

## Proficiency of Clinical Microbiology Laboratories in Iran for Performance of Susceptibility Testing of *Staphylococcus aureus* against Methicillin and Vancomycin

Farinaz Rashed Marandi<sup>1</sup>, Mohammad Rahbar<sup>1,2\*</sup>, Rogeih Sabourian<sup>1</sup>,  
Mahnaz Saremi<sup>1</sup> and Maryam Mir-Mohammad Ali Roodaki

<sup>1</sup>Department of Microbiology, Iranian Reference Health Laboratories,  
Ministry of Health and Medical Education, Tehran, Iran.

<sup>2</sup>Antimicrobial resistance center, Tehran University of Medical sciences, Tehran, Iran.

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Performance of external quality control is an important tool for monitoring results of susceptibility testing in microbiology laboratories. The aim of this study was to assess the ability of Iranian microbiology laboratories for detection of MRSA and performance of susceptibility testing. One strain of *Staphylococcus aureus* (ATCC 33591) resistant to methicillin and susceptible to vancomycin were sent to clinical microbiology laboratories. This isolate was blinded -coded, and laboratories were asked for identification unknown strain and susceptibility testing it to methicillin, vancomycin chloramphenicol, erythromycin and tetracycline by using their standard disk diffusion method guidelines. Of 2282 laboratories contacted, 1509(66.1%) agreed to participated in our study and sent back their results on time for analysis. Regarding to identification of *S. aureus* of 1509 laboratories, 1283(85%) were able to identify isolate to genus and species level. Of 1509 laboratories 1349 (89.4%) performed correct susceptibility testing for methicillin, 88 laboratories (5.8%) could not determined resistance of *S. aureus* to methicillin and 72(6.8%) laboratories did not performed susceptibility testing of *S. aureus* for methicillin. Of 1509 laboratories, 889 (58.9. %) correctly performed susceptibility testing of *S. aureus* to vancomycin and reported correct result. while 594( 39.4%) laboratories reported incorrect results, 26 (1.7%) laboratories did not performed susceptibility testing to vancomycin. In conclusion our study revealed that detection of MRSA in clinical microbiology laboratories is satisfactory but the majority of laboratories (about 40%) have difficulties in performance of susceptibility testing of *S. aureus* to vancomycin.

**Key Words:** *Staphylococcus aureus*, Methicillin, Vancomycin, Resistance, Microbiology Labs.

External quality assessment scheme (EQAS) is used in the sense of proficiency testing such as systematic evaluation by an external organization administering survey for participating laboratories, and the laboratories, being evaluated by their responses to survey. EQAS allows inter-

comparison between laboratories, detection of errors, and evaluation of the performance and suitability of some culture media, reagents, diagnostic kits and antibiotic susceptibility testing disks for the purpose they designed. EQAS is also useful tool for continuous education<sup>1,2</sup>. Antimicrobial susceptibility testing is one of the most important tasks of clinical microbiology laboratories for providing guidance to physicians for therapeutic options. This is also an important first step in providing surveillance data for use in local and national aggregate databases<sup>3,4,5</sup>.

\* To whom all correspondence should be addressed.  
Tel.: +98 21 3798670-1. Fax: +98213396373.  
E-mail: rahbar\_reflab@yahoo.com

Susceptibility testing is performed daily in clinical laboratories by standard methods. There are many different methods for susceptibility testing. However disk diffusion method has been extensively used for this purpose<sup>6,7,8,9</sup>. Quality assurance of antimicrobial susceptibility testing is commonly performed by using internal quality control protocols for monitoring of precision and accuracy of the methods. Additional external quality control assessment is necessary in quality assurance of identification and susceptibility testing methods<sup>2,10,11</sup>. In our county external quality control carries out by reference laboratories which distribute unknown strains to participating laboratories. This method has been used to compare ability of microbiology laboratories for detection and susceptibility testing both locally and national levels.

Methicillin resistant *Staphylococcus aureus* (MRSA) has become a serious clinical problem over the last decades and the ability to detect this resistance reliability and rapidly is now required of all clinical microbiology laboratories<sup>12,13</sup>. In the present study, we performed a multicenter study in Tehran Iran on proficiency of microbiology laboratories for identification and susceptibility testing of *S.aureus* as a major agent of hospital and community acquired infections.

laboratories in Tehran and other provinces. We asked all laboratories to return their result after two weeks receipt of samples. Instruction to the participating laboratories indicated that organism would be studied for identification and susceptibility testing against oxacillin (Methicillin), vancomycin, chloramphenicol, erythromycin and tetracycline. A report form was provided and asked to fill in quantitative (zone diameter) and qualitative (susceptible, intermediate, or resistant). The form also included a question to provide laboratory information, routine test methods for identification and susceptibility testing, source of culture media and antibiotics disks. We also asked laboratories to provide susceptibility testing results with interpretation report to the clinician (i.e. detection of MRSA). The antimicrobial susceptibility profile (reference values) of the *S.aureus* are studied by the Clinical and Laboratory Standards Institute (CLSI) disk diffusion method<sup>14</sup>. Zone sizes, and interpretative results received from participating laboratories were classified and given score according WHO guidelines. A descriptive statistical method was used for calculation of frequency and percentage. The maximum score for identification was three score of points and one score for susceptibility testing against each antibiotic.

