JOURNAL OF CLINICAL MICROBIOLOGY, June 2004, p. 2780–2782 Vol. 42, No. 6
0095-1137/04/$08.00+0 DOI: 10.1128/JCM.42.6.2780–2782.2004
Copyright © 2004, American Society for Microbiology. All Rights Reserved.

Evaluation of the Methicillin-Resistant *Staphylococcus aureus* (MRSA)-Screen Latex Agglutination Test for Detection of MRSA of Animal Origin

John H. Lee,[1]* Jae-Myung Jeong,[1] Yong-Ho Park,[2] Sung-Sun Choi,[1] Yong-Hwan Kim,[1] Joon-Seok Chae,[1] Jin-San Moon,[3] Hyun Park,[4] Shin Kim,[5] and Seong-Kug Eo[1]

College of Veterinary Medicine, Chonbuk National University, Chonju 561-756,[1] College of Veterinary Medicine, Seoul National University, Seoul 135-703,[2] National Veterinary Research and Quarantine Service, Anyang 430-016,[3] Northern Branch, Gyeongbuk Veterinary Service Laboratory, Andong 760-749,[5] and Department of Parasitology, College of Medicine, Wonkwang University, Iksan 570-749,[4] South Korea

Received 7 November 2003/Returned for modification 19 December 2003/Accepted 26 February 2004

Methicillin (oxacillin)-resistant staphylococci (MRS) have emerged as major clinical and epidemiological pathogens, and there have been frequent reports of MRS infections in the veterinary field. The MRSA-Screen latex agglutination test (Denka Seiken Co., Ltd., Tokyo, Japan) was compared with an oxacillin agar screen test, MIC determination, and *mecA* PCR assay, the "gold standard." In an analysis of 15 *mecA*-positive and 48 *mecA*-negative *S. aureus* animal isolates, as well as 9 *mecA*-positive and 147 *mecA*-negative, coagulase-negative staphylococcal animal isolates, the latex agglutination test surpassed the widely used oxacillin agar screen method and MIC determination, with a sensitivity and a specificity of 100%. The MRSA-Screen test is a reliable and rapid method of detecting MRS in the veterinary field.

Staphylococcus spp. cause severe diseases such as suppurative disease, mastitis, arthritis, and urinary tract infection by introducing numerous virulence factors such as extracellular toxins and enzymes into animal species (20). For humans, these organisms are important causes of food poisoning, pneumonia, wound infections, and nosocomial bacteremia (6). Staphylococcal isolates are frequently resistant to penicillinase-resistant penicillins. Organisms exhibiting this type of resistance are referred to as methicillin (oxacillin)-resistant staphylococci (MRS). These bacteria are also frequently resistant to most of the commonly used antimicrobial agents, including the aminoglycosides, macrolides, chloramphenicol, tetracycline, and fluoroquinolones (10). In addition, MRS strains should be considered to be resistant to all cephalosporins, cephems, and other β-lactams, such as ampicillin-sulbactam, amoxicillin-clavulanic acid, ticarcillin-clavulanic acid, piperacillin-tazobactam, and the carbapenems, regardless of the in vitro test results obtained with those agents (15).

There are many reports on MRS infections in dairy herds with mastitis, as well as in companion animals and horses (2, 4, 8, 9, 17, 18, 21). These reports indicate that MRS infections are problematic in the veterinary field, and these types of strains can be transmitted to humans (9). Therefore, infections by multidrug-resistant pathogens such as MRS require rapid and accurate diagnosis for elimination at an early stage because these strains can cause severe damage to infected sites and may be widespread in the environment. In most routine microbiological settings, detection of methicillin resistance among staphylococcal isolates is based on phenotypic assays such as a disk diffusion test and MIC determination. Genetic confirmation of positive findings based on detection of the *mecA* gene has also been reported (13, 19). However, these techniques are not yet generally available outside of reference laboratories. The methicillin resistance of *Staphylococcus* spp. is mediated by the *mecA* gene, which encodes penicillin-binding protein 2a (PBP2a) (3). A simple latex agglutination assay (MRSA-Screen; Denka Seiken Co., Ltd., Tokyo, Japan) was developed to detect methicillin-resistant *Staphylococcus aureus* (MRSA), which makes use of a specific monoclonal antibody directed toward the PBP2a antigen (14). This assay has the potential to detect MRSA in a routine microbiology setting because it combines high speed and excellent specificity and sensitivity. This assay has not been tested on isolates of animal origin. In this study, the MRSA-Screen latex agglutination test, which is a simple and rapid assay for detection of various MRS of animal origin, was evaluated to determine the sensitivity of the assay. This test was compared with an oxacillin agar screen test, MIC determination, and the *mecA* PCR assay, the "gold standard."

