did not screen for these enzymes. Respondents from the MYSTIC survey were more likely to screen for ESBLs, and were more concerned about the incidence of ESBL-producing bacteria than respondents from the omnibus survey. This is not surprising, as participants in surveillance studies may be more aware of the issue of antimicrobial resistance, which may be their initial reason for enrolling in a surveillance study.

In both surveys, many participants who screened for ESBLs did not identify them further, either because it was thought unnecessary, or because of lack of funding or facilities. Identification of the particular type of ESBL that a resistant organism produces will reveal the antibiotic resistance profile of the organism and may help clinicians to choose the most appropriate therapy [7]. Where high prevalences of ESBL producers are demonstrated as a result of surveillance studies or screening, an appropriate initial empirical therapy that covers ESBL-producing strains should be considered. The double-disk synergy test was the most common test used to detect ESBLs in both surveys, but the Etest ESBL screen was thought to be the most efficacious method. The most appropriate screening methodology should therefore be determined locally, according to local resources.

Overall, the surveys demonstrated that awareness of, and testing for, ESBLs is inconsistent. Since ESBLs have an influence on morbidity and mortality, and are associated with clinical failure, it is important to increase the level of awareness and frequency of testing for ESBLs.

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REFERENCES

1. Felmingham D, Gruneberg RN. A multicentre collaborative study of the antimicrobial susceptibility of community-acquired, lower respiratory tract pathogens 1992–1993: the Alexander Project. *J Antimicrob Chemother* 1996; **38**(suppl A): 1–57.
2. Pfaller MA, Jones RN, Doern GV, Kugler K, the SENTRY participants group. Bacterial pathogens isolated from patients with bloodstream infections: frequencies of occurrence and antimicrobial susceptibility patterns from the SENTRY antimicrobial surveillance program (United States and Canada, 1997). *Antimicrob Agents Chemother* 1998; **42**: 1762–1770.
3. Turner PJ. MYSTIC (Meropenem Yearly Susceptibility Test Information Collection): a global overview. *J Clin Microbiol* 2000; **46**: 9–23.
4. Paterson DL, Ko W, von Gottberg A *et al*. Outcome of cephalosporin treatment for serious infections due to apparently susceptible organisms producing extended-spectrum β-lactamases: implications for the clinical microbiology laboratory. *J Clin Microbiol* 2001; **39**: 2206–2212.
5. Lautenbach E, Patel JB, Bilker WB, Edelstein PH, Fishman NO. Extended-spectrum β-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae*: risk factors for infection and impact of resistance on outcomes. *Clin Infect Dis* 2001; **32**: 1162–1171.
6. Livermore DM. β-Lactamases in laboratory and clinical resistance. *Clin Microbiol Rev* 1995; **8**: 557–584.
7. Bush K. New β-lactamases in Gram-negative bacteria: diversity and impact on the selection of antimicrobial therapy. *Clin Infect Dis* 2001; **32**: 1085–1089.

RESEARCH NOTE

Evaluation of a cefoxitin disk diffusion test for the routine detection of methicillin-resistant *Staphylococcus aureus*

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ABSTRACT

Two oxacillin disk methods were compared with a cefoxitin disk diffusion test for detection of methicillin-resistant *Staphylococcus aureus* (MRSA), with PCR for *mecA* as the reference method. When tested with 115 MRSA and 350 methicillin-susceptible *S. aureus* isolates, the cefoxitin disk test (specificity 100%, sensitivity 96.5%) was superior to the oxacillin disk methods (specificity 99.1%, sensitivity 90.4%). Testing with both oxacillin and cefoxitin disks would give better sensitivity (100%) than the cefoxitin test alone, but at the expense of specificity (99.1%). The cefoxitin disk test required no special test conditions and would

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improve the reliability of routine tests for detection of MRSA.

Keywords Cefoxitin disk test, *mecA*, MRSA, susceptibility testing, *Staphylococcus aureus*

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Methicillin-resistant *Staphylococcus aureus* (MRSA) have become increasingly prevalent worldwide. Rapid and accurate identification of MRSA is required to help clinicians select appropriate antibiotic treatment and to avoid the spread of these strains [1–8]. However, there is no optimal phenotypic method for detecting methicillin resistance in *S. aureus* [2,8–10], and genotypic tests involving *mecA* gene detection by PCR, which are considered to be the reference [11–13], are not practical for routine use in clinical laboratories. Thus, there remains a need for a reliable test for MRSA that can be performed easily in routine situations. In this context, the use of cefoxitin rather than oxacillin for disk tests has been advocated [3–5]. Therefore, the present study compared the performance of disk diffusion tests with cefoxitin and oxacillin disks for the detection of MRSA.

