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Accuracy of Reporting of Methicillin-Resistant *Staphylococcus aureus* in a Provincial Quality Control Program: a 9-Year Study

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We report the results of a province-wide quality control program in which five methicillin-resistant *Staphylococcus aureus* strains were circulated to all Ontario laboratories (hospital, private, and public health laboratories) on nine occasions between 1980 and 1989. The level of expression of methicillin resistance in each of the isolates was determined by performing viable colony counts on serial dilutions of methicillin in agar, and each isolate was assigned to an expression class according to previous published criteria (A. Tomasz, S. Nachman, and H. Leaf, Antimicrob. Agents Chemother. 35:124–129, 1991). Over this time there was an improvement in the performance of laboratories in the recognition of three strains that were relatively easy to detect (strains B, C, and E). These strains were of expression class II, and 98% of laboratories reported correct identifications in 1986. Performance in identifying two strains (strains A and D) of expression class I remained poor. Strain A was circulated in two surveys in 1987 and 1989, and laboratories were sent a questionnaire requesting details of the methods used in those two surveys. The methods used by the laboratories were classified into three categories: disk diffusion, single-plate screening by agar incorporation, and automated methods, which included premanufactured MIC panels. Between the 1987 and 1989 surveys, there was no change in the performance of the disk diffusion test (60% correct on both occasions), but there was improvement in the sensitivity of the agar incorporation test (36% correct in 1987 and 84% correct in 1989) and in automated methods (43% correct in 1987 and 79% correct in 1989). Over a decade, there was overall improvement in the performance of laboratories in detecting easy-to-detect strains, but there were difficulties in detecting organisms of low expression class, and an organism of very low expression class should be designated as a control organism for routine testing of methicillin-resistant *S. aureus* isolates.

Methicillin-resistant *Staphylococcus aureus* (MRSA) has become a serious clinical problem over the last decade (4, 5, 8), and the ability to detect this resistance reliably and rapidly is now required of all clinical microbiology laboratories. The National Committee for Clinical Laboratory Standards (NCCLS) recommends three manual methods for MRSA detection (15, 16). These are, screening on an agar plate containing oxacillin (6 μg/ml), disk diffusion, and tube macro- and microdilution methods. Automated systems are also used for the detection of MRSA. These automated systems are based on the principle of MIC measurement. We present here the results of a 9-year study of the ability of a wide variety of laboratories to detect MRSA.

MATERIALS AND METHODS

The testing model. The Laboratory Proficiency Testing Program of Ontario is a provincial medical laboratory quality control program administered by the Ontario Medical Association. In the microbiology component of the program, freeze-dried cultures are sent at regular intervals to all Ontario laboratories licensed to practice microbiological procedures. Laboratories are required to report, within a deadline, the identities and relevant antimicrobial susceptibilities of organisms. Four of the five strains have been circulated twice and one strain has been circulated once since 1980 (Table 1). Participating laboratories include those in hospitals ranging in size from 23 to 1,000 beds, public health laboratories, and private laboratories.

***S. aureus* strains.** Five strains of *S. aureus* were used in the surveys. Strains A, B, D, and E were clinical strains isolated in Ontario laboratories. Strain C was NCTC 10442. For reference purposes, the susceptibilities of the strains to oxacillin and methicillin were determined by the NCCLS tube macrodilution method (16). The presence of the allele for Mec^r was demonstrated in all the strains by hybridization. Staphylococcal cells were lysed, and whole chromosomal DNA was extracted by standard phenol-chloroform-isopropanol extraction. Denatured DNA was applied to nitrocellulose filters (0.5 μg per slot) by using a slot blotter (Hybri-Slot; Bethesda Research Laboratories). Probe DNA was prepared as whole plasmid pG0158 DNA and was labelled with [α-^{32}P]dCTP by nick translation (13, 19, 23). Hybridization was carried out under standard aqueous conditions at 65°C; this was followed by washing at 65°C under conditions of high stringency. Hybridization was detected by autoradiography.

The β-lactamase test was performed by a previously described method (14).