Samples of feces, milk, feed material, and the joints, tracheas, uteri, and meat of cattle, pigs, and chickens were collected at slaughterhouses, meat processing facilities, and farms located throughout Korea, including Chungcheong, Gyeongsang, and Jeolra Provinces, between May 2001 and April 2003. The collected samples were inoculated into either *Staphylococcus* broth or tryptic soy broth with 70 mg of NaCl per ml and incubated at 35°C for 20 h with constant shaking. The inoculum was subcultured on Baird-Parker medium for 24 to 48 h at 35°C. The typical suspected colonies were tested for *Staphylococcus* spp. by conventional methods that included Gram staining, tests for colonial morphology, and tests for coagulase using

* Corresponding author. Mailing address: College of Veterinary Medicine, Chonbuk National University, Chonju 561-756, South Korea. Phone: 82-63-270-2553. Fax: 82-63-270-3780. E-mail: johnhlee@chonbuk.ac.kr.

TABLE 1. Diagnostic test results obtained for MRSA isolates from animals[a]

Isolate	Origin	*mecA* PCR	MRSA-Screen[b]	Oxacillin-Screen	MIC (μg/ml)
SA006	Chickens	+	+	+	16
SA023	Cattle	−	−[c]	+	4
SA030	Pigs	−	−	+	8
SA031	Pigs	−	−	+	4
SA032	Pigs	−	−[c]	+	4
SA033	Pigs	−	−	+	4
SA037	Pigs	−	−	+	2
SA046	Cattle	+	+	+	32
SA049	Chickens	−	−	+	1
SA055	Chickens	−	−	+	2
SA058	Chickens	−	−	+	4
SA095	Chickens	+	+	+	32
SA097	Chickens	+	+	+	32
SA106	Cattle	+	+	+	32
SA107	Cattle	−	−	+	4
SA108	Cattle	−	−	+	4
SA109	Cattle	+	+	+	16
SA120	Cattle	+	+	+	32
SA122	Cattle	+	+	+	32
SA123	Cattle	+	+	+	>128
SA124	Cattle	+	+	+	64
SA125	Cattle	+	+	+	8
SA126	Cattle	+	+	+	16
SA129	Cattle	−	−	+	8
SA130	Cattle	+	+	+	128
SA236	Cattle	+	+	+	64
SA237	Cattle	+	+	+	4
SA240	Cattle	−	−	+	2

[a] Symbols: +, positive; −, negative.
[b] All 35 *mecA*-negative, methicillin-susceptible *S. aureus* isolates were negative by the MRSA-Screen test, and the oxacillin MICs were less than 2 μg/ml.
[c] Slight agglutination of this strain became visible after 10- to 15-min reactions, while other positive reactions were visible within 3 min.

TABLE 2. Results obtained using the diagnostic tests on coagulase-negative MRS isolates from animals[a]

Species (no. of isolates)	No. of isolates		MRSA-Screen[b]	MIC (μg/ml)
	mecA negative	*mecA* positive		
S. epidermidis (45)	41		−	≤0.25
	2		−	≥4
		2	+	≥4
S. saprophyticus (37)	33		−	≤0.25
	1		−	≥4
		3	+	≥4
S. simulans (29)	24		−	≤0.25
	2		−	≥4
		3	+	≥4
S. xylosus (18)	14		−	≤0.25
	4		−	≥4
S. hominis (17)	15		−	≤0.25
	1		−	2
		1	+	≥4
S. lentus (10)	8		−	<0.25
	1		−	1
	1		−	≥4

[a] All of these coagulase-negative staphylococcal isolates were originated from cattle.
[b] Symbols: +, positive; −, negative.

