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PREVALENCE OF SUBCLINICAL MASTITIS AND ANTIBIOTIC RESISTANT BACTERIA IN THREE SELECTED CATTLE FARMS IN SERDANG, SELANGOR AND KLUANG, JOHOR

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SUMMARY

Sixty cows from three established cattle farms in Serdang and Kluang were randomly selected and examined for mastitis. A high (81.7%) prevalence of subclinical mastitis was seen amongst the animals examined as indicated by the California Mastitis Test (CMT). The prevalence of subclinical mastitis in the farms ranged from 75 to 95%. Thirteen bacterial genera were identified from the 126 isolates obtained. *Staphylococcus aureus* appeared to be the most predominant bacteria in the milk samples. This was followed by *Micrococcus* spp., *Corynebacterium* spp., *Staphylococcus epidermidis* and *Streptococcus* spp. These Gram-positive organisms made up almost 75% (95/126) of the isolates seen in the milk samples. Most of the organisms showed antibiotic resistance to tetracycline, chloramphenicol and sulphonamides. Fourteen (38.9%) of the *Staphylococcus aureus* isolates showed resistance to penicillin. The isolation of these bacterial species has given some insights into the distribution, pathogenicity and role of these organisms in bovine mastitis in Malaysia. The high prevalence of subclinical mastitis seen in all three farms warrants a more effective mastitis control measure.

Keywords: mastitis, antibiotic-resistant bacteria, cattle farms.

INTRODUCTION

Mastitis is one of the most important economic diseases affecting dairy herds. Losses are incurred through milk discard, cost of treatment, decreased milk yield and long-term effects from damage to the cow's udder. Subclinical mastitis in many instances accounts for the majority of the mastitic cases and a wide variety of microorganisms have been implicated as causative agents of bovine mastitis. Preventive programmes have been developed to reduce the incidence of mastitis. Even then, cases of mastitis do occur which require treatment and control. The emergence of antibiotic-resistant bacteria in bovine milk is a serious problem and it is therefore important that the antibiotic sensitivity test be fully utilized to determine the most suitable antibiotic for therapy. However, the correlation between antibiotic sensitivity in vitro and clinical response is often poor (Davidson *et al.*, 1982). This study was conducted in view of the relatively scanty information on mastitis and antibiotic resistant bacteria in milk in Malaysia. The microorganisms and their antibiotic-resistant patterns identified would be useful in the treatment and control of mastitis in Malaysia.

MATERIALS AND METHODS

Test animals

The study was carried out on 60 lactating cows from three established cattle farms in Serdang and Kluang and with 20 animals being randomly selected from each farm. The animals were mainly Friesian-Sahiwal crosses (85%) while a small percentage (15%) was made up of Jersey, Friesians and local Indian dairy crosses. All animals were machined-milked twice daily.

Collection of samples

The udders of the animals were washed with clean water and dried with a clean piece of cloth prior to examination for clinical mastitis. Milk was withdrawn from the four quarters into four test wells of the California mastitis test (CMT) plate (Wellcome, UK). Gross abnormalities of the milk were noted before subjecting the milk samples to the CMT for the presence of subclinical mastitis. Thirty ml of milk samples for bacteriological study were aseptically collected immediately after performing the CMT into sterile Bijou bottles, to be transported to the Bacteriology Laboratory at Universiti Putra Malaysia, Serdang. Samples from the farm in Kluang, Johor were packed in ice before being transported to the laboratory in UPM.

California mastitis test

Two milliliters of milk withdrawn from each quarter were placed in each well of the CMT plate and an equal amount of CMT test fluid was introduced. They were mixed thoroughly by gentle swirling of the test plate. The results were read after a few seconds and positive results were indicated by fine thread-like streaks or when the test mixture became mucilaginous and jelly-like. The test was considered negative when the test mixture remained fluid and there was no change in appearance.

