ANTIMICROBIAL SUSCEPTIBILITY OF Escherichia coli ISOLATED FROM MASTITIC BOVINE MILK

(SENSIBILIDADE ANTIMICROBIANA DE Escherichia coli ISOLADA DE LEITE BOVINO MASTÍTICO)

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SUMMARY

A total of 182 Escherichia coli strains isolated from 2,144 samples of mastitic bovine milk were tested for O serogroups. Fifty-seven strains with classical enteropathogenic E. coli (EPEC) serogroups O26, O55, O111, and O119 were examined for their susceptibility to eight antimicrobial agents. All were susceptible to gentamicin, but resistance to tetracycline (24.5%), nalidixic acid (17.5%), carbenicillin (15.7%), ampicillin (7.0%), amikacin (7.0%), cephalothin (2.0%) and streptomycin (1.7%) was observed. None of the E. coli strains studied showed resistance to all antimicrobial agents tested.


INTRODUCTION

Bovine mastitis is an infectious disease caused by various pathogens, which differ in virulence, frequency and persistence in the mammary gland. The proportion of mastitic infection due to Escherichia coli has increased in recent years, mainly as a result of improved control programs of mastitis caused by Gram-positive bacterial pathogens (FANG & PYORALA, 1996; JONES, 1986).

The extensive use of antibiotics has led to a heavy increase in antibiotic resistance in animal production worldwide (FRANKLIN, 1999). Antimicrobial therapy is usually used for E. coli mastitis even though its therapeutic value has not been proved in clinical trials (ERSKINE et al., 1991; PYORALA et al., 1994).

There is an increasing concern about the problem of antimicrobial resistance and about variability in antibiotic susceptibility patterns for bacteria isolated from cows, so it is prudent periodically to monitor bacterial isolates from cows with mastitis in a study population for the antibiotic susceptibility (BEZEK, 1998).

RESUMO

Um total de 182 cepas de Escherichia coli foram isoladas de 2,144 amostras de leite mastítico, e os sorogrupos analisados. Cinquenta e sete cepas apresentando os sorogrupos clássicos de E. coli enteropatogênica (EPEC) O26, O55, O111 e O119 foram examinados para verificar a sua sensibilidade a diversos agentes antimicrobianos. Todas as cepas eram sensíveis a gentamicina, mas apresentaram resistência a tetraciclina (24,5%), ácido nalidíxico (17,5%), carbenicilina (15,7%), ampicilina (7,0%), amikacina (7,0%), cefalotina (2,0%) e estreptomicina (1,7%). Nenhuma das cepas de E. coli estudadas mostrou resistência a todos os agentes antimicrobianos testados.

Because of the lack of information available (LANGENEGGER et al., 1970; LANGONI et al., 1991; MORENO et al., 1997) concerning the characteristics of the bovine mastitis due to E.coli in Brazil and the fact that an increased number of antibiotics has been used in antimicrobial therapy, we decided to investigate the incidence of E.coli mastitis and its antibiotic resistance spectra.

**MATERIAL AND METHODS**

**Samples and Bacteriological Examinations:** Milk samples (2,144) from cows in seven Brazilian states (Espírito Santo, Goias, Mato Grosso do Sul, Minas Gerais, Parana, Rio Grande do Sul, São Paulo), from March 1997 to August 1998, were collected aseptically. The teat ends were cleaned with alcohol (70%), moistened swabs and allowed to dry. After discarding the first few streams, the samples (2-4mL) were collected into sterile 10mL glass flasks and submitted to California Mastitis Test (CMT) according to the method proposed by SCHALM & NOORLANDER (1957) on a scale of 1-5 (KLASTRUP, 1975). CMT-positive samples were kept refrigerated at about 4 °C and dispatched to the laboratory without delay. Bacterial strains were isolated in MacConKey (Difco) and identified by standard bacteriological methods (KONEMAN et al., 1997). The tests performed included: Gram stain, triple sugar-iron agar, phenylalanine deaminase, Simmons citrate, methyl red, Voges-Proskauer, indole production, urease, ornithine and lysine decarboxylases, arginine dihydrolase, catalase, oxidase, nitrate reductase reaction, and fermentation of glucose, lactose, sucrose, arabinose, raffinose, rhamnose, trehalose, xylose, dulcitol, mannitol and inositol. Fermentation of carbohydrates was tested on phenol red broth base (Difco) supplemented with 1% of the appropriate carbohydrate. The results were read after 24 and 48 h of incubation at 37 °C.

