



M072-2 Infected finger swab: *Staphylococcus aureus*
(companion culture to G072)

HISTORY A simulated infected finger sample collected from a 45-year old male allergic to penicillin was sent to category A and B laboratories to culture and report as per their usual laboratory protocol. The companion smear for Gram staining G072 contained 4+ neutrophils and 3+ (11-50/oif) gram-positive cocci. It was anticipated that participants would correlate Gram smear results, isolate, identify, and report *Staphylococcus aureus* with appropriate antimicrobials.

CMPT QA Internal validation showed this sample contained a pure growth of *Staphylococcus aureus*, viable for 21 days. This strain was susceptible to methicillin (oxacillin), cephalothin/cefazolin, and trimethoprim-sulfamethoxazole and resistant to erythromycin and clindamycin.

GRADING (grade =4) IDENTIFICATION All 15 reference laboratories reported *Staphylococcus aureus* therefore this sample was acceptable for grading. Identification of this isolate posed no problem as all category A (n=75) and B (n=33) laboratories that processed the sample and submitted a result received a grade of 4 for correctly reporting *S. aureus*. One category B laboratory received a grade of zero for not submitting a result. The 5 laboratories that do not process this type of specimen were ungraded.

ANTIMICROBIAL SUSCEPTIBILITY TESTING (grade = 20) All 15 of the reference laboratories reported results for methicillin (oxacillin, cefoxitin) and trimethoprim-sulfamethoxazole (SXT) as susceptible and erythromycin and clindamycin as resistant; 12 reference laboratories reported cephalothin or cefazolin as susceptible, 3 did not submit a result. Therefore AST results were considered acceptable to grade. Only results for the antibiotics listed above were included in the grading. Laboratories that included the comment “*S. aureus* which are oxacillin sensitive can be presumed to be cephalothin or cefazolin sensitive” were considered acceptable. Laboratories that did not supply a result for any of these antibiotics received a grade of zero. Laboratories that routinely do not process this type of specimen were ungraded. Results received and grades assigned are shown in Table 1 on page 2.

Acceptable comments that accompanied clindamycin reporting included the comment suggested by the CLSI “This isolate is presumed to be resistant to clindamycin based on the detection of inducible clindamycin resistance. Clindamycin may still be effective in some patients” and “erythromycin resistant strains might develop resistance to clindamycin during prolonged therapy.” The category B laboratory that included “refer for D test” and no other comment or explanation with their clindamycin susceptible result received a grade of 1 as this comment could be confusing to some physicians.

Six laboratories reported susceptibility to a fluoroquinolone antibiotic, mainly levofloxacin. While these agents are effective therapy, laboratories should be aware that their overuse promotes the emergence of MRSA ².

GRADING (maximum grade = 24)

IDENTIFICATION: 100% of category A (n=75) and B (n=33) laboratories that processed the sample and submitted a result received a grade of 4/4.

AST: see Table 1 on page 2.

NOTES: Cloxacillin or a first-generation cephalosporin is the drug of choice for most *S. aureus* infections. However, where MRSA is a possibility, or the patient has a history of allergy to penicillin other agents such as clindamycin or trimethoprim-sulfamethoxazole may be used. Clindamycin has good bone and tissue penetration and is therefore often the drug used in these situations. A concern with the use of clindamycin is the induction of resistance that may occur during treatment. See **D zone test** on page 2.

CLINICAL SIGNIFICANCE There is little controversy about the significance of *S. aureus* in a wound specimen, particularly when interpreted along with the companion slide showing many polymorphonuclear WBCs and organisms with typical staphylococcal morphology. The companion Gram smear G072 supported this scenario.

S. aureus is a common cause of skin and soft tissue infections which can range from superficial localized infections such as impetigo and boils to involvement of deeper tissues. The recently described strains of Community Acquired – MRSA*, have been reported to cause more severe skin and soft tissue infections as cellulitis and abscess formation ¹. Superficial infections respond to local treatment, and antibiotics are not required. However, antibiotic therapy often in conjunction with surgical measures such as incision and drainage or debridement is required for deeper infections.

