prevalence was higher at 95 percent (Table 1).

The organisms that were isolated from bovine milk in the farms were indicated in Table 2. A total of 127 isolates were isolated, 44 from Farm A, 40 from Farm B and 43 from Farm C. Staphylococcus pyogenes seemed to be the predominant organism isolated in all the three farms. In Farm A, 27.3 percent of the isolates were Staphylococcus pyogenes, while in Farms B and C, it was 35 and 23.3 percent respectively. The second dominant isolate was different in each farm. In Farm A Streptococcus spp and Bacillus subtilis were the second most prevalent organisms isolated. Staphylococcus epidermidis was second in Farm B and Corynebacterium spp in Farm C. Corynebacterium spp however made up the third important organism isolated in Farm A but was negligible in Farm B. Only a few Streptococcus spp were isolated in Farms B and C. Micrococcus spp seemed to be quite important in both Farms B and C but only a few was isolated in Farm A.

Table 3 shows the overall antibiotic resistant patterns in the three farms. All the organisms tested, except for one Pseudomonas aeruginosa isolate showed complete sensitivity to Gentamycin. Out of 36 Staphylococcus pyogenes isolated, 14 showed resistance to Penicillin, nine to Chloramphenicol and seven to Sulfonamides. Only one isolate was resistant to Streptomycin. However, a smaller

number of Staphylococcus pyogenes was resistant to Tetracycline and Erythromycin. The Streptococcus spp isolated showed a high resistance to Sulfonamides and Tetracycline. Six out of the 10 isolates were resistant to Chloramphenicol, Penicillin, Streptomycin and Erythromycin. Out of seven Corynebacterium spp isolated, five were resistant to Chloramphenicol, four to Tetracycline and three to Erythromycin and Streptomycin. The Corynebacterium spp were also resistant to Sulfonamides and Penicillin. The Escherichia coli and Klebsiella pneumoniae isolated showed a complete resistance to Erythromycin. A high percentage of these isolates was also resistant to Ampicillin.

The antibiotic resistance patterns in the individual farms were tabulated in Tables 4, 5 and 6. In Farm A, the Staphylococcus pyogenes isolated was highly resistant to Chloramphenicol, Penicillin and Tetracycline while the Staphylococcus pyogenes isolated from Farm B was highly resistance to Penicillin only. However, in Farm C, the Staphylococcus pyogenes was resistant to Penicillin and Sulfonamides. All the Corynebacterium spp isolated from Farm A and one out of the three isolated from Farm C were resistant to Chloramphenicol. In Farm A, the Corynebacterium spp isolated was also resistant to Erythromycin and Streptomycin. The Streptococcus spp isolated from Farm A was highly resistant to Sulfonamides and Tetracycline but in Farm B the organism was only resistant to Chloramphenicol. The showed resistance to Sulfonamides, Chloramphenicol and Tetracycline in Farm C. All the organisms isolated from the three farms except one Pseudomonas aeruginosa isolate from Farm B were sensitive to Gentamycin.

Table 2 : The organisms isolated from the milk samples from  
the three study farms

| ORGANISMS                                | FARM A<br>( n = 44 )* | FARM B<br>( n = 40 )* | FARM<br>( n = 45 )* |
|--|-----------------------|-----------------------|---------------------|
| <u>Staphylococcus</u> <u>cyogenes</u>    | 12 (27.3%)            | 14 (35.0%)            | 10 (23.3%)          |
| <u>Staphylococcus</u> <u>epidermidis</u> | 1 ( 2.3%)             | 10 (25.0%)            | 2 ( 4.6%)           |
| <u>Streptococcus</u> spp                 | 6 (13.6%)             | 2 ( 5.0%)             | 2 ( 4.6%)           |
| <u>Corynebacterium</u> spp               | 5 (11.4%)             | 1 ( 2.5%)             | 10 (23.3%)          |
| <u>Micrococcus</u> spp                   | 4 ( 9.1%)             | 8 (20.0%)             | 8 (18.6%)           |
| <u>Pseudomonas</u> <u>aeruginosa</u>     | 1 ( 2.3%)             | 2 ( 5.0%)             | 2 ( 4.6%)           |
| <u>Escherichia</u> coli                  | ( 2.3%)               | 0                     | 3 ( 7.0%)           |
| <u>Klebsiella</u> <u>pneumoniae</u>      | ( 2.3%)               | 0                     | 2 (4. 6%)           |
| <u>Bacillus</u> subtilis                 | 6 (13.6%)             | 0                     | 1 ( 2.3%)           |
| <u>Acinetobacter</u> spp                 | 2 ( 4.5%)             | 0                     | ( 2.3%)             |
| Yeast                                    | 2 ( 4.5%)             | 1 ( 2.5%)             | 0                   |
| <u>Aeromonas</u> spp                     | 0                     | 0                     | ( 2.3%)             |
| <u>Yersinia</u> <u>enterocolitica</u>    | 0                     | 0                     | 1 ( 2.3%)           |
| <u>Nocardia</u> spp                      | 0                     | 2 ( 5.0%)             | 0                   |
| <u>Alcaligenes</u> <u>fecalis</u>        | 3 ( 6.8%)             | 0                     | 0                   |