**O-serotyping technique:** E.coli isolated on MacConkey agar Petri dishes and biochemically confirmed were submitted to slide-agglutination tests using polyvalent and monovalent sera against classic enteropathogenic E.coli (EPEC) serogroups O26, O55, O86, O111, O114, O119, O125, O126, O127, O128, O142 and O158. Commercially available antisera from Probac, Brazil (São Paulo) were used.

**Sensitivity Testing:** Antibiotic susceptibility testing was performed using the Kirby-Bauer disk diffusion method with Mueller Hinton agar plates (CRAIG, 1993). After culturing each E.coli strain on Brain Heart Infusion Agar (Difco), five isolated colonies were inoculated into 5.0 mL Tryptic Soy Broth (Difco) and incubated for 2 to 4 hours at 37°C. Turbidity of this log-phase suspension was adjusted to 0.5 McFarland before inoculation on Muller Hinton agar plates (Difco). The following disks (CEFAR) were tested: amikacin (30μg), ampicillin (10μg), carbenicillin (100μg), cephalothin (30μg), streptomycin (10μg), gentamicin (10μg), nalidixic acid (30μg) and tetracycline (30μg). After 16 to 18 h of incubation at 37°C, zones of inhibition around all disks were measured using reflected light and interpreted according to the NCCLS-National Committee for Clinical Laboratory Standards (1999) recommendations.

**RESULTS AND DISCUSSION**

Identification of bacteria that cause mastitis in cows and their antibiotic susceptibility patterns are necessary to select appropriate antibiotics for treatment. E.coli were isolated from 8.5% of all milk samples. Among the 182 E.coli isolated (Table1), 141 strains had the serogroup identified, twelve O-serogroups could be identified (77.5% of total). O-serogroup could not be determined with regard to 41 isolates.

Out of the 279 E.coli strains investigated by LINTON et al. (1979), 217 (77.1%) could be O-serotyped, and 67 different O-serotypes were found. This indicates that E.coli mastitis is not caused by a limited number of pathogenic strains. Many other authors similarly reported having found a wide range of serotypes in cattle (LINTON & ROBINSON, 1984; WILSON et al., 1992; MYAU et al., 1998).

Verocytotoxin-producing E.coli (VTEC) is a naturally occurring organism in the gut flora of cattle (CHAPMAN et al., 1997), and it is recognized as the most important cause of potentially life-threatening human diseases such as haemorrhagic colitis and haemolytic uraemic syndrome. However EPEC are an important cause of infant disease in the developing world (GOMES et al., 1989), and classical EPEC serogroups such as O111 and O119 have been recognized since a long time as the most important EPEC serogroups associated with infant diarrhea in Brazil (CAMPOS et al., 1994; ROSA et al., 1998), what represent a reason of concern considering the great number of O111 and O119 isolates from mastitic milk. These are preliminary results, and further serotyping work is needed to confirm that these are EPEC serotypes.

Outbreaks of foodborne illness caused by E.coli have been associated with consumption of milk, ice cream, kefir, cheese and other dairy products (PARK et al., 1973; GLATZ & BRUDVIG, 1980). JONES et al. (1967), in a study
of coliforms in Canadian pasteurized dairy products, found three EPEC serotype isolates. Also ABBAR & KADDAR (1991) reported the isolation of EPEC serotypes isolates from cheese kishfa and gaymerr from Iraq. In Brazil BARUFALDI et al. (1984) and more recently SILVA et al. (2001) isolated E.coli strains from pasteurized milk of type B, and 46 (22.1%) of these strains were serologically identified as EPEC. It is believed that the isolation of EPEC serogroups from milk represents a potential risk especially for children.

SMITH et al. (1985) reported the variation of percentage of E.coli mastitis in bovine between 1.3% and 3.0%, while PAAPE et al. (1996) reported a low percentage around 1.0%, indicating the correct management of the herd and prevention of the environmental contamination. In Brazil, however, we found a constant increment in the participation of E.coli in bovine mastitis, FERREIRA et al. (1981) 1.3%, LANGONI et al. (1991) 2.8%, MORENO et al. (1997) 7.1%, and 8.5% (this work). The explanation commonly used for this increasing incidence is reduced coccal subclinical mastitis, brought about by a combination of dry cow therapy and teat dipping, plus increased E.coli challenge related to unhygienic loose-housing conditions (BRAMLEY & DODD, 1984).

