

IN VITRO AND IN VIVO SUSCEPTIBILITY OF MASTITIS PATHOGENS ISOLATED FROM BOVIN MASTITIS TO ANTIMICROBIAL DRUGS

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SUMMARY

The in vitro sensitivity testing of mastitis pathogens, associated with udder infections in cows, against 12 chemotherapeutic agents, showed that all strains (100%) tested are sensitive to gentamicin. 96.12% to 70.87% of the isolates are sensitive to kanamycin, erythromycin, chloramphenicol, cloxacillin, streptomycin, tetracycline and ampicillin. All strains are resistant to colistin (except for *E. coli*) and sulfa drugs.

Multimast was found to be most efficacious in the treatment of bovine mastitis (89.28%), followed by Multiject Imm, Kanamast and Neomastitar (76.92%, 74.19% and 68.00%) respectively.

INTRODUCTION

Bovine mastitis is a major cause of economic loss in all countries where dairying is practised. The losses associated with the disease arise from reduced milk yield from infected quarters, loss of saleable milk during antibiotic therapy, culling of chronically affected cows and, occasionally, death in peracute cases.

Clinical cases of bovine mastitis are usually treated before precise culture and sensitivity test results are available. The therapeutic agent is chosen on the basis of the organisms likely to be responsible and their presumptive drug sensitivities. It can, therefore, be useful to gather individual antibiotic sensitivity test results to provide information on the current prevalence of resistant strains in a community⁽¹⁾.

For the effective treatment of mastitis, it is essential to use the antimicrobial drug to which the causative agent is sensitive. Where it is not possible to determine the sensitivity of the causative organisms prior to giving treatment, especially under field conditions, it is always desirable to have information on the sensitivity pattern of mastitis pathogens, particularly the isolates associated with recent udder infections.

Successful treatment of mastitis in dairy animals depends to a greater extent on the type of infection and the proper selection and administration of the drug⁽²⁾. A number of drugs/formulations are being periodically put into market by various manufacturers, all of them claiming superiority of their products. Before such drugs can be put into use, it is desirable to subject them to rigorous clinical trials.

In a view of this, a study was conducted to assess *in vitro* and *in vivo* susceptibility and pathogens, associated with the udder infections in cows, to the different antimicrobial drugs.

MATERIALS AND METHODS

The present study was conducted on 203 quarters (85 cows) showing evidence of clinical mastitis were examined clinically and bacteriologically..

Milk samples were collected from the affected quarters of each cow in sterile test tubes aseptically. A loopful of milk from each sample was inoculated on 5% sheep blood agar and MacConkey agar and incubated at

37°C for 24-72 hours. Cultures so obtained were identified according to their culture, morphological and biochemical characteristics as suggested by Carter⁽³⁾.

Sensitivity of mastitis pathogens was determined using Bauer-Kirby technique described by Bauer *et al.*⁽⁴⁾. For this purpose 12 different antimicrobial agents as listed in Table 1 were used. The antimicrobial discs used in this study were obtained from Difco Laboratories, Detroit Michigan U.S.A.

A total of 54 cows with 110 infected quarters were subjected to intramammary treatments with different antibiotics preparations as listed in Table 2. The drugs were selected according to the results of disc sensitivity test, taking into consideration its availability in Iraq. The dosage and administration were done according to the recommendations of the manufacturing concern. The treated quarters were re-examined clinically and bacteriologically after the 3rd, 5th and 7th days of treatment. Only those quarters were classed as "cured" in which no pathogen could be isolated on re-examination and their secretion were free from any abnormality on physical examination.

RESULTS

The results of sensitivity tests carried out on (103) isolates against 12 chemotherapeutic agents are prescuted in Table 1. It is evident from the results that all the isolates (100%) tested are sensitive to gentamicin. Considerably large number of isolates (96.12%) to (70.87%) are also sensitive to kanamycin, erythromycin, chloramphenicol, cloxacillin, streptomycin, tetracycline and ampicillin. All strains are resistant to colistin and (except *E. coli* strains) sulfa drugs.

Results of intramammary treatment with different antibiotic preparations are shown in Table 2 and 3.

DISCUSSION

In vitro test, result on (103) isolates recovered from bovine clinical mastitis, show that most of *S. aureus* strains (> 85%) were sensitive to

gentamicin, erythromycin, kanamycin, cloxacillin, chloramphenicol and streptomycin. Almost similar findings to some of the above mentioned antibiotics have been reported by^(5,6,7,8 and 9). More or less similar pattern of sensitivity of streptococcal strains to such antibiotics has been reported by (5, 10 and 11). However, variable results have been reported by (12, 13 and 14), regarding the sensitivity of streptococcal strains to penicillin, streptomycin, tetracycline and chloramphenicol. The sensitivity of the mastitis C. pyogenes isolates from other countries has not been reported to be so high to these antibiotics^(12 and 15), as observed in case of isolates from cases in this country. E. coli strains were found to be sensitive to gentamicin, erythromycin, kanamycin, streptomycin and colistin. This finding agrees with that of (16, 6 and 17). Sensitivity of Klebsiella spp. were similar to that of E. coli except for sensitivity to erythromycin and tetracycline.

