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Use of Cefoxitin-Based Selective Broth for Improved Detection of Methicillin-Resistant *Staphylococcus aureus*

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A cefoxitin-based selective broth was evaluated for its efficiency in detecting methicillin-resistant *Staphylococcus aureus* (MRSA) by the use of laboratory reference strains, clinical isolates of different clones, and clinical samples. The cefoxitin-based broth was proved to be more sensitive and rapid for the detection of MRSA strains, especially heterogeneously resistant strains, than the oxacillin-based broth.

The proportions of both hospital-acquired and community-acquired infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA) have steadily been increasing worldwide. Infections caused by MRSA result in lengthier hospital stays and rising health care costs and have a high attributable mortality rate (1, 3). Early recognition of patients colonized or infected with MRSA can have a direct impact on the selection of antibiotic therapy and the decision to initiate isolation procedures. An ideal method for MRSA detection should have a high sensitivity and a short time to the reporting of the results (20).

Resistance to methicillin in *Staphylococcus* spp. is primarily mediated by the presence of penicillin-binding protein 2a, encoded by the *mecA* gene. In certain MRSA strains, the *mecA* gene is heterogeneously expressed in vitro (18). Historically, these strains have been difficult to detect, and selective media have been used to facilitate the recovery of the resistant subpopulation in culture (2, 8, 10, 18). Previously, we have developed an MRSA screening assay based upon an oxacillin-based selective broth and real-time PCR (6). To increase the sensitivity for detection of heterogeneously resistant MRSA strains and to improve laboratory efficiency, a cefoxitin-based MRSA screening broth (FOXCA) was developed and compared with the previously described oxacillin-based broth (OXAA).

FOXCA broth was composed of Iso-Sensitest broth (Oxoid), 2.3% NaCl, 4 μg of cefoxitin/ml, 8 μg of colistin/ml, and 8 μg of aztreonam/ml. The addition of cefoxitin is principally aimed at inducing the expression of methicillin resistance (13) and inhibiting the growth of methicillin-susceptible *S. aureus* (MSSA). FOXCA broth contains a relatively high concentration of aztreonam to inhibit most isolates of the family *Enterobacteriaceae* and contains colistin, which is active against *Pseudomonas* spp. OXAA broth was prepared as described previously with 2 μg/ml of oxacillin and 1 μg/ml of aztreonam.

The broths were first tested with laboratory reference strains, i.e., *S. aureus* CCUG 31966 (a homogeneously resistant MRSA strain), *S. aureus* CCUG 46147 (a heterogeneously resistant MRSA strain), and *S. aureus* ATCC 29213 (a methicillin-sensitive strain), to determine the lowest inoculum needed to detect these strains in the presence of interfering strains, i.e., *Proteus mirabilis* ATCC 29245 and *Pseudomonas aeruginosa* ATCC 27853. The assays were performed in duplicate.

Each *S. aureus* strain was inoculated in the broths at 10-fold serial concentrations ranging from 10^0 to 10^5 CFU/ml. The interfering strains were inoculated into the broths at a constant concentration of 10^2 CFU/ml or 10^4 CFU/ml. The inoculated broths were incubated at 35 to 37°C for up to 2 days. A real-time PCR assay directed toward the *nuc* gene to identify samples containing *S. aureus* was performed with the overnight broths (6). Both the broths incubated overnight and the broths incubated for 2 days were subcultured onto *S. aureus* ID plates (10 μl; SAID; bioMérieux, France) for the identification of *S. aureus* (9), CLED plates (50 μl) for the recovery of *P. aeruginosa*, and blood-agar plates (50 μl) anaerobically to check the growth of *P. mirabilis*.

