Accepted Manuscript

Title: Evaluation of ceftiofur and cefquinome for phenotypic detection of methicillin resistance in *Staphylococcus aureus* using disk diffusion testing and MIC-determinations

Authors: Frank M. Aarestrup, Robert L. Skov

PII: S0378-1135(09)00337-X
DOI: doi:10.1016/j.vetmic.2009.07.005
Reference: VETMIC 4507

To appear in: VETMIC

Received date: 22-12-2008
Revised date: 30-6-2009
Accepted date: 3-7-2009

Please cite this article as: Aarestrup, F.M., Skov, R.L., Evaluation of ceftiofur and cefquinome for phenotypic detection of methicillin resistance in *Staphylococcus aureus* using disk diffusion testing and MIC-determinations, *Veterinary Microbiology* (2008), doi:10.1016/j.vetmic.2009.07.005

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.
Evaluation of ceftiofur and cefquinome for phenotypic detection of methicillin resistance in Staphylococcus aureus using disk diffusion testing and MIC-determinations

Frank M. Aarestrup,¹* Robert L. Skov²

¹: Community Reference Laboratory for Antimicrobial Resistance and WHO Collaborating Centre for Antimicrobial Resistance in Foodborne Pathogens, National Food Institute, Technical University of Denmark.

²: National Center for Antimicrobials and Infection Control, Statens Serum Institut.

* Corresponding author. Mailing address: National Food Institute, Technical University of Denmark, Bülowsvæj 27, DK-1790 Copenhagen V, Denmark. Phone: +45 72 34 60 00. Fax: +45 72 34 60 01. E-mail: fmaa@food.dtu.dk.
Abstract

Methicillin resistant *Staphylococcus aureus* (MRSA) have emerged in animals. Testing 98 meca-negative and 71 meca-positive *S. aureus* we compared the usefulness of ceftiofur and cefquinome to cefoxitin, for detection of MRSA and found that these cephalosporins are not as efficient as cefoxitin.

Key words: MRSA, detection, ceftiofur, cefquinome, cefoxitin
Introduction

Infections due to methicillin-resistant Staphylococcus aureus (MRSA) are an increasing problem worldwide inside and outside of hospitals (Grundmann et al., 2006). MRSA have during the last two decades emerged in pets and production animals (Huijsdens et al., 2006; Leonard and Markey, 2008; Weese et al., 2006; Witte et al., 2007). The colonization in animals has in several cases been implicated in infections in humans and MRSA should today be considered a zoonosis.

Phenotypic detection of MRSA has been problematic ever since its discovery in the early 1960s. Detection of the mecA gene or its product, penicillin binding protein (PBP2a), is considered the gold standard (Chambers, 1997) for MRSA confirmation. A number of studies have suggested that for sensitive and specific detection of mecA, susceptibility testing using cefoxitin is superior to most previously recommended phenotypic methods (Felten et al., 2002; Mougeot et al., 2001; Skov et al., 2003, 2006; Swenson and Tenover, 2005).

In veterinary medicine the cephalosporins ceftiofur and cefquinome are approved and used for several animal species in many countries worldwide. Many veterinary diagnostic laboratories will routinely test for susceptibility using the veterinary approved cephalosporins and not using cefoxitin specifically useful for detection of MRSA.

To our knowledge the ability of using ceftiofur or cefquinome for detection of MRSA have never been evaluated and this study reports the susceptibility of mecA-positive and mecA-negative S. aureus to ceftiofur and cefquinome using disk diffusion and MIC-determinations.

Materials and Methods
Strains. A total of 145 *S. aureus* previously reported strains were included in the study (Skov et al., 2006). The strains consisted of 95 *mecA*-negative consecutive blood culture isolates and 50 *mecA*-positive isolates from different patients. In addition, 24 *S. aureus* from pigs in 10 different farms were included. Of these 21 were *mecA* positive isolates of spa-type t034 and three *mecA* negative isolates of spa-types t034, t899 and t2462.

