
Lorenzo Filippin, Sandrine Roisin, Claire Nonhoff, Stien Vandendriessche, Amélie Heinrichs, Olivier Denis

National Reference Centre-Staphylococcus aureus, Department of Microbiology, Hôpital Erasme, Université Libre de Bruxelles, Brussels, Belgium

We evaluated the performance of the automated Vitek 2 system against disk diffusion for susceptibility testing of Staphylococcus aureus strains showing various resistance mechanisms to macrolides and lincosamides (ML). The Vitek 2 system showed 100% concordance with the D-zone test in detection of the most common resistance mechanisms to ML, including methylase and efflux systems.

Although structurally unrelated, macrolides, lincosamides, and streptogramins (MLS) have similar modes of action by binding to the peptidyl-transferase region of the 50S ribosomal subunit, which leads to inhibition of protein synthesis. Resistance to MLS in Staphylococcus aureus is mainly due to (i) active efflux by an efflux pump encoded by the msr(A) gene and conferring resistance to macrolides and type B streptogramins (MSB) or (ii) ribosomal target site modification due to methylases encoded by erm genes and conferring resistance to MLSB (1, 2, 3). MLSB phenotypes are either constitutive (cMLS), showing in vitro resistance to all MLSB agents, or inducible (iMLS) following exposure to a macrolide (4). iMLSB strains demonstrate in vitro resistance to 14- and 15-member macrolides but appear susceptible to 16-member macrolides, lincosamides, and type B streptogramins (5, 6, 7).

Among MLS, clindamycin (CLI) is most frequently used for therapy of staphylococcal infections, including osteomyelitis and skin and soft tissue infections (8). Its good oral bioavailability, tolerability, and excellent tissue penetration make it an important option for outpatient therapy and changeover after intravenous antibiotics (9). However, clindamycin treatment of infections caused by S. aureus strains with the iMLS phenotype may result in clinical failure due to the selection of constitutive mutants (10).

Clinical and Laboratory Standards Institute (CLSI) and EUCAST recommend the use of the double disk diffusion method (D-zone test) to reveal the presence of the iMLS phenotype in staphylococci (11, 12). Therefore, several automated systems provide a test for detection of inducible clindamycin resistance (ICR) in staphylococci. For instance, Vitek 2 added an Advanced Expert System (AES), which is able to interpret the ICR test and clindamycin susceptibility for S. aureus strains. The aim of the present study was to evaluate the performance of the Vitek 2 system against the disk diffusion method for the detection of various mechanisms of MLS resistance in a set of genotypically well characterized S. aureus isolates.

S. aureus strains were selected from a national reference collection composed of systematic national survey samples and isolates sent to the reference lab for outbreak investigations, exotoxin detection, or diagnostic problems (13). All strains were previously confirmed by a 16S rRNA-nuc-mecA triplex PCR and stored at −80°C for further analysis (14).

Susceptibility to MLS was phenotypically tested by MIC determination and genotypically confirmed by PCR. The MICs for erythromycin and clindamycin were determined by the agar dilution method (range, 0.06 to 128 mg/liter) using Mueller-Hinton II (MH) agar (Becton Dickinson, Heidelberg, Germany) (11). Multiplex PCR was performed as previously described with primers specific for erm(A), erm(B), erm(C), erm(T), and msr(A) (13, 14, 15).

Three sets of nonduplicated S. aureus strains (n = 110) including methicillin-resistant S. aureus (MRSA; n = 67) and methicillin-susceptible S. aureus (MSSA; n = 43) were included in this study.

(i) Strains harboring erm genes (n = 62). Among the strains harboring erm genes, 23 and 30 isolates carried the erm(A) and the erm(C) gene, respectively. Four isolates positive for the erm(B) gene and five isolates positive for erm(T) were also added. All strains showed high MICs (>128 mg/liter) to erythromycin (ERY); clindamycin MICs varied from 0.06 to 128 mg/liter (median value, 0.25 mg/liter).

(ii) Strains harboring the msr(A) gene (n = 12). Isolates harboring the msr(A) gene were resistant to erythromycin, with MICs of >128 mg/liter, but susceptible to clindamycin (MICs ranged from 0.12 to 0.25 mg/liter, with a median value of 0.12 mg/liter).

(iii) Susceptible isolates (n = 36). Susceptible isolates harbored no MLS resistance gene and showed MICs to both erythromycin and clindamycin ranging from 0.06 to 0.25 mg/liter.