Table 3 : The overall antibiotic resistance patterns of the bacterial isolates

| ORGANISMS                            | ANTIBIOTICS              |                          |                          |                           |                            |                            |                            |                           |                          |
|--------------------------------------|--------------------------|--------------------------|--------------------------|---------------------------|----------------------------|----------------------------|----------------------------|---------------------------|--------------------------|
|                                      | PENICILLIN<br>( 10 mcg ) | AMPICILLIN<br>( 10 mcg ) | GENTAMYCIN<br>( 10 mcg ) | SULFONAMID<br>( 300 mcg ) | CHLORAMPHENI<br>( 30 mcg ) | STREPTOMYCIN<br>( 10 mcg ) | TETRACYCLINE<br>( 30 mcg ) | ERYTHROMYCI<br>( 15 mcg ) | POLYMYXIN-B<br>( 300 U ) |
| <u>Staphylococcus pyogenes</u> (36)* | 14*                      | NA                       | 0                        | 7                         | 9                          | 1                          | 5                          | 3                         | NA                       |
| <u>Streptococcus</u> spp (10)        | 1                        | NA                       | 0                        | 6                         | 3                          | 1                          | 6                          | 1                         | NA                       |
| <u>Corynebacterium</u> spp (7)       | 1                        | NA                       | 0                        | 2                         | 5                          | 3                          | 4                          | 3                         | NA                       |
| <u>Pseudomonas aeruginosa</u> (8)    | NA                       | NA                       | 1                        | 2                         | 6                          | 7                          | 8                          | 7                         | 1                        |
| <u>Escherichia coli</u> (4)          | NA                       | 3                        | 0                        | 1                         | 1                          | 1                          | 2                          | 4                         | NA                       |
| <u>Klebsiella pneumoniae</u> (4)     | NA                       | 4                        | 0                        | 1                         | 0                          | 1                          | 0                          | 4                         | NA                       |

Table 4 : Antibiotic resistance patterns in Farm A

| ORGANISMS                           | ANTIBIOTICS              |                          |                          |                             |                               |                            |                            |                           |                          |
|-------------------------------------|--------------------------|--------------------------|--------------------------|-----------------------------|-------------------------------|----------------------------|----------------------------|---------------------------|--------------------------|
|                                     | PENICILLIN<br>( 10 mcg ) | AMPICILLIN<br>( 10 mcg ) | GENTAMYCIN<br>( 10 mcg ) | SULFONAMIDES<br>( 300 mcg ) | CHLORAMPHENICOL<br>( 30 mcg ) | STREPTOMYCIN<br>( 10 mcg ) | TETRACYCLINE<br>( 30 mcg ) | ERYTHROMYCI<br>( 15 mcg ) | FOLYMYXIN-B<br>( 300 U ) |
| <u>Staphylococcus pyogenes</u> (12) | 5                        |                          | 0                        | 2                           | 6                             | 1                          | 4                          | 1                         | NA                       |
| <u>Streptococcus</u> spp (6)        | 1                        | NA                       | 0                        | 5                           | 0                             | 1                          | 5                          | 1                         | NA                       |
| <u>Corynebacterium</u> spp (4)      | 1                        | NA                       | 0                        | 0                           | 4                             |                            | 2                          | 3                         | NA                       |
| <u>Escherichia coli</u> (1)         | NA                       | 1                        | 0                        | 1                           | 0                             | 1                          | 1                          | 1                         | NA                       |
| <u>Pseudomonas aeruginosa</u> (1)   |                          | NA                       | 0                        | 1                           | 1                             | 1                          | 1                          | 1                         | 0                        |
| <u>Klebsiella pneumoniae</u> (1)    | NA                       | 1                        | 0                        | 0                           | 0                             | 0                          | 0                          |                           | NA                       |