Heteroresistance population analysis and assignment of

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TABLE 1. Analysis of results obtained by laboratories in relation to the properties of five MRSA strains used in proficiency surveys between 1980 and 1989

Strain	Survey yr	No. of laboratories correctly reporting resistance/total no. of laboratories (%)	MIC (μg/ml)		β-Lactamase production	Expression class[a]
			Oxacillin	Methicillin		
A	1987 1989	132/230 (57) 147/216 (68)	4	8	±	I
B	1980 1984	164/225 (73) 211/226 (93)	16	64	+++	II
C	1980 1982	172/224 (77) 187/213 (88)	64	128	+++	II
D	1984 1985	152/230 (66) 101/221 (46)	8	32	±	I
E	1986	229/233 (98)	64	>128	+++	II

[a] Expression classes were derived from the data in Fig. 1.

strains to expression classes were performed by the methods previously described in detail (24). Briefly, serial 10-fold dilutions of an overnight broth culture, ranging from undiluted to 10^6, were all plated on serial 2-fold dilutions of methicillin in Trypticase soy agar (range, 0.8 to 800 μg/ml). The plates were incubated for 48 h at 35°C, and the colonies were counted after incubation. The curves shown in Fig. 1 were plotted from the colony counts, and each strain was assigned to an expression class as defined previously (24).

Strain A had an additional characteristic which may be significant. It grew on blood agar at a rate comparable to those of most strains of *S. aureus*, but growth on Mueller-Hinton agar and in Mueller-Hinton broth was slower. With this strain, close and careful examination was needed to obtain a result indicating resistance by the NCCLS disk diffusion test.

Methodology survey questionnaire. Following the 1989 survey, in which strain A was used, a questionnaire was sent to all participating laboratories requesting information on the methods used by each laboratory in the 1987 and 1989 surveys. Strain A was used for both of those surveys. Three methods were defined for analysis. These were the disk diffusion test, the single-plate agar incorporation screening test, and automated methods, which included systems that used preprepared MIC panels. Laboratories that used the disk diffusion method were asked to provide details of the disks, media, incubation temperatures, and incubation time that they used and the criteria that they used for the definition of resistance. Laboratories that used the agar incorporation screening plate were requested to provide details of the antibiotics, concentrations, agar media, salt additives, incubation times, and incubation temperatures that they used. Laboratories were considered to be noncompliant with NCCLS guidelines if any one of the variables reported above differed from the NCCLS recommendations (15, 16). Those laboratories that used automated or MIC methods were requested to provide details of the equipment and the methods that they used. We were not able to determine either the qualifications of the individuals completing the questionnaires or the accuracy of the information received.

RESULTS

The MICs for the strains were determined with oxacillin and methicillin (Table 1). Previous population analysis studies have used methicillin (2, 24), and for this reason we used this antibiotic for this part of our study. For strains A and D, the highest level of methicillin resistance in all cells was at a concentration of 2 μg/ml, and at 8 μg/ml the proportion of cells showing resistance was approximately 0.1% for strain D and approximately 0.01% for strain A. Resistance to 8 μg/ml was shown by 100% of cells of strains B, C, and E, and a significant number of cells were resistant to 100 μg/ml. By the criteria of Tomasz et al. (24), strains A and D are in expression class I and strains B, C, and E are in expression class II.

The five MRSA strains described in this report have been used in surveys on nine occasions since 1980 (Table 1). Four strains have been used twice, and one strain has been used once. Over the 9 years, from 1980 to 1989, there was a steady improvement in performance in relation to detecting strains B, C, and E, all of which were in expression class II. Strains B and C were each used twice, and there was a significant improvement in laboratory performance over time in detecting both strains. Strain E was correctly reported as resistant by 98% of laboratories in 1986 (Table 1). The results obtained with strains A and D (expression class I) contrast with the results obtained with strains B, C, and E. Strain A was correctly reported by only 68% of the laboratories as recently as 1989, although the laboratories showed a significant improvement in detecting this strain compared with the performance in 1987. Strain D was correctly reported by 46% of laboratories in 1985, although the 93% correct reporting of strain B in 1984 indicates that by that date most laboratories had become capable of recognizing a high-expression-class MRSA strain.

Usable questionnaires were returned by 174 of 230 (76%) of laboratories for the 1987 survey and by 180 of 216 (83%) of laboratories for the 1989 survey. There was no significant difference between the results reported by those laboratories that returned questionnaires and those that failed to do so.

