CTX-M1 ESBL-Producing Klebsiella pneumoniae subsp. pneumoniae Isolated from Cases of Bovine Mastitis

Escherichia coli and Klebsiella spp., followed by Serratia spp. and Enterobacter spp., are the most frequent Gram-negative pathogens isolated from bovine clinical mastitis (8). The incidence of mastitis due to such bacteria has increased in recent years (7). Klebsiella is usually referred to as particularly aggressive and prone to cause severe clinical mastitis, which responds poorly to treatment and is likely to have a fatal outcome (6, 13). Klebsiellae are defined as germs that easily produce enzymes like extended-spectrum β-lactamas (ESBL), thanks to their ability to survive longer than other Gram-negative rods in the environment and on the skin and, in particular, to allow ESBL genes to evolve (9). At the onset of clinical mastitis, it is impossible to identify the causative agent; thus, it is necessary to use broad-spectrum intramammary preparations which contain narrow- to extended-spectrum cephalosporins, alone or in combination with other antibiotics (1). The broad-spectrum cephalosporin cefotiofur is used as well as supportive systemic therapy (6).

Few recent works have studied Klebsiella isolation and ESBL detection in veterinary medicine (2, 16), and no data are available describing ESBL-mediated resistance in Klebsiella spp. isolated from bovine mastitis.

In this research, the antimicrobial susceptibility and ESBL production were evaluated in Klebsiella pneumoniae isolated from animals with bovine clinical mastitis and selected for their resistance to cefotiofur, a broad-spectrum cephalosporin. Susceptibility to a range of antimicrobials, including those approved for both human and animal use (Table 1), was determined by disk diffusion assay according to Clinical and Laboratory Standards Institute interpretative criteria (3). Escherichia coli ATCC 25922 and Staphylococcus aureus ATCC 25923 were used as quality control standards.

From March 2008 to March 2009, 140 klebsiellae were isolated from milk samples, representing 26.6% of the total number of Gram-negative isolates obtained from bovine clinical mastitis. The isolates were subcultured on MacConkey agar supplemented with 8 µg/ml of cefotiofur (5). Nine isolates, identified as Klebsiella pneumoniae, were able to grow in the presence of 8 µg of cefotiofur. All Klebsiella pneumoniae isolates were screened against the panel in Table 1 by the disk diffusion method and for ESBLs by the double-disk diffusion assay. A strain was considered an ESBL producer when it showed the expansion of an inhibition zone between a disk containing amoxicillin-clavulanate (20 and 10 µg) and disks containing, respectively, ceftazidime (30 µg) and cefotaxime (30 µg) placed 25 mm apart (10). All Klebsiella pneumoniae isolates were tested by PCR targeting the Ambler class A β-lactamase genes bla<sub>SHV</sub>, bla<sub>TEM</sub>, and bla<sub>CTX-M1</sub>, identified in Klebsiella pneumoniae animal isolates (16), using previously published primer sets (14, 15). Sequencing of amplified genes completed the study.

The complete resistance profiles and the PCR results are summarized in Table 1. Only isolate 205, identified as Klebsiella pneumoniae subsp. pneumoniae, had a positive result to the double-disk test and was resistant to all the β-lactams, including cefotaxime and ceftriaxone, and sensitive to amoxicillin-clavulanate. The amplification of a CTX-M class enzyme-encoding gene confirmed such a phenotype, and sequencing of the gene identified the bla<sub>CTX-M1</sub> group. To our knowledge, it is the first report about a CTX-M1 enzyme produced by a Klebsiella pneumoniae subsp. pneumoniae isolate in a case of bovine clinical mastitis. Isolate 205 also displayed a positive PCR result for genes bla<sub>SHV</sub> and bla<sub>TEM</sub> and so did isolates 204 and 251. A previous randomized search detected only ESBL-producing E. coli and no Klebsiella spp. (11).

The generally low level of in vitro resistance and the low prevalence of ESBL-producing strains (0.7%) should guarantee a good outcome of drug treatment for Klebsiella involved in mastitis, but antimicrobial resistance is not the only factor affecting therapy efficacy (1, 4). However, the described isolation must encourage that attention be paid to a phenomenon which could evolve and be enforced under the pressure of antimicrobial therapy, as the use of extended-spectrum cephalosporins in veterinary medicine may select ESBL producers (12). It is recommended to screen klebsiellae from cases of bovine mastitis in order to keep the prevalence of ESBL producers under control.

### TABLE 1. Comparison of resistance profiles of the nine isolates

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Resistance profile&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Presence of&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>bla&lt;sub&gt;SHV&lt;/sub&gt;</td>
</tr>
<tr>
<td>89</td>
<td>AMC CNM HAP NPZ RAX CFZ FOX STR</td>
<td>+</td>
</tr>
<tr>
<td>169</td>
<td>MAR CNM HAP NPZ RAX CFZ FOX TET</td>
<td>–</td>
</tr>
<tr>
<td>179</td>
<td>MAR CNM HAP NPZ RAX CFZ STR SXT</td>
<td>–</td>
</tr>
<tr>
<td>88</td>
<td>NPZ RAX STR KAN</td>
<td>–</td>
</tr>
<tr>
<td>119</td>
<td>HAP NDX RAX FOX FOX TET</td>
<td>–</td>
</tr>
<tr>
<td>187</td>
<td>CFT NPZ RAX STR KAN</td>
<td>+</td>
</tr>
<tr>
<td>204</td>
<td>CEF CNM HAP NPZ RAX CFZ FOX STR</td>
<td>+</td>
</tr>
<tr>
<td>205</td>
<td>CEF CNM HAP NPZ RAX CFZ CTX STR</td>
<td>+</td>
</tr>
<tr>
<td>251</td>
<td>CNM HAP RAX FOX FOX TET</td>
<td>–</td>
</tr>
</tbody>
</table>

<sup>a</sup> AMC, amoxicillin-clavulanate; CFT, cefotiofur; CFP, cepofur; CPF, cepofurazine; HAP, cepharolin; CNM, cefalonium; PH, penethamate hydriodide; MAR, marbofloxacin; DFX, dambofloxacin; NPZ, benzylenepicolin-nafillin-dihydrostreptomycin; RAX, rifaxim; CAZ, cepazidime; CTX, cefotaxime; CR<sub>0</sub>, cefotaxime; FOX, cefosfatin; CFZ, cefazolin; FEP, cefepime; IPM, imipenem; ATM, aztreonam; CH, cepesoxacin; PQR, streptomycin; GEN, gentamicin; KAN, kanamycin; TOR, tobramycin; TET, tetracycline; STX, trimethoprim-sulfamethoxazole.

<sup>b</sup> +, present; –, absent.
Nucleotide sequence accession number. The sequence of the CTX-M class enzyme-encoding gene identified in our study was added to GenBank (accession number HM921043).

REFERENCES


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