Table 5 : Antibiotic resistance patterns in Farm B

| ORGANISMS                           | ANTIBIOTICS              |                          |                          |                           |                              |                            |                            |                            |                          |
|-------------------------------------|--------------------------|--------------------------|--------------------------|---------------------------|------------------------------|----------------------------|----------------------------|----------------------------|--------------------------|
|                                     | PENICILLIN<br>( 10 mcg ) | AMPICILLIN<br>( 10 mcg ) | GENTAMYCIN<br>( 10 mcg ) | SULFONAMID<br>( 300 mcg ) | CHLORAMPHENICO<br>( 30 mcg ) | STREPTOMYCIN<br>( 10 mcg ) | TETRACYCLINE<br>( 30 mcg ) | ERYTHROMYCIN<br>( 15 mcg ) | FOLYMYXIN-B<br>( 300 U ) |
| <u>Staphylococcus pyogenes</u> (14) | 5                        | NA                       | 0                        | 2                         | 1                            | 0                          | 0                          | 1                          | NA                       |
| <u>Streptococcus spp</u> (2)        | 0                        | NA                       | 0                        | 0                         | 2                            | 0                          | 0                          | 0                          | NA                       |
| <u>Pseudomonas aeruginosa</u> (4)   | NA                       | NA                       | 1                        | 1                         | 2                            | 4                          | 4                          | 3                          | 1                        |

Table 6 : Antibiotic resistance patterns in Farm C

| ORGANISMS                           | ANTIBIOTICS              |                          |                          |                           |                               |                            |                            |                           |                          |
|-------------------------------------|--------------------------|--------------------------|--------------------------|---------------------------|-------------------------------|----------------------------|----------------------------|---------------------------|--------------------------|
|                                     | PENICILLIN<br>( 10 mcg ) | AMPICILLIN<br>( 10 mcg ) | GENTAMYCIN<br>( 10 mcg ) | SULFONAMID<br>( 300 mcg ) | CHLORAMPHENICOL<br>( 30 mcg ) | STREPTOMYCIN<br>( 10 mcg ) | TETRACYCLINE<br>( 30 mcg ) | ERYTHROMYCI<br>( 15 mcg ) | FOLYMI IN-B<br>( 300 U ) |
| <u>Staphylococcus pyogenes</u> (10) | 4                        | NA                       | 0                        | 3                         | 2                             | 0                          | 1                          | 1                         | NA                       |
| <u>Streptococcus spp</u> (2)        | 0                        | NA                       | 0                        | 1                         | 1                             | 0                          | 1                          | 0                         | NA                       |
| <u>Corynetacterium spp</u> (3)      | 0                        | NA                       | 0                        | 2                         | 1                             | 1                          | 2                          | 0                         | NA                       |
| <u>Pseudomonas aeruginosa</u> (3)   | NA                       | NA                       | 0                        | 0                         | 3                             | 2                          | 3                          | 3                         | 0                        |
| <u>Escherichia coli</u> (3)         | NA                       | 2                        | 0                        | 0                         | 1                             | 0                          | 1                          | 3                         | NA                       |
| <u>Klebsiella pneumoniae</u> (3)    | NA                       | 3                        | 0                        | 1                         | 0                             | 1                          | 0                          | 3                         | NA                       |

## DISCUSSION

The overall prevalence of mastitis in the three farms studied was 81.67 percent. This prevalence was relatively high when compared to previous studies by Koh and Joseph (1974) and Guzman et al (1982) who reported a prevalence of 60.1 and 70 percent respectively.

It was suggested by Blood et al (1983) that infection of the mammary glands occurred via the teat canal and the main source is the environment. Similarly, the environment is suspected to be the main source of infection and probably responsible for the high prevalence of mastitis in this study. Whittlestone (1962) mentioned the possible relationships between milking machine and mastitis. These possibilities are divided into two categories; those connected with the machine as a vector and those related to its traumatic effects. Thus, in this study, there is a possibility that the milking machine could be one of the factors contributing to the high prevalence of mastitis in these farms. The California mastitis test gives positive results when the leucocyte count exceeds 500,000 cells per ml. Cell count could to some extent increase with duration of lactation and with the age of the cow (Fetrow, 1980). Cows in the first week after calving or in the last stages of lactation give a strong positive reaction (Blood et al, 1983).