All strains were removed from −80°C and subcultured twice on Columbia agar before further phenotypic testing. A bacterial suspension equivalent to a 0.5 McFarland standard was prepared with isolated colonies from a fresh culture for Vitek 2 and disk diffusion testing. Vitek 2 AST-P610 cards (bioMérieux, Marcy l’Étoile, France) were inoculated according to the manufacturer’s instructions. AST-P610 cards contain two wells for ICR detection, one with 0.5 mg/liter of clindamycin and the other with a combi-
nation of 0.25 mg/liter of clindamycin and 0.5 mg/liter of erythromycin. In parallel, MLS resistance was detected by the disk diffusion method using the D-zone test as recommended by CLSI (11). Briefly, MH agar plates were inoculated with the same bacterial suspension as that for Vitek 2 testing. Disks (Bio-Rad, Marnes-la-Coquette, France) containing erythromycin (ERY; 15 μg) and clindamycin (CLI; 2 μg) were spaced 15 to 26 mm apart. After 16 to 18 h of incubation, strains showing flattening of the inhibition zone (D phenotype) around the clindamycin disk were interpreted as positive for the iMLS_R phenotype. Isolates resistant to both erythromycin and clindamycin were considered indicative of the cMLS_R resistance phenotype.

Of the 62 strains harboring erm genes, 35 showed the inducible phenotype and 27 showed the constitutive phenotype (Table 1). The erm(C) determinant (n = 15) was predominant among S. aureus strains showing the iMLS_R phenotype. Eight strains harboring the erm(A) gene were also positive for this phenotype as well as two erm(B)-positive isolates and five erm(T)-positive isolates. The sensitivity of both the D-zone test and the Vitek 2 system for the detection of the iMLS_R phenotype was 100%. Among isolates displaying the cMLS_R phenotype, the majority harbored erm(A) (n = 15). Two and 10 strains harboring the erm(B) and the erm(C) genes, respectively, also showed a constitutive resistance phenotype. All isolates with cMLS_R demonstrated in vitro resistance to erythromycin and clindamycin and were correctly detected by the Vitek 2 system and by the disk diffusion method. The MS_R phenotype was found only for isolates harboring msr(A) (n = 12). These were well detected by both disk diffusion and the Vitek 2 system. Finally, all susceptible strains (n = 36) showed no resistance to MLS as demonstrated by any susceptibility testing methods.

The Vitek 2 system showed 100% agreement with the D-zone test for the detection of MLS resistance in S. aureus isolates and was in complete concordance with the detected resistance genes. The median time to final susceptibility reporting based on the Vitek 2 system, 8 h 15 min (range, 8 h 3 min to 8 h 34 min), was significantly lower than the median time for D-zone testing (18 h) (P value, <0.001; Wilcoxon matched sample test).

The incidence and mechanisms of MLS resistance vary widely by geographic region and even from hospital to hospital, with some studies showing high local incidence of either constitutive or inducible MLS_R resistance in staphylococcal isolates (16, 17, 18, 19). The D-zone test is recommended by both CLSI and EUCAST for the detection of MLS resistance. Automated systems developed reliable and rapid testing with the aim to accurately detect the MLS resistance mechanisms. Several articles compared automated system and D-zone testing for detection of MLS resistance among clinical isolates of S. aureus and coagulase-negative staphylococci (CNS) (20, 21, 22, 23). In these studies, the sensitivity and specificity of ICR detection in automated systems (Vitek 2 and Phoenix [Becton Dickinson]) ranged from 91 to 100% and from 99.5 to 100%, respectively. In contrast, our results showed complete agreement between the Vitek 2 system and the D-zone test for detection of MLS resistance phenotypes in S. aureus isolates well characterized for the presence of various methylases or an efflux system. The lower sensitivity of previous studies might be explained by the incorporation of CNS. Furthermore, for the detection of ICR, CLSI recommends a single well containing both 4 mg/liter erythromycin and 0.5 mg/liter clindamycin. A possible reason, in these articles, for the failed detection of ICR with the Vitek 2 system might be due to a concentration of erythromycin insufficient to induce the erm gene. The shortened incubation time in the Vitek 2 system might also contribute to the failure to detect ICR in some S. aureus strains.

In conclusion, results of both Vitek 2 and D-zone tests were in complete agreement with the genotypic testing. The Vitek 2 system was able to differentiate between iMLS_R resistance phenotype and macrolide resistance due to an active efflux mechanism. The fully automated Vitek 2 ICR test is a good alternative to the D-zone test, providing faster results in a working day. Further studies are needed to confirm these results and to clarify the medical added value of rapid testing such as the Vitek 2 ICR test.

ACKNOWLEDGMENTS

We thank Sylvianne Rottiers and Emilie Vandaele for molecular testing and Pascale Buidin, Geneviève Hay, Nathalie Legros, and Christine Thiroux for assistance in phenotypic testing.

REFERENCES