The antibiotic susceptibility patterns indicate that Kanamast was able to cure 100 percent of the quarters infected with streptococcal and C. pyogenes strains. The 100 percent over all recovery rate in the present study agrees with findings of other investigators^(18, 19 and 20). Accordingly, the present study suggests that Kanamast is a good choice for therapy against streptococcal and C. pyogenes strains.

Multimast, Neomastitar and Multiject are more or less effective treatment against streptococcal, C. Pyogenes, E. coli and Klebsiella spp. strains. In case of Staph aureus infections, only 50 to 76.92 percent of the infected quarters could be cured. This finding is approximate to the result of other workers^(2 and 21).

The low recovery rate of Staph aureus strains against the different mastitis therapy may be due to the Staph aureus become walled off in the udder parenchyma by thick, fibrous scar tissue⁽²²⁾ so that the antibiotic cannot reach the pathogen. Staph aureus can survive, in some instances, within lemphocytes so that such pathogen may not come into contact with the drug and therefore not killed⁽²³⁾. In addition to that, the production of Betalactomase is an important mechanism conferring resistance to penicillin, by Staph aureus⁽²⁴⁾.

The in vivo results are not comparable with in vitro results. The reason for this failure is that the antibiotic act in the presence of degenerated tissue, pus, serum, etc., the change in the pH and the presence of other biochemical

Table 1 : In Vitro sensitivity of mastitis pathogens to different antimicrobial agents

| Species | No. of strains examined | Sensitivity | | | | | | | | | | | | | | | | | | | | | | |
|------------------------|-------------------------|-----------------|----------------|----------------|---------------|----------------|---------------|---------------|---------------|------------------|---------------|---------------|--------|---------------|--------|-------------|--------|----------|--------|-------------|--------|--|--------|--------|
| | | Genta-mycin | | Erythro-mycin | | Kana-mycin | | Cloxa-cillin | | Chloram-phenicol | | Strep-tyocin | | Tetra-cycline | | Peni-cillin | | Neomycin | | Ampi-cillin | | Coli-sulpha-metho-xazole + Trimetho-prim | | |
| | | 10 mcg | 15 mcg | 30 mcg | 1 mcg | 30 mcg | 10 mcg | 30 mcg | 10 mcg | 30 mcg | 10 mcg | 30 mcg | 10 mcg | 30 mcg | 10 mcg | 30 mcg | 10 mcg | 30 mcg | 10 mcg | 30 mcg | 10 mcg | 30 mcg | 10 mcg | 30 mcg |
| <u>S. aureus</u> | 37 | 37 (100.00) | 37 (100.00) | 37 (100.00) | 33 (89.50) | 35 (94.50) | 32 (86.49) | 29 (78.30) | 25 (67.57) | 20 (54.05) | 26 (70.27) | 13 (35.13) | | | | | | | | | | | | |
| <u>S. agalactiae</u> | 23 | 23 (100.00) | 23 (100.00) | 23 (100.00) | 19 (82.61) | 20 (86.96) | 18 (78.26) | 18 (78.26) | 14 (60.89) | 13 (56.52) | 16 (69.57) | 8 (34.78) | | | | | | | | | | | | |
| <u>S. dysgalactiae</u> | 14 | 14 (100.00) | 13 (92.85) | 14 (100.00) | 12 (85.71) | 14 (100.00) | 11 (78.57) | 10 (71.43) | 11 (78.57) | 9 (64.29) | 10 (71.43) | 6 (42.86) | | | | | | | | | | | | |
| <u>S. uberis</u> | 6 | 6 (100.00) | 6 (100.00) | 6 (100.00) | 6 (100.00) | 6 (100.00) | 5 (83.33) | 4 (66.67) | 3 (50.00) | 2 (33.33) | 5 (83.33) | 0 (0.0) | | | | | | | | | | | | |
| <u>E. coli</u> | 10 | 10 (100.00) | 9 (90.00) | 10 (100.00) | 5 (50.00) | 10 (100.00) | 9 (90.00) | 6 (60.00) | 2 (20.00) | 6 (60.00) | 4 (40.00) | 2 (20.00) | | | | | | | | | | | | |
| <u>C. pyogenes</u> | 8 | 8 (100.00) | 8 (100.00) | 8 (100.00) | 6 (75.00) | 7 (87.50) | 6 (75.00) | 5 (62.50) | 5 (62.50) | 5 (62.50) | 6 (75.00) | 0 (0.0) | | | | | | | | | | | | |
| <u>Klebsiella spp.</u> | 5 | 5 (100.00) | 3 (60.00) | 4 (80.00) | 2 (40.00) | 5 (100.00) | 4 (80.00) | 2 (40.00) | 0 (0.0) | 3 (60.00) | 0 (0.0) | 4 (80.00) | | | | | | | | | | | | |
| Total | 103 | 103 (100.00) | 99 (96.12) | 102 (99.03) | 63 (61.17) | 95 (92.23) | 85 (83.49) | 74 (71.84) | 60 (58.26) | 58 (56.31) | 73 (70.87) | 31 (30.10) | | | | | | | | | | | | |