Therapeutic value of antibiotics to treat coliform mastitis remains uncertain. The results of several trials have failed to show a beneficial effect of antibiotics compared with untreated controls or with administration of drugs without activity against coliforms in vitro (PYORALA & SYVAJARVI, 1987; ERSKINE et al., 1991; VAN EENANNAAM et al., 1995). The development of resistance to antimicrobials used in animal may present a hazard to humans when the resistant bacteria can cause disease in humans and can be transmitted via contaminated food (MCKELLAR, 1998).

Fifty-seven of 141 (40%) E.coli isolates were tested for antibiotic susceptibility. The frequency and patterns of resistance to the antibiotics are shown in Table 2.

Bacterial resistance to antibiotics varies widely (BARROW & HILL, 1989; BEZEK, 1998) and depends on country, use of drugs and infection control practice. However, the extensive use of antibiotics has led to a heavy increase in antibiotic resistance in animal production worldwide (MCKELLAR, 1998).

The occurrence of multi-resistant strains may be a response to the selective pressure caused by the abusive addition of antibiotics to animal rations. However the antibiotic sensitivity patterns observed were somewhat different from those observed by other Brazilian authors (LANGONI et al., 1991; MORENO et al., 1997), who found

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**Table 1** - Distribution and frequency of *Escherichia coli* O-serogroup isolated from bovine mastitis, in seven Brazilian states (1997-1998).

<table>
<thead>
<tr>
<th>O-SEROGROUPS</th>
<th>N*</th>
<th>%</th>
</tr>
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<tbody>
<tr>
<td>111</td>
<td>16</td>
<td>8.8</td>
</tr>
<tr>
<td>26</td>
<td>16</td>
<td>8.8</td>
</tr>
<tr>
<td>125</td>
<td>14</td>
<td>7.7</td>
</tr>
<tr>
<td>158</td>
<td>14</td>
<td>7.7</td>
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<tr>
<td>119</td>
<td>13</td>
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</tr>
<tr>
<td>55</td>
<td>12</td>
<td>6.6</td>
</tr>
<tr>
<td>86</td>
<td>12</td>
<td>6.6</td>
</tr>
<tr>
<td>127</td>
<td>11</td>
<td>6.0</td>
</tr>
<tr>
<td>142</td>
<td>10</td>
<td>5.5</td>
</tr>
<tr>
<td>114</td>
<td>09</td>
<td>5.0</td>
</tr>
<tr>
<td>126</td>
<td>07</td>
<td>3.8</td>
</tr>
<tr>
<td>128</td>
<td>07</td>
<td>3.8</td>
</tr>
<tr>
<td><strong>OTHER O-SEROGROUPS</strong></td>
<td><strong>41</strong></td>
<td><strong>22.5</strong></td>
</tr>
</tbody>
</table>

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**Table 2** - Patterns and phenotypes of drug resistance amongst 57 strains of *Escherichia coli* isolated from bovine mastitis in seven Brazilian states (1997-1998).

<table>
<thead>
<tr>
<th>PATTERNS</th>
<th>PHENOTYPES</th>
</tr>
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<tbody>
<tr>
<td>Resistant to none (31) *</td>
<td>Sensitive (31)</td>
</tr>
<tr>
<td>Resistant to 1 drug (12)</td>
<td>Ap (1)</td>
</tr>
<tr>
<td>Te (2)</td>
<td>Ca (2)</td>
</tr>
<tr>
<td>Na (3)</td>
<td></td>
</tr>
<tr>
<td>Resistant to 2 drugs (10)</td>
<td>Na - St (1)</td>
</tr>
<tr>
<td>Na - Ce (2)</td>
<td></td>
</tr>
<tr>
<td>Am - Ca (3)</td>
<td></td>
</tr>
<tr>
<td>Na - Te (4)</td>
<td></td>
</tr>
<tr>
<td>Resistant to 3 drugs (4)</td>
<td>Ap - Ca - Te (1)</td>
</tr>
<tr>
<td>Am - Ca - Te (3)</td>
<td></td>
</tr>
</tbody>
</table>

* Number of strains is given in parentheses.

an increased incidence of resistance in E. coli population, including a more extended multidrug resistance. In this study, 54.4% of the strains tested were sensitive to all antimicrobial agents analyzed and only four strains showed resistance to three antibiotics among the eight tested, so the resistance patterns were not so upsetting. However the constant monitoring of resistance patterns of clinical isolates is necessary to choose suitable antibiotics for therapeutic use, in order to avoid the selection of multidrug-resistant strains.

**ACKNOWLEDGEMENTS**

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