In total, 465 isolates of *S. aureus* were obtained from individual patients in 2001 and 2002 at two Tunisian teaching hospitals. The isolates were identified by conventional methods (Gram-positive cocci, catalase-positive, mannitol-fermenting and DNase-positive), and were confirmed as *S. aureus* by their ability to coagulate rabbit plasma (bioMérieux, Marcy l'Etoile, France) and produce clumping factor (Staphyslide test; bioMérieux). Isolates were characterised as MRSA or methicillin-susceptible *S. aureus* (MSSA) following DNA extraction with the InstaGene Matrix (Bio-Rad Laboratories, Marnes La Coquette, France) and PCR for the *mecA* gene with primers MEC1 (5′-AAAATCGATGGTAAAGGTTGGC-3′) and MEC2 (5′-AGTTCTGCAGTACCGCATTT-GC-3′) [14]. Amplification was performed with a GeneAmp PCR System 2400 (Perkin-Elmer, Norwalk, CT, USA) with 50 μL of reaction buffer containing 5 μL of bacterial DNA extract, 200 μM of each dNTP, 2.5 mM $MgCl_2$, 20 pmol of each primer and 0.5 U of Ampli*Taq* polymerase (Promega, Lyon, France).

Oxacillin susceptibility testing was performed with 5-μg oxacillin disks incubated at 30°C for 24 h on Mueller–Hinton agar, and with 5-μg oxacillin disks incubated at 37°C for 24 h on Mueller–Hinton agar supplemented with NaCl 4% w/v, in accordance with the guidelines of the Comité de l'Antibiogramme de la Société Française de Microbiologie (CA-SFM) [15]. Susceptibility to cefoxitin was determined without the special conditions used for oxacillin testing [15]. A suspension of organisms adjusted to 0.5× MacFarland standard was diluted 1:100 and inoculated on to Mueller–Hinton agar by streaking over the agar surface. Cefoxitin 30-μg disks were applied and the plates were incubated at 37°C for 24 h. An isolate was considered to be an MRSA strain if the cefoxitin inhibition zone diameter was ≤21 mm [3]. *S. aureus* strains ATCC 43300 (heterogeneous oxacillin resistance) and ATCC 25923 (oxacillin-susceptible) were used as quality control strains.

Of the 465 isolates tested, 115 were *mecA*-positive by PCR, and 350 were negative (Table 1). The two oxacillin disk methods with Mueller–Hinton agar incubated at 30°C, and with Mueller-Hinton agar supplemented with NaCl 4% w/v incubated at 37°C, agreed with each other, but falsely identified 11 isolates as oxacillin-susceptible (sensitivity 90.4%) and three isolates as oxacillin-resistant (specificity 99.1%) in comparison with PCR. The cefoxitin disk test detected oxacillin resistance correctly in all but four isolates (sensitivity 96.5%), and there were no false-resistant results (specificity 100%). The 11 resistant isolates reported as susceptible by the oxacillin method were different from the four

Table 1. Susceptibility of *Staphylococcus aureus* isolates tested with two oxacillin disk diffusion methods and a cefoxitin disk diffusion method in comparison with *mecA* PCR

		Cefoxitin method		Oxacillin methods[a]	
mecA PCR	Number of isolates	Number susceptible	Number resistant	Number susceptible	Number resistant
Negative	350	350	0	347	3
Positive	115	4	111	11	104

[a]Both oxacillin disk methods used (see Materials and methods) gave identical results.

resistant isolates reported as susceptible by the cefoxitin method. Hence, in total, there were 18 discordant results (three with MSSA and 15 with MRSA) between the oxacillin disk methods and the cefoxitin disk method. Combining the results of tests with both cefoxitin and oxacillin would give a sensitivity of 100% and a specificity of 99.1%. The greater reliability of tests with cefoxitin disks confirmed earlier studies which showed that cefoxitin disk tests, without modification to conditions to improve expression of resistance, are as reliable or more reliable than oxacillin disk tests for the detection of methicillin resistance in *S. aureus* [3–5].