[*Note: Historically, resistance to the penicillinase-stable penicillins, has been referred to as ‘methicillin resistance’; thus the acronyms MRSA (for ‘methicillin-resistant *S. aureus*’) or MRS (for ‘methicillin-resistant staphylococci’) are still commonly used even though methicillin is no longer the agent of choice for testing or treatment.]

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Resistance to macrolide, lincosamide and streptogramin B (MLS_B) antibiotics most commonly occurs as a result of acquisition of erythromycin resistance methylase (erm) genes. These genes encode enzymes that alter the ribosomal binding sites of these antibiotics. MLS_B resistance may be constitutive or inducible. Strains with MLS_B constitutive resistance will demonstrate resistance to MLS antibiotics on in vitro testing. Strains with inducible resistance will be resistant to the macrolides (erythromycin), but appear susceptible to the lincosamides

(clindamycin). Erythromycin is an effective inducer of clindamycin resistance and this factor is used in the “D zone test” to determine the presence of inducible clindamycin resistance in both staphylococci and beta-hemolytic streptococci.

The “D zone test” is a disk approximation test performed by the Kirby Bauer Method where a 2 µg clindamycin disk is placed 15 mm away from the edge of a 15 µg erythromycin disk. Following overnight incubation strains that have inducible resistance will show flattening of the clindamycin zone in the area next to the erythromycin disc “D zone”. Clindamycin is reported as resistant on these isolates³. *S. aureus* isolates showing susceptibility to clindamycin and no “D zone” [negative] are reported as susceptible to clindamycin. Isolates may be resistant to macrolides via an efflux mechanism encoded by the *msr A* gene; these strains show resistance to erythromycin only³. As noted under AST, CLSI also suggests that a comment be added “This isolate is presumed to be resistant based on detection of inducible clindamycin resistance. Clindamycin may still be effective in some patients⁴.” Table 2 summarizes testing and results.

Rates of inducible clindamycin resistance varies in different geographical locations, and is seen with MSSA and MRSA in both community and hospital-acquired infections⁵. Therefore it is important that all laboratories perform D zone testing on all staphylococci and on invasive isolates of beta-hemolytic streptococci⁶ (other than pharyngeal) resistant to erythromycin. While there are reports of treatment failure with the use of clindamycin, in surgical wound infections⁷ and deep-seated infections such as osteomyelitis⁸ and endocarditis,⁹ clindamycin

use may still be an option in wound infections⁸. In these situations when clinicians are alerted to the possibility of treatment failure they can follow the patient more closely to ensure adequate response.

REFERENCES

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Table 1. M072-2 AST reported and methods noted by category A and B laboratories.

Anti microbial	Methicillin/Oxacillin		Cephalothin/Cefazolin		SXT		Erythromycin		Clindamycin	
	Lab	A	B	A	B	A	B	A	B	A
Grade 4	73 (S)	31 (S)	60 (S) 3 comment	20 (S)	69 (S)	29 (S)	69 (R)	27 (R)	61 (R) 5 comment	19 (R); 1 comment; 1(S)*
3	/	/	/	/	/	/	4 (I)	3 (I)	0	0
1	/	/	/	/	/	/	/	/	/	1 refer for D test, no other comment
0	2 (NR)	3 (NR)	12 (NR)	14 (NR)	6 (NR)	5 (NR)	2 (NR)	1 (S), 3 (NR)	6 (S), 3 (NR)	8 (S); 4 (NR)
Ungraded (snrp)	2	3	2	3	2	3	2	3	2	3

1 (clindamycin—S) but noted refer for D test to determine if inducible; NR = no report; snrp=sample not routinely processed

Table 2. *S. aureus* mechanisms of resistance to erythromycin and clindamycin and the D zone test.

Key: R=resistant, S=susceptible	Mechanism of Resistance	
	Efflux	Ribosomal Target Modification
Antimicrobial		Constitutive ↔ Inducible
Erythromycin	R	R
Clindamycin	S	S
D Zone Test	Negative	Positive (i.e., S → R)