rabbit plasma tubes (Sigma, St. Louis, Mo.). These were also tested by the API STAPH IDENT system (Biomerieux, Lyon, France). The agar screen test for the oxacillin resistance of *S. aureus* was performed in accordance with NCCLS recommendations (16) with Mueller-Hinton agar containing 4% NaCl and 6 μg of oxacillin per ml. The MIC of oxacillin was determined by an agar dilution method in accordance with NCCLS recommendations (16) with an inoculum of 10^4 CFU/spot on Mueller-Hinton agar containing 4% NaCl and oxacillin at concentrations ranging from 0.5 to 128 μg/ml for *S. aureus* and 0.25 to 8 μg/ml for the coagulase-negative staphylococci. The resistance breakpoints of the oxacillin MICs for the *S. aureus* and coagulase-negative staphylococci were greater than or equal to 4 and 0.5 μg/ml, respectively. A previously described method was used for whole-cell DNA extraction (12). Two microliters of supernatant was used as the template. The presence of the *mecA* gene was demonstrated by PCR. Amplification of the *mecA* gene was performed with the primers *mecA1* and *mecA2* (13). The control organisms included *S. aureus* ATCC 43300, *S. aureus* ATCC 25923, and *S. epidermidis* ATCC 12228. The MRSA-Screen latex agglutination assay was performed in accordance with the manufacturer's protocol. Agglutination was assessed visually within 3 min.

Staphylococcus species were isolated from various samples associated with animals and identified. An agar screen test with oxacillin was performed to determine the phenotypic methicillin (oxacillin) resistance of *S. aureus*. *S. aureus* isolates from 28 samples from animal were resistant to oxacillin (Table 1). Thirty-five methicillin-susceptible *S. aureus* isolates were also collected. A total of 156 coagulase-negative staphylococcal isolates consisting of 45 *S. epidermidis*, 37 *S. saprophyticus*, 29 *S. simulans*, 18 *S. xylosus*, 17 *S. hominis*, and 10 *S. lentus* isolates were tested (Table 2). Of the 28 MRSA animal isolates, 15 were found to be *mecA* positive and 13 were found to be *mecA* negative by PCR (Table 1). All of the 35 methicillin-susceptible *S. aureus* isolates were *mecA* negative. All of the 15 *mecA*-positive MRSA isolates were also positive by the MRSA-Screen latex agglutination test. The reactions were determined within 3 min. All of the *mecA*-negative MRSA and methicillin-susceptible *S. aureus* isolates were negative by the MRSA-Screen test, with the exception of two *mecA*-negative isolates that yielded weak latex agglutination after the 10- to 15-min reactions. These two isolates had highly viscous characteristics than the other types of *S. aureus*. Of the 156 coagulase-negative staphylococci, 9 contained the *mecA* gene while 147 did not (Table 2). Regardless of the different species, all 9 *mecA*-positive, coagulase-negative isolates were positive by the MRSA-Screen test and all of the 147 *mecA*-negative isolates were negative. Therefore, the results of the MRSA-Screen latex agglutination test for PBP2a agreed with those of the *mecA* PCR. With the PCR as the gold standard method, the MRSA-Screen latex agglutination test demonstrated 100% (24 of 24) sensitivity and 100% (195 of 195) specificity. The oxacillin agar screen test for *S. aureus* identified all of the 15 *mecA*-positive isolates, for a sensitivity of 100%. However, it yielded 13 false-positive results for the 48 *mecA*-negative *S. aureus* isolates tested in this study, for a specificity of 72.9% (Table 1). The oxacillin MICs for the *mecA*-positive MRSA isolates determined by the oxacillin agar screen test ranged from 4 μg/ml to greater than 128 μg/ml, and the MICs for the *mecA*-negative MRSA determined by the oxacillin agar screen test were within

relatively lower, ranging from 1 to 16 μg/ml (Table 1). The MICs for the *mecA*-negative and methicillin-susceptible *S. aureus* isolates determined by the oxacillin agar screen test were all less than 2 μg/ml. MIC determination for *S. aureus* showed a sensitivity and a specificity of 100% (15 of 15) and 81.3% (39 of 48), respectively. The MICs for the 9 *mecA*-positive, coagulase-negative staphylococci were all greater than 0.5 μg/ml, and the MICs for 12 of the 147 *mecA*-negative, coagulase-negative staphylococci were also greater than 0.5 μg/ml (Table 2). The MIC for the coagulase-negative staphylococci showed a sensitivity and a specificity of 100% (9 of 9) and 91.8% (135 of 147), respectively.