Methicillin resistance in *S. aureus* is associated with the production of an altered penicillin-binding protein, PBP2a, encoded by the *mec* gene complex [8,10]. Laboratory methods have been developed to enhance the expression of methicillin resistance in staphylococci by modifications to test conditions, including supplementation of media with NaCl and prolonging the incubation period to 24 h. Phenotypic methods for detecting MRSA remain controversial [12,16,17], and MRSA strains are not always identified correctly. Problems in detection of MRSA may be caused by low-level expression of oxacillin resistance in some strains [12,16,17]. One study that included heterogeneous strains found that disk diffusion methods had low sensitivity [8]. There are also difficulties in differentiating MRSA from borderline oxacillin-resistant *S. aureus* (BORSA) strains [10,18]. As shown in this and other studies, no phenotypic method is completely reliable for the detection of MRSA [2,6,8,10,12,19].

Errors in the detection of oxacillin resistance can have serious adverse clinical consequences [9]. False-susceptibility results may result in treatment failure and the spread of MRSA if appropriate infection control measures are not applied. Conversely, false-resistance results may increase health care costs following unnecessary isolation precautions, and may lead to overuse of glycopeptides [20]. Hence, accurate identification of MRSA is necessary. Detection of the *mecA* gene (or PBP2a) is recognised to be the most accurate method for detecting methicillin resistance in *S. aureus* [10,13]. However, use of PCR assays is generally limited to specialised reference laboratories, and neither method is used widely for routine methicillin susceptibility tests in diagnostic laboratories. The cefoxitin disk susceptibility test appeared to be a useful procedure in that it is easy to perform in routine laboratories and has greater accuracy than oxacillin disk tests. Combining the results of cefoxitin and oxacillin tests would improve the sensitivity of the cefoxitin test alone, but the specificity would be reduced. The cefoxitin disk test has the potential for wider use in diagnostic laboratories.

REFERENCES

1. Yamazumi T, Marshall SA, Wilke WW, Diekema DJ, Pfaller MA, Jones RN. Comparison of the Vitek Gram positive susceptibility 106 card and MRSA screen latex agglutination test for determining oxacillin resistance in clinical bloodstream isolates of *Staphylococcus aureus*. *J Clin Microbiol* 2001; **39**: 53–56.
2. Brown DFJ, Walpole E. Evaluation of the Mastalex latex agglutination test for methicillin resistance in *Staphylococcus aureus* grown on different screening media. *J Antimicrob Chemother* 2001; **47**: 187–189.
3. Mougeot C, Guillaumat-Tailliet J, Libert JM. *Staphylococcus aureus*: new detection of intrinsic resistance using the diffusion method. *Path Biol* 2001; **49**: 199–204.
4. Skov R, Clausen M, Larsen AR, Frimodt-Moller N, Olsson-Liljequist B, Kahlmeter G. Evaluation of a cefoxtin 30 μg disc on iso-sensitest agar for detection of methicillin-resistant *Staphylococcus aureus*. *J Antimicrob Chemother* 2003; **52**: 204–207.
5. Felten A, Grandy B, Lagrange PH, Casin I. 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* 2002; **40**: 2766–2771.
6. Louie L, Matsumura SO, Choi E, Louie M, Simor AE. Evaluation of three rapid methods for detection of methicillin resistance in *Staphylococcus aureus*. *J Clin Microbiol* 2000; **38**: 2170–2173.
7. Merlino J, Leroi M, Bradbury R, Veal D, Harbour C. New chromogenic identification and detection of *Staphylococcus aureus and* methicillin resistant *S. aureus*. *J Clin Microbiol* 2000; **38**: 2378–2380.
8. Cavassini M, Wenger A, Jaton K, Blanc DS, Bille J. Evaluation of MRSA screen, a simple anti-PBP-2a slide latex agglutination kit, for rapid detection of methicillin resistance in *Staphylococcus aureus*. *J Clin Microbiol* 1999; **37**: 1591–1594.
9. Ribeiro J, Vieira FD, King T, D'arezzo JB, Boyce JM. Misclassification of susceptible strains of *Staphylococcus aureus* as methicillin resistant *S. aureus* by a rapid automated susceptibility testing system. *J Clin Microbiol* 1999; **37**: 1619–1620.
10. Gerberding JL, Miick C, Liu HH, Chambers HF. Comparison of conventional susceptibility tests with direct detection of penicillin binding protein 2a in borderline oxacillin-resistant strains of *Staphylococcus aureus*. *Antimicrob Agents Chemother* 1991; **35**: 2574–2579.