For the homogeneously resistant MRSA strain (*S. aureus* CCUG 31966), the sensitivities for *nuc* gene detection and colony recovery on the SAID plates were comparable for the two broths, whereas prolonged incubation was required for the OXAA broth to obtain the best sensitivity for colony recovery (Table 1). For the heterogeneously resistant MRSA strain (*S. aureus* CCUG 46147), the sensitivity of OXAA for *nuc* gene detection and strain recovery was much lower than that of FOXCA (Table 1). The interfering strains did interfere with the detection of either the homogeneous or the heterogeneous MRSA strain (the sensitivity for *nuc* gene detection was increased 10- to 100-fold when no interfering strains were cocultured), although the detection of the heterogeneously resistant MRSA strain was influenced more by the presence of the interfering strains.

The growth of the MSSA strain (*S. aureus* ATCC 29213) was inhibited in both FOXCA and OXAA broths, and the strain could not be detected upon subculture when the original inocula were less than 10^5 and 10^4 CFU/ml, respectively (Table 1).

P. aeruginosa and *P. mirabilis* are common interfering bacterial species that coexist in the clinical specimens used to screen for MRSA. As shown in this study, the recovery of *S. aureus* became worse with the presence of *P. mirabilis* and *P. aeruginosa* in the culture. Recently, a study also indicated that the growth of *S. aureus* is influenced by the coculture of *P. aeruginosa* in an in vivo model (11). As expected, the interfer-

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TABLE 1. Lowest inocula of *S. aureus* in the broths from which the *nuc* gene can be detected after overnight incubation and *S. aureus* can be discovered on SAID plates subcultured from broths incubated overnight and for 2 days

Strain	Lowest inoculum (CFU/ml) for:					
	Detection of *nuc* gene		Discovery of *S. aureus* on SAID plates			
			FOXCA		OXAA	
	FOXCA	OXAA	Overnight incubation	2 days of incubation	Overnight incubation	2 days of incubation
MRSA CCUG 31966	10	10	10	10	10^2	10
MRSA CCUG 46147	10^2 (10)[a]	10^3	10^2 (10)	10^2 (10)	10^4 to 10^5	10^3
MSSA ATCC 29213	10^5	10^5 (10^4)	10^5	10^5	10^4	10^4

[a] The results with the interfering strains at a concentration of 10^2 CFU/ml are given in parentheses when the data differ from those obtained with the interfering strains at a concentration of 10^4 CFU/ml.

ing strains tested were recovered from samples inoculated in the OXAA broth but not in the FOXCA broth.

In the next experimental setting, a collection of 40 clinical MRSA isolates with distinct genotypes was tested. This group of MRSA strains represents different MRSA clones that were detected in southern Stockholm, Sweden, from 1996 to 2004 and in which the major MRSA genetic lineages known to exist in the world were also represented, as characterized by multilocus sequence typing and staphylococcal chromosomal cassette *mec* typing (4, 5, 14, 16). Each strain tested was inoculated in FOXCA and OXAA broths in parallel at 10 to 10^2 CFU/ml, which are the lower limits for the detection of MRSA strains, as determined by the use of reference strains. All 40 clinical isolates together with the 2 MRSA reference strains became positive for *nuc* gene detection and colony recovery on plates after enrichment in FOXCA broth overnight. When the isolates were inoculated in OXAA broth, 4 of the 40 isolates as well as the heterogeneously resistant MRSA strain (*S. aureus* CCUG 46147) were negative for *nuc* gene detection, and no growth was observed on SAID plates for these strains in which *nuc* was undetectable, together with a *nuc*-positive isolate. Three of the clinical isolates in which *nuc* was undetectable and strain CCUG 46147 still failed to grow on plates when incubation in OXAA broth was prolonged to 2 days (Table 2). Two MSSA reference strains, ATCC 25923 and ATCC 29213, included in the experimental setting were negative for both *nuc* gene detection and colony recovery after enrichment in both broths.