Bacteriological examination

Bacteriological examination consisted of isolation and identification of the organisms and testing the isolates for antibiotic sensitivity. Isolation was done on blood and MacConkey agar (BBL, Maryland USA) incubated at 37°C for 12 h or overnight whilst identification was based on Cowan and Steel's Manual for the Identification of Medical Bacteria (Cowan, 1974). Representative colonies for each isolate were maintained on nutrient agar slant for antibiotic sensitivity test.

The antibiotic sensitivity test

The antibiotic sensitivity test was performed on selected isolates according to the modified Kirby-Baeur disc technique (Baeur *et al.*, 1966). Five colonies of each isolate were grown in 0.5 ml tryptose soy broth (BBL, Maryland USA). The growth was standardized to the opacity of 1% barium chloride and an aliquot was streaked onto a Mueller-Hinton agar to obtain a bacterial lawn. The antibiotics tested were (a) the penicillin group: penicillin G (10 mcg), ampicillin (10 mcg), erythromycin (15 mcg), (b) the aminoglycosides: streptomycin (10 mcg), gentamycin (10 mcg), (c) the broad spectrum group: chloramphenicol (30 mcg), tetracyclines (30 mcg), (d) polymxin B (300 U), and e) sulfonamide compound (300 mcg). The choice of antibiotics was in accordance with current antibiotics used in therapy, particularly for humans. The polymyxin B was mainly to test the *Pseudomonas aeruginosa* isolates. The diameter of the zone of inhibition was measured using a pair of calipers and the sensitivity of the isolate against each antibiotic was determined based on the Kirby-Baeur interpretation chart.

RESULTS

As determined by the CMT, the overall prevalence of subclinical mastitis in the three studied farms was found to be very high (81.7% or 49/60 cows). There was no significant difference in prevalence between the three farms which ranged from 75 to 95%. However, almost all (95%) animals tested in Farm C had subclinical mastitis (Table 1).

Table 1: Prevalence of subclinical mastitis in the three studied farms as determined by the California Mastitis Test

Farms	No. of animals examined	CMT positives
A	20	15 (75%)
B	20	15 (75%)
C	20	19 (95%)
Total	60	49 (81.7%)

A total of 126 bacterial isolates were obtained from the 60 milk samples. Again, the number of isolates obtained did not differ significantly from one farm to the other (Table 2). Altogether 13 bacterial genera were identified but only four were significant. These four genera were the *Staphylococcus, Streptococcus, Micrococcus and Corynebacterium*. The Gram-positive organisms were the major group of bacteria involved in mastitis in this study. *Staphylococcus aureus* was shown to be the predominant organism isolated in all three farms. It made up between 23% (10/42) in Farm C to 35% (14/40) in Farm B, with a 28.6% overall prevalence of *S. aureus* in all three farms (Table 2).

The three bacterial genera that have been frequently reported causing mastitis are namely, *Staphylococcus, Streptococcus, and Corynebacterium*. In this study, the isolates from the three genera were observed to be resistant to the broad spectrum group of antimicrobials: chloramphenicol, tetracycline and the sulphonamides. Antibiotic sensitivity test on selected isolates obtained from this study indicated that a significant number of the *S. aureus* isolates were resistant to penicillin. The coliforms (*E. coli* and *Klebsiella* spp.) on the other hand, were seen to be resistant to ampicillin and erythromycin which are the antibiotics commonly used in humans and therefore suggesting public health importance. *Pseudomonas aeruginosa*, which is known to be not affected by a majority of antibiotics, is sensitive to gentamycin and polymyxin-B as expected.

