Selected Biological Properties of Enterotoxigenic Staphylococci Isolated from Milk

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ABSTRACT

A total of 79 strains of staphylococci isolated from milk of mastitic cows in different Ninevah localities was divided into three groups based on biochemical activity. Group A was classified as Staphylococcus aureus. The resistance of these strains to antibiotics was: penicillin, 47.17%, streptomycin, 20.75%; erythromycin, 9.43%; and chloramphenicol, 5.66%. Most of the strains were resistant to the international phage set but were sensitive to phages 78 and 102 of the bovine phage set. Five strains produced enterotoxin C and two strains produced enterotoxin D.

Staphylococci are a major problem for the dairy industry. They cause heavy economic losses in terms of a decrease in milk production and an increase in costs for veterinary services and culling of the mastitic animals. Earlier workers reported that infection of bovine mammary glands represents a source of enterotoxigenic strains of Staphylococcus aureus (8,14,18). Consequently, further attention has been concentrated on staphylococcal strains of bovine origin as a potential source of human enterotoxicosis. These problems may result from supplying milk from mastitic cows to the dairy plants. Because of the high incidence of bovine mastitis among animals in Iraq (13), it was appropriate to search for enterotoxigenic organisms in cows infected with mastitis. The object of this investigation was to determine the biochemical properties, antibiotic sensitivity and enterotoxigenicity of staphylococci isolated from mastitic cows.

MATERIALS AND METHODS

The present study was based on the analysis of 165 milk samples from 142 mastitic cows presented to the college clinics, University of Mosul, Iraq, from different parts of Ninevah province. The samples were processed immediately for the isolation of microorganisms by plating on mannitol-salt-agar (Biomerieux) and Baird-Parker agar (Difco). Colonies exhibiting the morphological characteristics of staphylococci were tested for pigmentation, production of coagulase, hemolysin and heat-stable nuclease and utilization of mannitol (anaerobic), phosphatase and acetoin. Production of enterotoxin was by the sac-culture method of Donnelly (6), and enterotoxin detection by the microslide method (16). The sensitivity of the microslide method in our hands was 2.0 μg/ml. Reference enterotoxins A, B, C and D and their corresponding specific antisera were kindly supplied by M.S. Bergdoll, Food Research Institute, University of Wisconsin-Madison. Phage typing was carried out with the phages of the international basic set for typing bovine staphylococci as recommended by Davidson (4) and by some phages of the international basic set for phage-typing of human staphylococci. The sensitivities of the strains to penicillin, erythromycin, streptomycin, tetracycline, cephaloridine, chloramphenicol, methicillin, kanamycin and neomycin were tested using antibiotic sensitivity discs (Oxoid) as described by Bailey and Scott (1).

RESULTS AND DISCUSSION

A total of 79 strains were isolated from the 165 milk samples under study. On the basis of anaerobic growth and fermentation of glucose, all strains were considered to be staphylococci (2).

The results of the examination of the staphylococcal strains for biochemical activities are presented in Table 1. They were divided into three groups, A, B and C, based on their activities. The properties of the 53 strains (67.1%) in group A were those of S. aureus except that some did not produce heat-stable nuclease (4 strains), lacked pigment (3 strains) or were acetoin-negative (2 strains). They were considered to be S. aureus because of their similarity in other properties to this species. Previous investigators also have isolated mutants of S. aureus which had lost some of their physiological characteristics (7,15). The eight strains (10,12) included in group B has properties similar to those of strains described by Devriese (5). They were classified as Staphylococcus hyicus because their key properties corresponded biochemically with sub-group III (bipotype 2) of Staphylococcus epidermidis (3). The members of group C (22.8%) were classified on the basis of their properties as S. epidermidis.
Juneja (11) reported a high prevalence of clinical and sub-clinical staphylococcal mastitis in dairy herds and the presence of *S. aureus* in raw milk from the mastitic animals. The present survey of mastitic milk in various parts of Ninevah province showed that staphylococci were responsible for 55.6% of the mastitis cases. Similar results have been reported by other investigators (9,17). This incidence may be due to several factors: unhygienic handling of the teats, milking by hand and improper use of milking machines.