11. Thean YT. A comparison of PCR detection of *mecA* with two standard methods of oxacillin disk susceptibility testing for coagulase negative staphylococci. *J Med Microbiol* 2002; **51**: 83–85.
12. Sakoulas G, Gold HS, Venkataraman L, Degirolami PC, Eliopoulos GM, Qian Q. Methicillin resistant *Staphylococcus aureus*: comparison of susceptibility testing methods and analysis of *mecA* positive susceptible strains. *J Clin Microbiol* 2001; **39**: 3946–3951.
13. Tokue Y, Shoji S, Satoh K, Watanabe A, Motomiya M. Comparison of a polymerase chain reaction assay and a conventional microbiologic method for detection of methicillin resistant *Staphylococcus aureus*. *Antimicrob Agents Chemother* 1992; **36**: 6–9.
14. Murakami K, Minamide W, Wada K, Nakamura E, Teraoka H, Watanabe S. Identification of methicillin resistant strains of staphylococci by polymerase chain reaction. *J Clin Microbiol* 1991; **29**: 2240–2244.
15. Comité de l'Antibiogramme de la Société Française de Microbiologie. Communiqué, 2003. http://www.sfm.asso.fr/
16. Swenson JA, Williams PP, Killgore G, O'Hara CM, Tenover FC. Performance of eight methods, including two new rapid methods, for detection of oxacillin resistance in a challenge set of *Staphylococcus aureus*. *J Clin Microbiol* 2001; **39**: 3785–3788.
17. Swenson JA, Spargon J, Tenover FC, Ferraro MJ. Optimal inoculation methods and quality control for the NCCLS oxacillin agar screen test for detection of oxacillin resistance in *Staphylococcus aureus*. *J Clin Microbiol* 2001; **39**: 3781–3784.
18. Resende CA, Figueiredo AM. Discrimination of methicillin resistant *Staphylococcus aureus* from borderline resistant and susceptible isolates by different methods. *J Med Microbiol* 1997; **46**: 145–149.
19. Nicolas F, Bantar C, Canigia F, Relloso S, Bianchini H, Smayevsky J. Comparison of several methods to determine methicillin resistance in *Staphylococcus aureus*. *Diagn Microbiol Infect Dis* 2000; **36**: 91–93.
20. Hiramatsu K. The emergence of *Staphylococcus aureus* with reduced susceptibility to vancomycin in Japan. *Am J Med* 1998; **104**(suppl): S7–S10.

RESEARCH NOTE

Penetration of fusidic acid and rifampicin into cerebrospinal fluid in low-grade inflammatory meningitis caused by *Staphylococcus epidermidis*

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ABSTRACT

Cerebrospinal fluid (CSF) concentration–time curves of rifampicin and fusidic acid were studied in a patient with post-operative meningitis caused by *Staphylococcus epidermidis*. The patient was treated with this combination of antimicrobial agents because of a severe hypersensitivity reaction to vancomycin. Peak CSF concentrations of rifampicin exceeded the MIC by > 60-fold, while those of fusidic acid just reached the MIC. CSF concentrations of fusidic acid were relatively stable within the range reported for patients with uninflamed meninges, but serum levels were surprisingly low. An increase in the metabolism of fusidic acid induced by rifampicin cannot be excluded.

Keywords Cerebrospinal fluid penetration, fusidic acid, meningitis, rifampicin

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Data on the pharmacokinetics of rifampicin and fusidic acid in cerebrospinal fluid (CSF) are limited. Therefore, it was of interest to study the drug concentration–time curves in a patient with post-operative ventricular drain-associated *Staphylococcus epidermidis* meningitis. The patient was treated with this combination because of a severe hypersensitivity reaction to vancomycin, which is used commonly for the treatment of post-surgical nosocomial meningitis [1,2].

The patient was a 55-year-old man with a massive sub-arachnoid haemorrhage and acute hydrocephalus. The aneurysm was embolised and CSF drainage was started. Three weeks later, the patient developed meningitis. The CSF showed marked pleocytosis and yielded an isolate of *S. epidermidis* that was resistant to all antibiotics tested except vancomycin, rifampicin and fusidic acid. Despite ventricular catheter removal and treatment with intravenous vancomycin for 1 week, the patient's condition deteriorated. A