Staphylococcal strains may vary in different hosts and epidemiological potential. This study included *S. aureus* isolates, as well as coagulase-negative staphylococcal isolates, in order to evaluate animal staphylococcal strains with various genetic backgrounds. However, regardless of the different species, the latex agglutination test detected the PBP2a antigen in the genetically diverse MRS strains and approached the accuracy of the PCR assay for *mecA* with 100% sensitivity and specificity. Therefore, the latex agglutination test can be applicable to animal MRS strains. The mechanism of methicillin resistance in staphylococcal strains is poorly understood but is believed to involve an interaction of PBP2a and various gene products such as those encoded by the *fem* (factor essential for methicillin resistance) genes, which are involved in cell wall peptidoglycan synthesis (1, 3, 5).

Despite the standardized recommendations for the susceptibility testing of MRS given by the NCCLS (16), many of the isolates in this study that did not carry *mecA* were phenotypically resistant to methicillin according to the oxacillin agar screen test and MIC determination. The phenotypic expression of resistance can vary depending on the growth conditions (e.g., the temperature or osmolarity of the medium), making susceptibility testing of MRS by standard microbiological methods potentially difficult (3). Therefore, predicting the presence of *mecA* may be problematic with these susceptibility tests for methicillin resistance in *S. aureus*.

mecA-positive, methicillin-susceptible MRS strains become resistant after being incubated with methicillin (3). Induction by β-lactams appears to increase PBP2a, particularly in coagulase-negative staphylococci (7). Other studies have shown that the exposure of several *mecA*-positive, phenotypically methicillin-susceptible *S. aureus* isolates to β-lactams results in an increase in the MIC of oxacillin well above the resistance level. This is despite the fact that the initial susceptibility tests revealed that these isolates were vulnerable (11). This suggests that an induction step with β-lactams in conventional phenotypic susceptibility assays for detecting MRS strains is needed.

The PCR assay is considered to be the gold standard for the detection of MRSA. However, this method is too time-consuming and expensive to be practical in a clinical microbiology laboratory. The latex agglutination test has the advantage of a rapid turnaround time from the isolation of an organism to the determination of susceptibility. The test provided the results within 20 min and easily managed the processing of large numbers of samples simultaneously.

This study was supported by the Technology Development Program for Agriculture and Forestry, Ministry of Agriculture and Forestry, and by the Brain Korea 21 Project in 2003, Republic of Korea.