DISCUSSION

The overall prevalence of subclinical mastitis in the three farms studied was 81.7% and this is relatively higher than the earlier prevalence reported (Koh and Joseph, 1974; Hussain and Othman, 1984). *Staphylococcus aureus* was the predominant organism isolated from the milk samples in this study. Of the 126 organisms isolated, 36 (28.3%) were *S. aureus*, which was similar to the findings (26.2%) of Koh and Joseph (1974). Staphylococci are the most common cause of mastitis and this is not surprising as their habitats are the skin, mouth, upper respiratory tract and the mammary gland itself. Infection can be easily spread by milking, licking and suckling of the mammary gland. Jain (1979) had suggested that as

Table 2: Microorganisms isolated from milk samples obtained from the three studied farms

Organisms	Farm A	Farm B	Farm C	Overall
Staphylococcus aureus	12 (27%)	14 (35%)	10 (24%)	36 (28.6%)
Micrococcus spp.	4 (9%)	8 (20%)	8 (19%)	20 (15.9%)
Corynebacterium spp.	5 (11%)	1 (3%)	10 (24%)	16 (12.7%)
Staphylococcus epidermidis	1 (2%)	10 (25%)	2 (5%)	13 (10.3%)
Streptococcus spp.	6 (14%)	2 (5%)	2 (5%)	10 (7.9%)
Bacillus subtilis	6 (14%)	0 (2%)	1 (2%)	7 (5.5%)
Pseudomonas aeruginosa	1 (2%)	2 (5%)	2 (5%)	5 (3.9%)
Escherichia coli	1 (2%)	0	3 (7%)	4 (3.2%)
Klebsiella. pnuemoniae	1 (2%)	0	2 (5%)	3 (2.4%)
Acinetobacter spp.	2 (5%)	0	1 (2%)	3 (2.4%)
Yeasts	2 (5%)	1 (3%)	0	3 (2.4%)
Yersinia enterocolitica	0	0	1 (2%)	1 (0.8%)
Nocardia spp.	0	2 (5%)	0	2 (1.6%)
Alcaligenes faecalis	3 (7%)	0	0	3 (2.4%)
Total number of isolates	44	40	42	126 (100%)

Table 3: The antibiotic resistance pattern of the major bacteria isolated from the milk samples

	No. of Isolates Obtained	Penn***	Amp	Genta	Sulfo	Chloram	Strep	Tetra	Eryth	PolyB
FARM A										
Staphylococcus aureus	12	5* (42%**)	NT	0	2 (17%)	6 (50%)	1 (8%)	4 (33%)	1 (8%)	NT****
Streptococcus spp.	6	1 (17%)	NT	0	5 (83%)	0	1 (17%)	5 (83%)	1 (17%)	NT
Corynebacterium spp.	4	1 (25%)	NT	0	0	4 (100%)	2 (50%)	2 (50%)	3 (75%)	NT
Pseudomonas aeruginosa	1	NT	NT	0	1 (100%)	1 (100%)	1 (100%)	1 (100%)	1 (100%)	0
Escherichia coli	1	NT	1 (100%)	0	1 (100%)	0	1 (100%)	1 (100%)	1 (100%)	NT
Klebsiella. pneumoniae	1	NT	1 (100%)	0	0	0	0	0	1 (100%)	NT
FARM B										
Staphylococcus aureus	14	5 (36%)	NT	0	2 (14%)	1 (7%)	0	0	1 (7%)	NT
Streptococcus spp.	2	0	NT	0	0	2 (100%)	0	0	0	NT
Pseudomonas aeruginosa	4	NT	NT	1 (25%)	1 (25%)	2 (50%)	4 (100%)	4 (100%)	3 (75%)	1 (25%)
FARM C										
Staphylococcus aureus	10	4 (40%)	NT	0	3 (30%)	2 (50%)	0	1 (10%)	1 (10%)	NT
Streptococcus spp.	2	0	NT	0	2 (100%)	1 (50%)	1 (50%)	2 (100%)	0	NT