Further investigation of the MRSA isolates in which *nuc* was undetectable revealed that the original inoculum of these isolates in the OXAA broth must have been at least 10^3 to 10^4 CFU/ml to be detectable by the *nuc*-specific PCR and not lower than 10^4 to 10^5 CFU/ml to be recoverable on the plates. However, these isolates were readily found to be MRSA when they were inoculated in FOXCA broth at an inoculum of 10 to 10^2 CFU/ml. According to the results of susceptibility testing, these strains were actually low-level-resistant MRSA strains. The MICs (Etest; AB Biodisk, Solna, Sweden) of cefoxitin for the four strains were 8, 8, 4, and 12 μg/ml respectively.

TABLE 2. Detection of the *nuc* gene and recovery of *S. aureus* colonies on SAID plates of 40 clinical MRSA isolates with an original inoculum of 10 to 100 CFU/ml in the broths

Broth	No. of MRSA isolates		
	nuc gene detected[a]	Colonies recovered	
		Broths incubated overnight	Broths incubated for 2 days
FOXCA	40	40	40
OXAA	36	35	37

[a] For broths incubated overnight.

Cefoxitin susceptibility testing of the 40 representative MRSA strains revealed that 55% (22 of 40) of them had MICs ≥32 μg/ml, with 35% (14 of 40) being categorized as intermediate according to the guidelines of the CLSI (formerly the NCCLS) (12), and 10% (4 of 40) of them had MICs ≤8 μg/ml; the lowest MIC was 4 μg/ml. The breakpoint of cefoxitin for *S. aureus* is 4 μg/ml, according to the Swedish Reference Group for Antibiotics. Based on the facts presented above, the concentration of cefoxitin in the FOXCA broth was set at 4 μg/ml in the present study. The evaluation performed in this study revealed that all the low-level-resistant MRSA strains, including the one with an MIC of 4 μg/ml, were readily detectable after enrichment in FOXCA broth, while it was more difficult for MSSA to survive in FOXCA broth than in OXAA broth.

FOXCA broth was introduced in our routine laboratory for screening for MRSA in March 2005. In the 1 month from the time of application of FOXCA broth, a total of 5,564 samples were sent to our laboratory for screening for MRSA. After overnight enrichment in FOXCA broth and a PCR assay for the *nuc* gene (6), 90% (5,001 of 5,564) of the specimens could be reported to be negative for MRSA and excluded from further investigation. Among the remaining 10% of the specimens (n = 563), 34 MRSA isolates (34 of 563 [6%]) were detected by further investigation (9). In comparison, a total of 5,127 clinical samples were screened for MRSA during the 1 month before the introduction of the new MRSA-selective broth. Some 88.7% (4,546 of 5,127) of the samples were excluded after the first screening step, i.e., enrichment in OXAA broth followed by the *nuc*-specific PCR. Twenty-seven of 581 samples (4.6%) were finally found to contain MRSA. The positive predictive value for the detection of MRSA after enrichment in FOXCA is higher than that after enrichment in OXAA (6% versus 4.6%), although the difference is not statistically significant. However, the difference between the exclusion rates of the two broths (90% versus 88.7%) after the first screening step is statistically significant by chi-square analysis ($P < 0.05$). Moreover, with the application of the FOXCA

broth, the prolonged incubation (2 days versus 1 day) was no longer needed, which saves much labor and time.

The rate of resistance to β-lactam antibiotics varies among and within staphylococcal strains. Some strains show heteroresistance, with only a minor population of the cells being obviously resistant, while the huge majority apparently remain susceptible (7, 10, 18), which makes detection challenging. Cefoxitin is a cephamycin antibiotic and has been described as an inducer of methicillin resistance (13). The performance of cefoxitin either as a disk or as a supplement in agar medium for the detection of MRSA has been confirmed extensively (7, 15, 17, 19). Accordingly, the findings of this study proved that the cefoxitin-based FOXCA broth was more sensitive and rapid for the detection of MRSA, especially for heterogeneously resistant strains, than the oxacillin-based broth.

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