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

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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

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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

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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

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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 80 *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 87 *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 98 *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

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

Discussion

MRSA have recently emerged as a cause of infections and a coloniser in pets and food animals. The 3rd generation cephalosporin ceftiofur and 4th 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 *mecA* negative and *mecA* positive isolates for both ceftiofur and cefquinome were over-lapping. 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 ≥ 8 mg/L or inhibition zone ≤ 17 mm) (CLSI, 2008), for a large number of *mecA*-positive isolates will not results 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 *mecA* negative and positive isolates than MIC determinations. For ceftiofur and cefquinome the two populations were however, still over-lapping 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

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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.

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Conflict of interest

None to declare.

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References

Altman, G. 1990. Practical Statistic in Medical Research. Chapman & Hall/CRC. ISBN-13 978-0-412-27630-9.

Chambers, H. F. 1997. Methicillin resistance in staphylococci: molecular and biochemical basis and clinical implications. Clin. Microbiol. Rev. 10, 781-791.

Clinical and Laboratory Standards Institute. 2006a. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically, 7th Edition. Approved Standard. M07-A7. Wayne, PA.

Clinical and Laboratory Standards Institute. 2006b. Performance standards for antimicrobial disk susceptibility tests; approved standard – ninth edition. M2-A9. Clinical and Laboratory Standards Institute, Wayne, PA.

Clinical Laboratory Standards Institute. 2008. Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for bacteria Isolated from Animals, 3rd Edition, Approved Standard. M31-A3. Wayne, PA.

Felten, A., Grandry, B., Lagrange, P. H., Casin, I. 2002. Evaluation of three techniques for detection of low-level methicillin-resistant *Staphylococcus aureus* (MRSA): a disk diffusion method with cefoxitin and moxalactam, the Vitek 2 system, and the MRSA-screen latex agglutination test. J. Clin. Microbiol. 40, 2766-2771.

Grundmann, H., Aires-de-Sousa, M., Boyce, J., Tiemersma, E. 2006. Emergence and resurgence of meticillin-resistant *Staphylococcus aureus* as a public-health threat. Lancet 368, 874-885.

Accepted Manuscript

ACCEPTED MANUSCRIPT

Huijsdens, X. W., van Dijke, B. J., Spalburg, E., van Santen-Verheuvel, M. G., Heck, M. E., Pluister, G. N., Voss, A., Wannet, W. J., de Neeling, A. J. 2006. Community-acquired MRSA and pig-farming. Ann. Clin. Microbiol. Antimicrob. 10, 26.

Leonard, F. C., Markey, B. K. 2008. Meticillin-resistant *Staphylococcus aureus* in animals: A review. Vet. J. 175, 27-36.

Luhofer, G., Böttner, A., Hafez, H.M., Kaske, M., Kehrenberg, C., Kietzmann, M., Klarmann, D., Klein, G., Krabisch, P., Kühn, T., Richter, A., Sigge, C., Traeder, W., Waldmann, K.H., Wallmann, J., Werckenthin, C., Schwarz, S.; Arbeitsgruppe "Antibiotikaresistenz". 2004. [Proposals of the working group "Antibiotic resistance" for the configuration of microtitre plates to be used in routine antimicrobial susceptibility testing of bacterial pathogens from infections of large food-producing animals and mastitis cases]. Berl. Munch. Tierarztl. Wochenschr. 117, 245-251.

Mougeot, C., Guillaumat-Tailliet, J., Libert, J. M. 2001. *Staphylococcus aureus*: new detection of intrinsic resistance using the diffusion method. Pathol. Biol. (Paris) 49,199-204.

Skov, R., Smyth, R., Clausen, M., Larsen, A. R., Frimodt-Moller, N., Olsson-Liljequist, B., Kahlmeter, G. 2003. Evaluation of a cefoxitin 30 µg disc on Iso-Sensitest agar for detection of methicillin-resistant *Staphylococcus aureus*. J. Antimicrob. Chemother. 52, 204-207.

Skov, R., Smyth, R., Larsen, A. R., Bolmstrôm, A., Karlsson, A., Mills, K., Frimodt-Møller, N., Kahlmeter, G. 2006. Phenotypic detection of methicillin resistance in *Staphylococcus aureus* by disk diffusion testing and Etest on Mueller-Hinton agar. J. Clin. Microbiol. 44, 4395-9.

Swenson, J. M., Tenover, F. C. 2005. Results of disk diffusion testing with cefoxitin correlate with presence of *mecA* in *Staphylococcus* spp. J. Clin. Microbiol. 43,3818-3823.

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ACCEPTED MANUSCRIPT

Weese, J. S., Dick, H., Willey, B. M., McGeer, A., Kreiswirth, B. N., Innis, B., Low, D. E. 2006. Suspected transmission of methicillin-resistant *Staphylococcus aureus* between domestic pets and humans in veterinary clinics and in the household. Vet. Microbiol. 115, 148-155.

Witte, W., Strommenger, B., Stanek, S., Cuny, C. 2007. Methicillin-resistant *Staphylococcus aureus* ST398 in Humans and Animals, Central Europe. Emerg. Infect. Dis. 13, 255-258.

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

Antimicrobial agent	*mecA*	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

*: performed by Etest.

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Table 1 Ceftiofur, cefquinome and cefoxitin zone diameters of *mecA* positive and *mecA* negative *Staphylococus aureus*

Antimicrobial agent	mecA	No. isolates	Number of isolates with a zone diameter of:																													
			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		1	2	9	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	3	7	11	8	9	4	3	6	3	2		2						1									

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