Table 3: Continued

	No. of isolates obtained	Penn***	Amp	Genta	Sulfo	Chloram	Strep	Tetra	Eryth	PolyB
Corynebacterium spp.	3	NT	ND	0	0	1 (66%)	0	1 (66%)	3 (100%)	NT
Pseudomonas aeruginosa	3	NT	ND	0	0	3 (100%)	2 (66%)	3 (100%)	3 (100%)	0
Escherichia coli	3	NT	2 (66%)	0	0	1 (33%)	0	1 (33%)	3 (100%)	NT
Klebsiella pneumoniae	3	NT	3 (100%)	0	1 (33%)	0	1 (33%)	0	3 (100%)	NT
OVERALL										
Staphylococcus aureus	36	14 (39%)	NT	0	7 (19%)	9 (25%)	1 (3%)	5 (14%)	3 (8%)	NT
Streptococcus spp.	10	1 (10%)	NT	0	6 (60%)	3 (30%)	1 (10%)	6 (60%)	1 (10%)	NT
Corynebacterium spp	7	1 (14%)	NT	0	2 (29%)	5 (71%)	3 (43%)	4 (57%)	3 (43%)	NT
Pseudomonas aeruginosa	8	NT	NT	1 (13%)	2 (25%)	6 (75%)	7 (88%)	8 (100%)	7 (88%)	1 (13%)
Escherichia coli	4	NT	3 (75%)	0	1 (25%)	1 (25%)	1 (25%)	2 (50%)	4 (100%)	NT
Klebsiella pneumoniae	4	NT	4 (100%)	0	1 (25%)	0	1 (25%)	0	4 (100%)	NT

* Number in cells indicate the number of isolates resistant to the individual antibiotic tested.
** Number in brackets indicate the percentage of isolates resistant to the individual antibiotic.
*** Antibiotics tested: Penn (Penicillin), Amp (Ampicillin), Genta (Gentamycin), Sulfo (Sulphonamides), Chloram (Chloramphenicol), Strep (Streptomycin), Tetra (Tetracyclines), Eryth (Erythromycin), Poly B (Polymyxin B).
**** NT (Not tested)

S. aureus had the capacity to penetrate tissues producing deep seated foci, intramammary antibiotic therapy quite often failed to eradicate staphylococcal mastitis. All these factors may possibly contribute to the high prevalence of *S. aureus* infection in these study farms. The prevalence of mastitis between the farms was not significantly different (Table 1). The slight difference observed could be due to the different environment and management practices in each farm.

Other major organisms isolated in this study were *Staphylococcus, epidermidis, Streptococcus* spp., *Micrococcus* spp. and *Corynebacterium* spp. These Gram-positive organisms made up almost 75% (95/127) of the isolates obtained and were the main cause of mastitis in a similar study by Watts (1988). This is not surprising as this group of bacteria is usually found in the animal's environment where they can easily infect mammary glands and cause mastitis. Bovine mastitis due to yeast infections has been reported to be associated with contaminated antibiotic preparations and infusion equipment (Richard *et al.*, 1980). In this study, a low prevalence (3/126) of yeast infection was evident which possibly indicates the low rate of treatment using antibiotic infusions.

The antibiotic sensitivity of selected isolates particularly the three common genera mentioned above, the *Pseudomonas* and the coliforms were determined in this study. It was observed that a significant number of *S. aureus* tested was resistant to penicillin, chloramphenicol, tetracycline and the sulphonamides. Although the number of *Pseudomonas aeruginosa* isolates tested was small, the organism demonstrated some resistance to the antibiotics that were known to be effective against it, i.e. polymyxin B and gentamycin. Overall, nearly all of the bacterial species found in this study had some degree of resistance to tetracycline.

It is suggested by Blood *et al.* (1983) that infection of the mammary gland occurs via the teat canal and the main source is the animal and environment. Thus, for control and prevention of mastitis, strict hygienic measures are essential. A complete epidemiological study needs to be organized to delineate the role of microorganisms in bovine mastitis and to aid development of improved control methods. Epidemiological studies have been developed on effective mastitis control measures, such as post-milking teat antisepsis and dry-cow antibiotic therapy (Dodd, 1986). In one study, *S. aureus* herd infection level was reduced only after determining that transmission occurred primarily during the milking process, and then developing specific preventive methods against it (Watts, 1988).

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