Susceptibility testing. The 145 previously reported strains were already tested for susceptibility to cefoxitin by Etest and disk diffusion (Skov et al., 2006). The 24 isolates from pigs were tested for cefoxitin susceptibility using the same methodology. All 169 strains were tested by disk diffusion and micro broth dilution using cefquinome and ceftiofur. Disk diffusion was done with 30-µg disks (Oxoid, Basingstoke, United Kingdom) on Mueller-Hinton BBL II agar (Becton Dickinson, Heidelberg, Germany). MIC determination was done in home made micro-titre plates containing two-fold dilutions of the antibiotic in Mueller-Hinton-II broth prepared the day prior to use according to CLSI standards (CLSI, 2006a). The micro-titre plates were prepared with 50 µL and contained two times the desired final concentration.

For each strain an inoculum was standardized to 0.5 McFarland turbidity. This was used for inoculation of the agar plates. For MIC-determinations 10 µL of this inoculum was transferred to a tube with 10 mL MH-II broth and each micro-titre well was inoculated with 50 µL to achieve a final volume of 100 µL. Both agar plates and micro-titre plates were incubated at 35°C and read after 18 to 19 hours in accordance with standards by CLSI (CLSI, 2006ab). *S. aureus* ATCC 25923 and ATCC 29213 were included at all runs for quality control of disk diffusion and MIC-determinations, respectively.

Statistical analysis. To evaluate the performance of the different cephalosporins compared to *mecA*-PCR as the gold standard, an optimum cut-off values separating *mecA*-positive and
mecA-negative isolates were chosen and kappa for the different cephalosporins calculated (Altman, 1990).

Results

Minimum inhibitory concentration. MICs are shown in Table 1. In the cases were the Etest results for cefoxitin were not the same as a two-fold dilution MIC the strain result were assigned the closest value above. Cefoxitin MICs ranged from 0.5 to 4 mg/L for mecA negative and from 4 to >16 mg/L for mecA positive isolates. Ceftiofur MICs ranged from 0.25 to 8 mg/L for mecA negative isolates and from 1 to >16 mg/L for mecA positive isolates. Cefquinome MICs ranged from 0.25 to 4 mg/L for mecA negative isolates and from 1 to 16 mg/L for mecA positive isolates.

Using a cut-off value of >1 mg/L for ceftiofur mecA negative isolates were categorised as susceptible (SP = 0.82), 62 mecA positive isolates categorised as resistant (SE = 0.87) and kappa were 0.68. Using a cut-off value of >1 mg/L for cefquinome mecA negative isolates were categorised as susceptible (SP = 0.89), 57 mecA positive isolates categorised as resistant (SE = 0.80) and kappa were 0.68. For cefoxitin and using a cut-off of >4 mg/L, all mecA negative isolates were categorised as susceptible (SP = 1), 67 mecA positive as resistant (SE = 0.94) and kappa were 0.95.

Disk diffusion. Results for cefoxitin, ceftiofur and cefquinome disks incubated 18 to 19 h at 35°C are shown in Table 2. Cefoxitin zones ranged from 6 to 27 mm for mecA positive isolates and from 25 to 34 mm for mecA negative isolates. Ceftiofur zones ranged from 6 to 30 mm for mecA positive isolates and from 19 to 36 for mecA negative isolates. Cefquinome
zones ranged from 6 to 30 mm for \textit{mecA} positive isolates and from 23 to 36 for \textit{mecA} negative isolates.

Using <25 mm as the interpretive zone diameter for ceftiofur gave a specificity of 96%, a sensitivity of 93% and a kappa of 0.89. For cefquinome a cut-off value of <27 mm gave a specificity of 94%, a sensitivity of 92% and a kappa of 0.86. For cefoxitin a cut-off value of <22 mm gave a specificity of 100%, a sensitivity of 99% and a kappa of 0.99.

\textbf{Discussion}

MRSA have recently emerged as a cause of infections and a coloniser in pets and food animals. The 3\textsuperscript{rd} generation cephalosporin ceftiofur and 4\textsuperscript{th} generation cefquinome are widely used for treatment of infections in animals in many countries worldwide and routine susceptibility testing might typically be done using these agents.