The majority of laboratories used the disk diffusion method for strain detection in both surveys, and this method was associated with a 60% correct result in both surveys

TABLE 2. Accuracy of results and methods used in 1987 and 1989 surveys for strain A[a]

Method	Survey yr	No. of laboratories correctly reporting resistance/total no. of laboratories (%)	P for difference between years (χ^2)
Disk diffusion	1987	61/101 (60)	>0.05
	1989	52/86 (60)	
Agar incorporation	1987	5/14 (36)	<0.05
	1989	16/19 (84)	
Automated MIC	1987	15/35 (43)	<0.05
	1989	34/43 (79)	
More than one method	1987	15/24 (63)	>0.05
	1989	23/32 (72)	

(Table 2). There was a significant improvement in the performance of the agar incorporation screening test between 1987 and 1989. The number of laboratories reporting the use of an automated MIC system as a single method were 24 in 1987 and 32 in 1989. There was, overall, a statistically significant improvement in the performances of the laboratories that used automated MIC systems, but 11 different MIC systems were represented within this category, 9 of which used an MIC system as the sole detection method, and the numbers of laboratories that used any one system were too few to permit valid comparisons. In both surveys the performance of laboratories that used more than one method was no better than the performance of laboratories that used a single method.

The level of compliance with NCCLS standards for those laboratories that used the disk diffusion and agar screening techniques was as follows. For those laboratories that complied with NCCLS standards, 62 obtained a correct result and 33 obtained an incorrect result ($P > 0.05$; χ^2 test). For those laboratories that did not comply with NCCLS standards, 72 obtained a correct result and 53 obtained an incorrect result ($P > 0.05$; χ^2 test). Full compliance with NCCLS standards was reported for agar incorporation and disk diffusion by 47 of 115 (41%) laboratories in 1987 and by 48 of 105 (46%) laboratories in 1989. There was no correlation between adherence to NCCLS conditions and the accuracy of reporting.

Table 3 shows the distribution of correct and incorrect results reported by hospital laboratories in relation to the number of hospital beds. There was a significant association between the size of the laboratory as defined by the hospital bed number and the accuracy of reporting.

TABLE 3. Accuracy of reporting by hospital laboratories in relation to size (1989 survey)

No. of hospital beds	No. of laboratories with correct result/ total no. of laboratories (%)
1–100	23/40 (58)
101–200	18/28 (64)
201–300	16/23 (70)
301–400	18/20 (90)
401–500	9/15 (60)
501–600	6/6 (100)
601–700	5/5 (100)
701–800	1/2 (50)
801–900	2/2 (100)
901–1,000	2/2 (100)

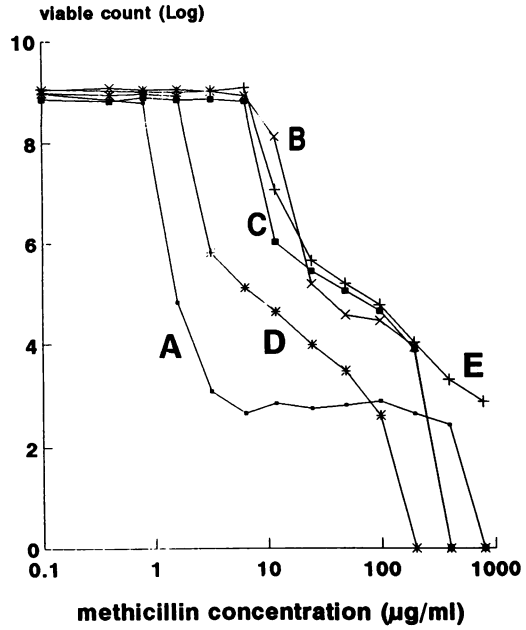

FIG. 1. *S. aureus* population analysis. Serial dilutions (10^0 to 10^6) were spread (0.1 ml per plate) onto serial doubling dilutions of methicillin in Trypticase soy agar from 0.8 to 800 μg/ml. Plates were incubated for 48 h.

DISCUSSION

Figure 1 shows the expression level for methicillin resistance of each of the five strains used in the survey. The improvement in reporting of the relatively easier to detect class II strains (strains B, C, and E) over the 9 years of the study (Table 1) is probably attributable to the dissemination of awareness of the importance of MRSA. The Laboratory Proficiency Testing Program of Ontario distributes with the evaluations comments on each test organism, and other educational efforts such as teleconferences are sponsored; this may have played a part in increasing general awareness concerning MRSA. Detection of strains A and D, however, continued to be problematic, and after receiving the results of the 1989 survey, the committee decided to investigate the methods being used by the participating laboratories in the 1987 and 1989 surveys to determine whether the incorrect results for strain A might be method related.