Table 2: Efficacy of antibiotics in treatment of clinical cases of mastitis.

| Name of drug | No. of animal affected | No. of quarter affected | animal | | quarter | | cured | |
|--------------------------|------------------------|-------------------------|--------|-------|---------|-------|-------|--|
| | | | No. | % | No. | % | | |
| Kanamast ⁽¹⁾ | 14 | 31 | 10 | 71.43 | 23 | 74.19 | | |
| Multimast ⁽²⁾ | 13 | 28 | 11 | 8.62 | 25 | 89.28 | | |
| Neomastitar | 15 | 25 | 10 | 66.67 | 17 | 68 | | |
| Multiject ⁽⁴⁾ | 12 | 26 | 9 | 75 | 20 | 76.92 | | |
| IMM | | | | | | | | |

1. Infusion containing kanamycin sulfate 1.60g - benzyl - penicillin procaine 3.000.000 iu - diphenhydramine hydrochloride 4 g .
2. Infusion containing neomycin 250 g - streptomycin 250 g - penicillin G procaine 100.000 iu - oxytetracycline hydrochloride 50 g and prednisolone 10 mg .
3. Infusion containing procaine penicillin 500 mg - neomycin base 300 mg .
4. Infusion containing procaine penicillin 100.000 iu - streptomycin 100 mg - neomycin 100 mg and prednisolone 10 mg

Table 3: The response of mastitis pathogens to the different intramammary infusions

| Organisms involved | Kanamast | | Multimast | | Neomastitar | | Multiject IMM | |
|--------------------------|-----------------------|---------|-----------------------|---------|-----------------------|---------|-----------------------|---------|
| | No. of quarters cured | % cured | No. of quarters cured | % cured | No. of quarters cured | % cured | No. of quarters cured | % cured |
| <u>Staph aureus</u> | 9/14 | 64.28 | 10/13 | 76.92 | 5/10 | 50 | 9/13 | 69.23 |
| <u>Str. agalactiae</u> | 5/5 | 100 | 3/3 | 100 | 4/5 | 80 | 3/4 | 75 |
| <u>Str. dysgalactiae</u> | 3/3 | 100 | 4/4 | 100 | 2/3 | 66.67 | 2/3 | 66.67 |
| <u>Str. uberis</u> | 1/1 | 100 | 2/2 | 100 | 2/2 | 100 | 1/1 | 100 |
| <u>E. coli</u> | 2/4 | 50 | 3/3 | 100 | 2/2 | 100 | 3/3 | 100 |
| <u>C. pyogenes</u> | 2/2 | 100 | 2/2 | 100 | 1/2 | 50 | 1/1 | 100 |
| <u>Klebsiella spp</u> | 1/2 | 50 | 1/1 | 100 | 1/1 | 100 | 1/1 | 100 |
| Total | 23/31 | 74.19 | 25/28 | 89.28 | 17/25 | 68 | 20/26 | 76.92 |

substances can alter the efficacy of an antibiotic⁽²¹⁾; the presence of oedema and inflammatory products to a certain extent, obstruct the diffusion of antibiotics by compression or blockage of the milk duct system⁽²³⁾.

The present study suggests that, regardless of the in vitro sensitivity, the final test must be in the in vivo eradication of infection. For antibacterial therapy of mastitis to be successful, the active drug must reach the bacteria of the focus of infection in concentrations exceeding the minimal inhibitory concentration, and this must be maintained for the appropriate time necessary to break the production and toxinproducing cycle of the causative pathogen. The pathology of the udder tissue caused by mastitis and the consequent effect there of on the pharmacokinetic properties of mastitis drugs, rather than widespread antibiotic resistance seem to be the major reasons for therapy failures. The answer to the mastitis problem lies in the prevention of new intramammary and teat canal infections which may cause mastitis, rather than the treatment of existing mastitic infections.

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قابلية تأثير الجراثيم المعزولة من حالات التهاب الضرع السريري في الإبصار للمضادات البكتيرية في الزجاج والحد

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الخلاصة

اظهرت نتائج هذه الدراسة بأن قابلية تأثير الجراثيم المعزولة من حالات التهاب الضرع السريري في الإبصار لـ 12 مضاد بكتيري كانت جميعها حساسه (100%) للحتاميسن 70.87%-96.12% من العزلات كانت حساسه للمضاد كنامايسين ارثرومايسين , كلورمفينيكول كلوكساسيلين, ستربتومايسين , تتراسايكلين وامبيسلين . جميع العزلات اظهرت مقاومه للمضاد كولستين (باستثناء العصيات القولونية) وادوية السلفا.

كما اظهرت هذه الدراسة بان عقار الملتيماست كان اكثر فعاله (89.28%) في علاج حالات التهاب الضرع السريري في حين كانت فعاله عقار الملتيجكت، الكناماست والنيوماستايتز هي 76.92% ، 74.19% ، 68% على التوالي.