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Authors: Frank M. Aarestrup, Robert L. Skov

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

4 Frank M. Aarestrup,^{1*} Robert L. Skov²

5

6 1: Community Reference Laboratory for Antimicrobial Resistance and WHO Collaborating
7 Centre for Antimicrobial Resistance in Foodborne Pathogens, National Food Institute,
8 Technical University of Denmark.

9 2: National Center for Antimicrobials and Infection Control, Statens Serum Institut.

10

11 * Corresponding author. Mailing address: National Food Institute, Technical University of
12 Denmark, Bülowsvej 27, DK-1790 Copenhagen V, Denmark. Phone: +45 72 34 60 00. Fax:
13 +45 72 34 60 01. E-mail: fmaa@food.dtu.dk.

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17

18 **Abstract**

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

23

24

25 **Key words:** MRSA, detection, ceftiofur, cefquinome, cefoxitin

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27 **Introduction**

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

34 Phenotypic detection of MRSA has been problematic ever since its discovery in the early
35 1960s. Detection of the *mecA* gene or its product, penicillin binding protein (PBP2a), is
36 considered the gold standard (Chambers, 1997) for MRSA confirmation. A number of studies
37 have suggested that for sensitive and specific detection of *mecA*, susceptibility testing using
38 cefoxitin is superior to most previously recommended phenotypic methods (Felten et al.,
39 2002; Mougeot et al., 2001; Skov et al., 2003, 2006; Swenson and Tenover, 2005).

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

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

47

48 **Materials and Methods**

49 **Strains.** A total of 145 *S. aureus* previously reported strains were included in the study (Skov
50 et al., 2006). The strains consisted of 95 *mecA*-negative consecutive blood culture isolates and
51 50 *mecA*-positive isolates from different patients. In addition, 24 *S. aureus* from pigs in 10
52 different farms were included. Of these 21 were *mecA* positive isolates of spa-type t034 and
53 three *mecA* negative isolates of spa-types t034, t899 and t2462.

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

64 For each strain an inoculum was standardized to 0.5 McFarland turbidity. This was used for
65 inoculation of the agar plates. For MIC-determinations 10 μ L of this inoculum was transferred
66 to a tube with 10 mL MH-II broth and each micro-titre well was inoculated with 50 μ L to
67 achieve a final volume of 100 μ L. Both agar plates and micro-titre plates were incubated at
68 35°C and read after 18 to 19 hours in accordance with standards by CLSI (CLSI, 2006ab). *S.*
69 *aureus* ATCC 25923 and ATCC 29213 were included at all runs for quality control of disk
70 diffusion and MIC-determinations, respectively.

71 **Statistical analysis.** To evaluate the performance of the different cephalosporins compared to
72 *mecA*-PCR as the gold standard, an optimum cut-off values separating *mecA*-positive and

73 *mecA*-negative isolates were chosen and kappa for the different cephalosporins calculated
74 (Altman, 1990).

75

76 **Results**

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

84

85 Using a cut-off value of >1 mg/L for ceftiofur 80 *mecA* negative isolates were categorised as
86 susceptible (SP = 0.82), 62 *mecA* positive isolates categorised as resistant (SE = 0.87) and
87 kappa were 0.68. Using a cut-off value of >1 mg/L for cefquinome 87 *mecA* negative isolates
88 were categorised as susceptible (SP = 0.89), 57 *mecA* positive isolates categorised as resistant
89 (SE = 0.80) and kappa were 0.68. For cefoxitin and using a cut-off of >4 mg/L, all 98 *mecA*
90 negative isolates were categorised as susceptible (SP = 1), 67 *mecA* positive as resistant (SE =
91 0.94) and kappa were 0.95.

92

93 **Disk diffusion.** Results for cefoxitin, ceftiofur and cefquinome disks incubated 18 to 19 h at
94 35°C are shown in Table 2. Cefoxitin zones ranged from 6 to 27 mm for *mecA* positive
95 isolates and from 25 to 34 mm for *mecA* negative isolates. Ceftiofur zones ranged from 6 to
96 30 mm for *mecA* positive isolates and from 19 to 36 for *mecA* negative isolates. Cefquinome

97 zones ranged from 6 to 30 mm for *mecA* positive isolates and from 23 to 36 for *mecA* negative
98 isolates.

99

100 Using <25 mm as the interpretive zone diameter for ceftiofur gave a specificity of 96%, a
101 sensitivity of 93% and a kappa of 0.89. For cefquinome a cut-off value of <27 mm gave a
102 specificity of 94%, a sensitivity of 92% and a kappa of 0.86. For cefoxitin a cut-off value of
103 <22 mm gave a specificity of 100%, a sensitivity of 99% and a kappa of 0.99.

104

105 **Discussion**

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

110

111 In our study, the MIC-values for *mecA* negative and *mecA* positive isolates for both ceftiofur
112 and cefquinome were over-lapping. Using cefoxitin it was possible to separate the two
113 populations almost entirely. It is important to note that the current CLSI break points for
114 resistance to ceftiofur (resistance, MIC \geq 8 mg/L or inhibition zone \leq 17 mm) (CLSI, 2008),
115 for a large number of *mecA*-positive isolates will not results in detecting MRSA. Similar
116 problems exist with the provisional which has been suggested for respiratory pathogens for
117 cefquinome (Luhofner et al., 2004). We found that disk diffusion testing gave a better
118 separation between *mecA* negative and positive isolates than MIC determinations. For
119 ceftiofur and cefquinome the two populations were however, still over-lapping and these two
120 cephalosporins were not as efficient as cefoxitin in detecting MRSA. None-the-less if zone
121 diameters of 25 mm for ceftiofur and 27 mm for cefquinome were used most isolates would

122 be categorised correctly and MRSA would with a great certainty be found also in the clinical
123 laboratory.

124

125 **Conclusion**

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

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131 **Acknowledgement**

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134

135

136 **Conflict of interest**

137 None to declare.

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

Antimicrobial agent	<i>mecA</i>	Number of isolates	Number of isolates with a MIC (mg/L) of:							
			0.25	0.5	1	2	4	8	16	>16
Ceftiofur	Negative	98	5	33	42	15	1	2		
	Positive	71			9	23	28	3	4	4
Cefquinome	Negative	98	4	46	37	8	3			
	Positive	71			14	38	13	4	2	
Cefoxitin*	Negative	98		2	2	60	34			
	Positive	71					4	10	7	50

191 *: performed by Etest.