Staphylococcus pyogenes was the predominant organism isolated from the milk samples in this study. Out of 127 organisms isolated, 36 (28.3%) were Staphylococcus pyogenes. Similar findings were reported by several workers; Madariaga-aguilar and Lopez-alvarez,

1979, in Mexico; Sarma and Boro, 1980 in India and Koh and Joseph, 1974 in Malaysia. Staphylococcus pyogenes was also the predominant organism isolated from the three farms studied in this study.

Staphylococci, in particular Staphylococcus pyogenes are the most common cause of intramammary infection (McDonald, 1977). The principal reservoir of Staphylococcus pyogenes are the udder and teat skin and the milk of infected glands (Spencer and Lasmanis, 1952). Infection can spread during milking, and the infection rate increases with age (Schalm and Wood, 1953). Jain (1977) suggested that Staphylococcus pyogenes has the capacity to penetrate tissues producing deep seated foci, hence intramammary antibiotic therapy quite often fails to eradicate Staphylococcal mastitis. All these factors may possibly contribute to the high numbers of Staphylococcus pyogenes isolated in this study.

The other organisms isolated in this study were Micrococcus spp, Corynebacterium spp, Staphylococcus epidermidis, Streptococcus spp and Bacillus subtilis. Beside Staphylococcus pyogenes, Sarma and Boro (1980) also isolated Corynebacterium spp, Streptococcus spp and Pseudomonas aeruginosa. The same organisms were isolated by Verma and Mishra (1977). Madariaga-aguilar and Lopez-alvarez (1979) also managed to isolate Staphylococcus epidermidis and Bacillus subtilis. Similar findings were reported by Koh and Joseph (1974). The findings in this study seemed to show similarity with the findings of other workers.

The prevalence of organisms isolated from the individual farms showed a slightly different pattern. This difference could have

arised from different management practise and different environment present in each farm.

With the exception of Streptomycin, when the antibiotic resistance patterns of Staphylococcus pyogenes in this study is compared with previously published reports (House and Manley, 1974; Frost and O'Boyle, 1981) , it does not appear that marked changes in the patterns over the past few years have occurred.

In this study, the resistance of Staphylococcus pyogenes to Streptomycin is 2.8 percent. Other reports (House and Manley, 1974; Frost and O'Boyle, 1981) on Streptomycin resistance vary from 23 percent to 52 percent. It was observed that most of the Staphylococcus pyogenes showed resistance to Penicillin and Chloramphenicol. This is in agreement with other workers (Davidson et al, 1982). There is a slight increase in the percentage of resistance to Penicillin from what is reported by Koh and Joseph (1974), who observed 22 percent of the organism was resistant to Penicillin. An increase in antibiotic resistance has not been reported following unsuccessful treatment of Staphylococcus pyogenes infection (Higgs et al, 1967). Failure to eliminate the organism following therapy has been related to the formation of microabscesses within the infected glands which prevents contact of antibiotics and the organisms (Poutrel, 1978). There are also other factors that can influence the outcome of antibiotic therapy.

The antibiotic resistance patterns of Streptococcus spp isolated, is similar to previous reports (Verma and Mishra, 1977). The Pseudomonas aeruginosa isolated also showed similar antibiotic

resistant patterns to previous report (Koh and Joseph ,1974).

Pseudomonas aeruginosa is difficult to destroy because of its inherent resistance to antibiotics and its ability to acquire extra-chromosomal tolerance. This organism abounds in unsanitary condition (Huber, 1977). On the other hand, Streptococcal organisms are probably the most sensitive to antibiotics especially Penicillin (Huber, 1977). This could account for the lesser number of the organism isolated when compared with that of Koh and Joseph (1974), who isolated 28 percent of the organism in their study.

The antibiotic resistance patterns of Escherichia coli is similar to that reported by Davidson et al (1982) but the patterns of Klebsiella spp differs from that reported by the same worker. Both organisms were resistant to Ampicillin and Erythromycin.

Gram negative bacteria, especially the family Enterobacteriaceae represent a difficult group of organisms to treat during the infectious processes of bovine mastitis. The organisms have the ability to rapidly developed remarkable tolerance to antibiotics via extrachromosomal, plasmid or R factor mediated resistance development (Huber, 1977). Escherichia coli has the ability to develop resistance via extrachromosomal transmission.