REFERENCES

1. **Archer, G. A., and D. M. Niemeyer.** 1994. Origin and evolution of DNA associated with resistance to methicillin in staphylococci. Trends Endocrinol. Metab. **2:**343–348.
2. **Cefai, C., S. Ashurst, and C. Owens.** 1994. Human carriage of methicillin-resistant *Staphylococcus aureus* linked with pet dog. Lancet **344:**539–540.
3. **Chambers, H. F.** 1997. Methicillin resistance in staphylococci: molecular and biochemical basis and clinical implications. Clin. Microbiol. Rev. **10:**781–791.
4. **Hartmann, F. A., S. S. Trostle, and A. A. O. Klohnen.** 1997. Isolation of methicillin-resistant *Staphylococcus aureus* from a postoperative wound infection in a horse. J. Am. Vet. Med. Assoc. **221:**590–592.
5. **Hiramatsu, K.** 1995. Molecular evolution of MRSA. Microbiol. Immunol. **39:**531–543.
6. **Horan, T., D. Culver, and W. Jarvis.** 1988. Pathogens causing nosocomial infections: preliminary data from the national nosocomial infections surveillance system. Antimicrob. Newsl. **5:**65–67.
7. **Hussain, Z., L. Stoakes, S. Garrow, S. Longo, V. Fitzgerald, and R. Lannigan.** 2000. Rapid detection of *mecA*-positive and *mecA*-negative coagulase-negative staphylococci by an anti-penicillin binding protein 2a slide test. J. Clin. Microbiol. **38:**2051–2054.
8. **Koterba, A., J. Torchia, C. Silverthorne, R. Ramphal, A. M. Merritt, and J. Manucy.** 1986. Nosocomial infections and bacterial antibiotic resistance in a university equine hospital. J. Am. Vet. Med. Assoc. **189:**185–191.
9. **Lee, J. H.** 2003. Methicillin (oxacillin)-resistant *Staphylococcus aureus* strains isolated from major food animals and their potential transmission to humans. Appl. Environ. Microbiol. **69:**6489–6494.
10. **Mandell, G., J. Douglas, and R. Bennett (ed.).** 1995. Principles and practices of infectious diseases, 4th ed. Churchill Livingstone, Ltd., Edinburgh, United Kingdom.
11. **Martineau, F., F. J. Picard, N. Lansac, C. Menard, P. H. Roy, M. Ouellette, and M. G. Bergeron.** 2000. Correlation between the resistance genotype determined by multiplex PCR assays and the antibiotic susceptibility patterns of *Staphylococcus aureus* and *Staphylococcus epidermidis*. Antimicrob. Agents Chemother. **44:**231–238.
12. **Melter, O., I. Santos Sanches, J. Schindler, M. Aires de Sousa, R. Mato, V. Kovarova, H. Zemlickova, and H. de Lencastre.** 1999. Methicillin-resistant *Staphylococcus aureus* clonal types in the Czech Republic. J. Clin. Microbiol. **37:**2798–2803.
13. **Murakami, K., W. Minamide, K. Wada, E. Nakamura, H. Teraoka, and S. Watanabe.** 1991. Identification of methicillin-resistant strains of staphylococci by polymerase chain reaction. J. Clin. Microbiol. **29:**2240–2244.
14. **Nakatomi, Y., and J. Sugiyama.** 1998. A rapid latex agglutination assay for the detection of penicillin-binding protein 2′. Microbiol. Immunol. **42:**739–743.
15. **National Committee for Clinical Laboratory Standards.** 1997. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standards. NCCLS document M7–A4. National Committee for Clinical Laboratory Standards, Wayne, Pa.
16. **National Committee for Clinical Laboratory Standards.** 2003. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standards 6th edition. NCCLS document M07-A6. National Committee for Clinical Laboratory Standards, Wayne, Pa.
17. **Seguin, J. C., R. D. Walker, J. P. Caron, W. E. Kloos, C. G. George, R. J. Hollis, R. N. Jones, and M. A. Pfaller.** 1999. Methicillin-resistant *Staphylococcus aureus* outbreak in a veterinary teaching hospital: potential human-to-animal transmission. J. Clin. Microbiol. **37:**1459–1463.
18. **Tomlin, J., M. J. Pead, D. H. Lloyd, S. Howell, F. Hartmann, H. A. Jackson, and P. Muir.** 1999. Methicillin-resistant *Staphylococcus aureus* infections in 11 dogs. Vet. Rec. **16:**60–64.
19. **Udo, E. E., I. A. al-Obaid, L. E. Jacob, and T. D. Chugh.** 1996. Molecular characterization of epidemic ciprofloxacin- and methicillin-resistant *Staphylococcus aureus* strains colonizing patients in an intensive care unit. J. Clin. Microbiol. **34:**3242–3244.
20. **Waldvogel, F. A.** 1990. *Staphylococcus aureus*, p. 1489–1510. *In* G. L. Mandell, R. G. Douglas, and J. E. Bennett (ed.), Principles and practices of infectious disease, 3rd ed. Churchill Livingstone, New York, N.Y.
21. **Zadoks, R., W. van Leeuwen, H. Barkema, O. Sampimon, H. Verbrugh, Y. H. Schukken, and A. van Belkum.** 2000. Application of pulsed-field gel electrophoresis and binary typing as tools in veterinary clinical microbiology and molecular epidemiologic analysis of bovine and human *Staphylococcus aureus* isolates. J. Clin. Microbiol. **38:**1931–1939.