The method analysis (Table 2) shows that between 1987 and 1989, the laboratories improved significantly in their conduct of the agar incorporation screen test, but the disk test was unreliable in both surveys. Previous studies have reported that sensitivities for both the agar screening method (7, 21) and the disk test (3, 10, 21) are close to 100% for MRSA when either agar dilution or broth microdilution is used as the "gold standard." A study involving 40 laboratories and using the results generated in a single reference laboratory by multiple methods as the gold standard reported a sensitivity of 99% for the agar screening method with both methicillin and oxacillin. The sensitivity of the disk diffusion test was 100% with an oxacillin disk (1 μg) and 97.2% with a 5-μg methicillin disk (11). In another multi-

center study involving six laboratories, the disk diffusion test with a 1-μg oxacillin disk failed to detect 64% of MRSA strains after 24 h of incubation (1). In most studies the sensitivity of both agar screening and disk diffusion approaches but does not reach 100%, with one exception (1), implying the existence of a small minority of strains which are difficult to detect. Our study, we would suggest, focused on two such strains (strains A and D) and demonstrated the widespread difficulty of detecting these two strains, particularly by the disk diffusion test. In previously reported proficiency surveys (6, 6a, 12), 96.8 (6, 6a) to 100% (12) of participants reported a correct resistance result for MRSA, but the expression classes of the strains were not known.

High-level β-lactamase production has been shown to be an additional mechanism of borderline methicillin resistance (14). We did not evaluate the extent to which β-lactamase production may have contributed to the methicillin resistance of strains B, C, and E, but it is unlikely to have played a significant role in the resistance of strains A and D, in view of the weak reactions shown by these strains (Table 1).

There was no apparent correlation in our study between the accuracy of results and compliance with NCCLS standards (see Results), although it is interesting that in 1989 the three laboratories reporting incorrect results by the agar incorporation method were all noncompliant with NCCLS standards. Previously, it was reported (17) that fewer than 60% of laboratories in a single U.S. state were fully compliant with NCCLS standards in MRSA testing, and that study did not investigate the relationship between the accuracy of results and compliance with standard methods. Some departures from standard conditions, such as shortening the incubation period from 24 to 18 h, would be expected to give a false susceptibility result. Other technical errors, such as using an inappropriately low concentration of antibiotic in a screening plate, would generate a report of false resistance. In our study, we examined the error in only one direction, namely, false susceptibility, and for this reason we cannot perform a complete analysis of the impact of technological errors on the accuracy of the results. We suggest, however, that the majority of laboratories would not be able to demonstrate data to justify departure from consensus standards, and we adhere to the position that standardized methods should be used.

The results obtained with the automated and MIC methods are of interest. Early studies of automated methods showed poor performance in the detection of MRSA (1, 5, 9). In our study, there was poor performance of these methods in 1987, but the performance had significantly improved by 1989. We suggest that this may be due to improvements in both the quality of the equipment specifications and improvements in the skill and understanding of the laboratory personnel. In our study, many types of equipment and tests were represented in this category and the numbers of laboratories that used any one method were too few to permit comparisons between the different systems.

Table 3 shows that larger laboratories were significantly more accurate in their MRSA reporting than smaller laboratories (χ^2 test for trend analysis). There are many possible reasons for this, including greater experience associated with larger volumes or more specialized direction and supervision of microbiology services.

We suggest that one simple precaution can be used to increase the accuracy of MRSA testing, namely, the designation of a low-expression-class MRSA isolate as a standard control organism for routine testing. Concerns have recently been raised that some organisms with the Mec^r phenotype may meet the criteria to be considered susceptible (22), although this is a matter of dispute (20). There is also the possibility that strains with the inducible penicillin-binding protein PBP 2a may not reveal themselves on routine testing (18). If these observations are confirmed, it may become necessary in the future to revise methods for MRSA detection.

Our data demonstrate a conflict which can arise in proficiency testing programs. For the purposes of regulation and licensing, easily characterized organisms which represent a minimal acceptable standard are needed. However, these programs also have educational and quality improvement roles, and for these purposes more difficult challenges are more appropriate. For MRSA, a correct result is the only result compatible with an adequate standard of care, and the present study demonstrates either a need for technical improvement in the participating laboratories or a need for a modification of standard methods.

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