The antibiotic resistant patterns of Staphylococcus pyogenes in the three farms were rather similar with most of the organisms showed resistance to Penicillin and Chloramphenicol. However, in Farm A, the organism showed a higher resistance to Chloramphenicol than in other farms. It was also resistant to Tetracycline while those in Farms B and C were resistant to Sulfonamides beside Penicillin. The patterns

in Farms B and C were similar. The difference in Farm A may be due to differences in management practises and environment.

#### SUMMARY

The prevalence of mastitis in the three farms studied was very high (81.67%). A total of 127 organisms were isolated. Staphylococcus pyogenes was predominantly isolated.

Most of the Staphylococcus pyogenes was resistant to Penicillin and Chloramphenicol. Streptococcus spp was resistant to Sulfonamides and Tetracycline whilst Corynebacterium spp was resistant to Chloramphenicol. Pseudomonas aeruginosa was resistant to most of the antibiotics tested. Escherichia coli and Klebsiella spp were resistant to Ampicillin and Erythromycin. Most of the organisms were sensitive to Gentamycin. The antibiotic resistance patterns of Staphylococcus pyogenes in Farms B and C were similar.

Antibiotic sensitivity test should be carried out before any treatment to determine the most appropriate antibiotics for therapy. Efficient and effective methods of sanitation including teat dipping are vitally important in maximising effective antibiotic utilization. Indiscriminate use of antibiotics should be avoided since it could result in an increase percentage of antibiotic resistant bacteria. The use of antibiotic combination in therapy could also be considered

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APPENDIX - A

California Mastitis Test

1. Milk was withdrawn from the four quarters into the four test wells. Each well on the test plate received a sample of milk from only one quarter. Excess milk was poured-off from the test plate leaving about two mls of milk in each well.
2. An equal amount of the test fluid (Wellcome) was added to each well. The milk and the test fluid were mixed thoroughly by gentle swirling of the test plate.
3. The result was read after a few seconds and a positive result was indicated by fine threadlike streaks or when the test mixture became mucilaginous and jelly-like. The test was considered as negative when the test mixture remained fluid and there was no change in its appearance.

APPENDIX - B

The modified Kirby-Bauer Disc Technique

1. Five to six colonies of the selected isolates were inoculated into 0.5 ml of sterile Tryptose-Soya Broth (Oxoid), and incubated at 37°C for 24 hours.
2. The concentration of the bacterial cells was standardized to that of one percent Barium sulphate solution using sterile distilled water.
3. A sterile cotton swab was used to pick up the inoculum and it is then streaked onto a preheated Mueller-Hinton Agar plate (Oxoid). The streaking was repeated several times to ensure a complete lawn of bacterial growth.
4. Seven types of antibiotics discs (Oxoid) were placed at equidistant from each other onto the inoculated Mueller-Hinton Agar plate.
5. The plates were incubated aerobically at 37°C for 24 hours. The zones of inhibition were measured with a ruler and the sensitiveness was interpreted as given in Appendix -C.

APPENDIX - C

The Kirby-Bauer interpretation chart for Antibiotic Sensitivity Test (Bauer et al, 1966)

| ANTIBIOTIC                       | DISC POTENCY | INHIBITION ZONE (mm) |              |            |
|----------------------------------|--------------|----------------------|--------------|------------|
|                                  |              | RESISTANCE           | INTERMEDIATE | SENSITIVE  |
| Ampicillin                       | 10 mcg       | 11 or less           | 12 - 13      | 14 or more |
| Erythromycin                     | 15 mcg       | 13 or less           | 14 - 17      | 18 or more |
| Chloramphenicol                  | 30 mcg       | 12 or less           | 13 - 17      | 18 or more |
| Gentamycin                       | 10 mcg       |                      |              | 13 or more |
| Penicillin G<br>(Staphylococcus) | 10 mcg       | 20 or less           | 21 - 28      | 29 or more |
| Penicillin G<br>(others)         | 10 mcg       | 11 or less           | 12 - 21      | 22 or more |
| Streptomycin                     | 10 mcg       | 11 or less           | 12 - 14      | 15 or more |
| Sulfonamides                     | 300 mcg      | 12 or less           | 13 - 16      | 17 or more |
| Polymyxin-B                      | 300 U        | 8 or less            | 9 - 11       | 12 or more |

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