In our study, the MIC-values for \textit{mecA} negative and \textit{mecA} positive isolates for both ceftiofur and cefquinome were overlapping. Using cefoxitin it was possible to separate the two populations almost entirely. It is important to note that the current CLSI break points for resistance to ceftiofur (resistance, MIC $\geq$ 8 mg/L or inhibition zone $\leq$ 17 mm) (CLSI, 2008), for a large number of \textit{mecA}-positive isolates will not result in detecting MRSA. Similar problems exist with the provisional which has been suggested for respiratory pathogens for cefquinome (Luhofer et al., 2004). We found that disk diffusion testing gave a better separation between \textit{mecA} negative and positive isolates than MIC determinations. For ceftiofur and cefquinome the two populations were however, still overlapping and these two cephalosporins were not as efficient as cefoxitin in detecting MRSA. None-the-less if zone diameters of 25 mm for ceftiofur and 27 mm for cefquinome were used most isolates would
be categorised correctly and MRSA would with a great certainty be found also in the clinical laboratory.

Conclusion

In conclusion, this study shows that cefoxitin gave a better separation between mecA positive and mecA negative *S. aureus* than ceftiofur and cefquinome. Thus, even though these two latter cephalosporins might be used, cefoxitin is recommended for detection of MRSA in routine susceptibility testing for disk diffusion and MIC testing.
Acknowledgement

This study was supported by the European Commission and grant 274-05-0117 from the Danish Research Agency.

Conflict of interest

None to declare.
References


Table 1  Ceftiofur and cefquinome MICs of *mecA* positive and *mecA* negative *Staphylococcus aureus* determined by broth dilution.

<table>
<thead>
<tr>
<th>Antimicrobial agent</th>
<th><em>mecA</em></th>
<th>Number of isolates</th>
<th>Number of isolates with a MIC (mg/L) of:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.25</td>
</tr>
<tr>
<td>Ceftiofur</td>
<td>Negative</td>
<td>98</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Positive</td>
<td>71</td>
<td>9</td>
</tr>
<tr>
<td>Cefquinome</td>
<td>Negative</td>
<td>98</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Positive</td>
<td>71</td>
<td>14</td>
</tr>
<tr>
<td>Cefoxitin*</td>
<td>Negative</td>
<td>98</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Positive</td>
<td>71</td>
<td>4</td>
</tr>
</tbody>
</table>

*: performed by Etest.
Table 1  Ceftiofur, cefquinome and cefoxitin zone diameters of *mecA* positive and *mecA* negative *Staphylococcus aureus*

| Antimicrobial agent | meca No. isolates | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 | 31 | 32 | 33 | 34 | 35 | 36 |
|---------------------|-------------------|---|---|---|---|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|
| Ceftiofur            | Negative          | 98 | 3 | 1 | 3 | 3 | 1 | 3 | 4 | 10 | 18 | 18 | 15 | 11 | 5 | 3 |    |    |    |    |    |    |    |    |    |    |    |    |    |
|                     | Positive          | 71 | 5 | 2 | 1 | 2 | 3 | 11 | 2 | 7 | 5 | 13 | 6 | 8 | 1 | 1 | 3 | 1 |    |    |    |    |    |    |    |    |    |    |    |
| Cefquinome          | Negative          | 98 | 2 | 1 | 3 | 3 | 2 | 6 | 12 | 27 | 16 | 16 | 4 | 4 | 2 |    |    |    |    |    |    |    |    |    |    |    |    |    |
|                     | Positive          | 71 | 1 | 1 | 2 | 1 | 2 | 12 | 3 | 8 | 9 | 8 | 7 | 6 | 5 | 3 | 2 | 1 |    |    |    |    |    |    |    |    |    |    |    |
| Cefoxitin           | Negative          | 98 | 5 | 14 | 32 | 24 | 17 | 4 | 1 | 1 |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
|                     | Positive          | 71 | 4 | 2 | 3 | 3 | 7 | 11 | 8 | 9 | 4 | 3 | 6 | 3 | 2 | 2 | 1 |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
