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Inducibly Cefoxitin-Resistant *Macrococcus*-Like Organism Falsely Identified as Methicillin-Resistant *Staphylococcus aureus* on CHROMagar with Oxacillin▽

In a screening study for methicillin-resistant *Staphylococcus aureus* (MRSA), nasal, pharyngeal, and rectal swabs were collected from healthy dogs at the University of Saskatchewan. Multiple solid media, including CHROMagar Staph aureus (CHROMagar, Paris, France), were inoculated; *S. aureus* grows as distinctive mauve colonies on CHROMagar Staph aureus (8). The inclusion of 4 μg/ml oxacillin (Sigma-Aldrich, St. Louis, MO) in the medium allows for the selective culture of MRSA.

Colonies typical of *S. aureus*, grown from a nasal swab on CHROMagar plus oxacillin, were presumptively identified as MRSA. While this isolate was oxidase negative, other biochemical characteristics were inconsistent with *S. aureus* including the following: no hemolysis on 5% sheep blood agar (Becton, Dickinson and Company, Sparks, MD) and lack of DNase, hyaluronidase, coagulase, and acetoin production.

Analysis of the 16S rRNA gene (GenBank accession no. GU994136) using previously published primers revealed 99% similarity to a previously deposited *Macrococcus caseolyticus* sequence (1, 6). However, analysis of the *cpn60* gene (GenBank accession no. GU994137), which has superior discrimination of staphylococci and macrococci, found only 89% similarity with the same *Macrococcus* entry (1, 10, 12). Phenotypic methicillin resistance was confirmed with oxacillin disk testing; when cefoxitin disks (30 μg) were used, a target zone of inhibition was seen (Fig. 1) and postulated to indicate inducible resistance. Target phenotypes resulting from inducible resistance are reported for MRSA with nafcillin and coagulase-negative staphylococci with imipenem (2, 3). Growth inside the inhibitory zone occurred when oxacillin or amoxicillin-clavulanate disks were placed adjacent to cefoxitin disks, indicating more potent resistance induction by these drugs (Fig. 2). Using a commercially prepared nitrocefin test (Hardy Diagnostics, Santa Maria, CA), the isolate was found to produce β-lactamase which may be responsible for the resistance seen. The contrast and brightness of both images were enhanced for clarity.

Methicillin resistance has been found in *M. casolyticus*, the most closely related organism identified, where a gene with 72% homology to the *S. aureus mecA* gene was identified (1). Perhaps due to a similarly low homology, *mecA* was not amplified from our isolate with previously published primers (5). It is not known whether the inducible cefoxitin resistance in this isolate was due to extended-spectrum β-lactamase production or a *mecA*-like mechanism. It is possible that like *S. aureus* ATCC 43300, this isolate produces both altered penicillin binding proteins and β-lactamase (4, 9). While the mechanism leading to this putative inducible resistance phenotype is unknown, this may be an example of "Eagle-type" resistance. Eagle-type resistance describes bacterial growth at high, but paradoxically not low, drug concentrations; in the case of

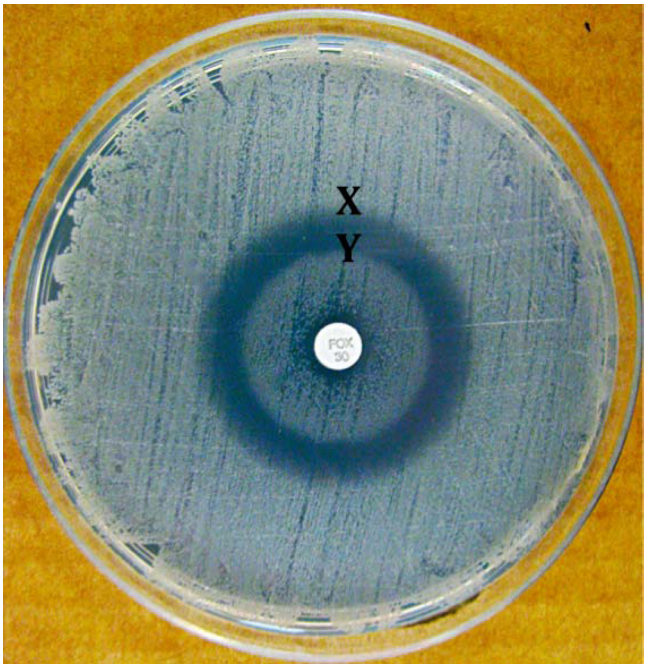

FIG. 1. Target zone of inhibition associated with cefoxitin disk (FOX) (30 μg) on antibiotic-free Mueller-Hinton agar. The cefoxitin concentration at X is the MIC resulting in a zone of clearing, while induction of resistance at supra-MIC concentrations Y accounts for the inner zone of growth.

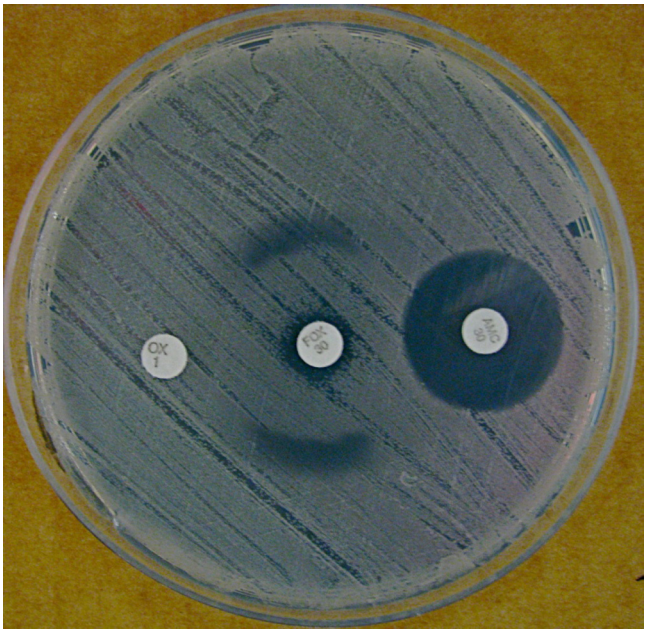

FIG. 2. Blunting of the cefoxitin zone of inhibition, suggesting resistance induction by oxacillin (OX) (1 μg) and amoxicillin-clavulanate (AMC) (30 μg).

MRSA, "derepression" of the *mecA* gene is purportedly causal (7, 11).

This report highlights the need for confirmatory testing when using selective/differential media for the identification of *S. aureus* (MRSA), as false-positive results may have therapeutic or infection control implications. Additional tests, including analysis of the *cpn60* sequence may be indicated, particularly in bacterial species in veterinary animals, where colonizing bacterial populations are ill defined compared to those in humans (12). Finally, as with inducible clindamycin among *S. aureus*, further testing is essential to accurately characterize isolates with unusual resistance phenotypes (4).

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