The resistance patterns of all organisms tested against antibiotics are given in Table 2. *S. aureus* showed maximum resistance to penicillin (47.2%) followed by resistance to streptomycin (20.8%) and erythromycin (9.4%); only a very small percentage of isolates showed resistance to chloramphenicol (5.7%). This is in accordance with the observation of Garcia et al. (8). Higher resistance to chloramphenicol would have been expected because this antibiotic is the drug of choice for treatment of bovine staphylococcal mastitis (13). The presence of penicillin-resistant staphylococci in bovine mastitis milk is not uncommon; however, its incidence differs from location to location (10).

It has been reported that milk can act as a source for dissemination of antibiotic-resistant staphylococci (12). Therefore, it is advisable to initiate treatment of mastitis only after conducting the antibiotic sensitivity test to avoid dissemination of resistant staphylococci from diseased animals to healthy ones. This is important also to enhance control of foodborne staphylococcal intoxication, which appears to be on the increase. Nevertheless, the misguided and often inappropriate use of antibiotics in dairy husbandry is an underlying factor in the appearance of resistant strains and may contribute to their epidemic spread. Practices such as these are jeopardizing the success of measures aimed at reducing the incidence of the resistant staphylococci.

**TABLE 1. Biochemical characteristics of staphylococcal strains causing mastitis.**

<table>
<thead>
<tr>
<th>Group and No. of strains</th>
<th>Coagulation of plasma</th>
<th>Pigmentation</th>
<th>Hemolytic effect</th>
<th>Heat-stable nuclease</th>
<th>Mannitol anaerobically</th>
<th>Phosphatase</th>
<th>Acetoin</th>
<th>Phage typable</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (53)</td>
<td>53</td>
<td>50 (94.43%)</td>
<td>53</td>
<td>49 (92.45%)</td>
<td>53</td>
<td>53</td>
<td>51 (96.23%)</td>
<td>40 (75.47%)</td>
</tr>
<tr>
<td>B (8)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>8</td>
<td>0</td>
<td>8</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>C (18)</td>
<td>0</td>
<td>5 (27.77%)</td>
<td>7 (38.88%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>14 (77.77%)</td>
<td>0</td>
</tr>
</tbody>
</table>

**TABLE 2. Percentages of strains resistant to each antibiotic.**

<table>
<thead>
<tr>
<th>Antibacterial agent</th>
<th>μg of agent used</th>
<th><em>S. aureus</em> (n = 53)</th>
<th><em>S. hyicus</em> (n = 8)</th>
<th><em>S. epidermidis</em> (n = 18)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Penicillin G(P)</td>
<td>10a</td>
<td>25 (47.17%)</td>
<td>3 (37.5%)</td>
<td>5 (27.77%)</td>
</tr>
<tr>
<td>Methicillin(Met)</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Kanamycin(K)</td>
<td>30</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Streptomycin(S)</td>
<td>10</td>
<td>11 (20.75%)</td>
<td>1 (12.5%)</td>
<td>2 (11.11%)</td>
</tr>
<tr>
<td>Tetracycline(TE)</td>
<td>30</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Erythromycin(E)</td>
<td>15</td>
<td>5 (9.43%)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Cephaloridine(CR)</td>
<td>30</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Chloramphenicol(C)</td>
<td>30</td>
<td>3 (5.66%)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Neomycin(N)</td>
<td>30</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

*a* I.U. of agent used.

Phage typing of the 53 *S. aureus* strains (Table 3) showed that 74.5% of them were typable with phages of both sets. Twenty-four (45.3%) and ten (18.9%) strains were most susceptible to phage 78 and 102, respectively. Only nine strains (17.0%) were typable at 100 × RTD with phages of the human set all of which gave a weak reaction. Most of these strains were sensitive to phages 116, 3A, 71 and 55 of group II. Among the mastitis strains from many countries, a significant number were found to be most sensitive to phage 78. The enterotoxigenic strains isolated in this study produced enterotoxin C (5 strains) and enterotoxin D (2 strains). Similar findings were reported by other workers (8,14).

Use of milk from mastitic cows for manufacturing of dairy products may play a role in food poisoning, and thus may be hazardous to human health. Mastitis should be controlled by appropriate treatment; an effective qual-
ity control should be in effect during production, processing and distribution. Proper handling of the milk is important in preventing any staphylococci that may be present from growing and producing enterotoxin. Even though the staphylococci are destroyed during pasteurizing, any enterotoxin present would survive this treatment.

ACKNOWLEDGMENTS

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REFERENCES