## MATERIALS AND METHODS

One strain of *Staphylococcus aureus* (ATCC 33591) were chosen and coded as strain 1. It was selected from strain collection of Iranian reference laboratories collection. This strain lyophilized and distributed to 2282 clinical

## RESULTS

Of 2282 laboratories contacted, 1509 (66.1%) agreed to participated in our study and sent back their results on time. The remaining 773 (33.9) laboratories did not participated in our survey. Regarding to identification of *S.aureus* of

**Table 1.** EQAS susceptibility testing results of *S.aureus* to Vancomycin and Oxacillin

Region	Oxacillin			Vancomycin		
	Correct answer	Incorrect answer	No answer	Correct answer	Incorrect answer	No answer
Tehran	295 (68.6%)	135 (31.17%)	1 (0.23%)	402 (93.27%)	21 (4.87%)	8 (1.86%)
Other provinces	594 (55.1%)	459 (42.6%)	25 (2.3%)	947 (87.8%)	67 (6.2%)	64 (6%)
Total (1509)	889 (58.9%)	594 (39.4%)	26 (1.7%)	1349 (89.4%)	88 (5.8%)	72 (4.8%)

1509 laboratories 129 (8.54%) obtained the maximum score (Three score), 1350 laboratories (89.4%) obtained intermediate score (1-2.5) and the remaining 30 (1.98%) obtained zero score of points in otherwise this group did not identified correctly *S. aureus*. The mean score for identification of *S. aureus* in country was 1.91 (Tehran 2.01 vs. 1.91 other provinces). Of 1509 laboratories 1349 (89.4%) performed correct susceptibility testing for methicillin 88 laboratories (5.8%) could not determined resistance of *S. aureus* to methicillin and 72 laboratories did not performed susceptibility testing of *S. aureus* for methicillin. Mean score of susceptibility testing for methicillin was: 0.89 for country, 0.93 for Tehran and 0.87 for other provinces. Proficiency in performance of susceptibility testing for vancomycin varied between laboratories. Of 1509 laboratories, 594 (55.1%) correctly performed susceptibility testing of *S. aureus* according to vancomycin and reported correct result, while 594 (39.4%) laboratories reported incorrect results (the mean error) and 26 laboratories 1.7% did not performed susceptibility testing according to vancomycin. Mean score of susceptibility testing for vancomycin was: 0.59 for country, 0.68 for Tehran (Table 1).

## DISCUSSION

Accurate determination of resistance pattern and the underlying mechanisms of resistance are of crucial importance, not only for treatment of patients but also from public health perspective<sup>5</sup>. This study specifically addressed the issue of whether laboratories using routine methodologies were able to test *Staphylococcus aureus* for its susceptibility to methicillin and vancomycin. Misidentification of the infecting organism, and over or under-reporting of resistance can have serious consequences for the patient, resulting in the prescription of less than optimal antimicrobial agents. Susceptibility testing results provided in clinical microbiology laboratories are also invaluable as a data source for use in surveillance systems. In this respect, incorrect identification and susceptibility testing results can have ramification that go beyond the individual patient<sup>3,4</sup>. In evaluating the microbiology laboratories in Islamic Republic of Iran it was supposed that the laboratories were functioning

within an acceptable range. Unfortunately our results did not confirm this assumption, and there was a wide range of capabilities of the laboratories for identification and susceptibility testing of different species microorganisms. In a previous study by Abbassi *et al* they evaluated the results of 10<sup>th</sup> external quality control assessment results which carried out in reference laboratory of Iran in summer of 2002. They distributed five species bacteria (each laboratories two unknown organism) among 487 microbiology laboratories in Tehran and districts. Of 487 laboratories they received answers from 437 (89.7%) laboratories. Of 291 laboratories 224 (77%) produced correct answer for identification of *S. saprophyticus*, Of 146 laboratories 102 (69.85) for *C. freundii* Of 114 laboratories, 34 (30%) for *Acinetobacter baumannii*. Of 146 laboratories 37 (25.3%) for *E. faecalis* and of 177 laboratories 63 (35.6%) for *E. agglomerans*<sup>15</sup>. In other study which carried out in Feb 2007 21st run of proficiency testing of Iranian microbiology laboratories carried out by Iranian reference health laboratories. In this survey two unknown microorganisms including *Salmonella paratyphi* B and *Staphylococcus aureus* were submitted to 1305 microbiology laboratories. Of 1305 laboratories, 1122 (86%) laboratories participated in survey and 183 (14%) laboratories did not participated in the program. Of 1122 laboratories, 523 (46.6%) laboratories identified *S. paratyphi* B correctly. The results of susceptibility testing of *S. paratyphi* B were relatively satisfied for nalidixic acid, ciprofloxacin and trimethoprim-sulfamethoxazole. However the results of susceptibility testing for tetracycline and ampicillin were unsatisfied and only 578 (52.5%) and 558 (49.7%) of laboratories reported correct answer for tetracycline and ampicillin respectively. Regarding to identification of *Staphylococcus aureus* of 1122 laboratories 767 (68.4%) laboratories identified this organism correctly<sup>1</sup>.

Results of EQAS programs in other countries have been shown that many laboratories have not satisfactory results for susceptibility testing in EQAS surveys. In a study by Tenover *et al* in CDC, they assessed ability of 130 laboratories to detect emerging antimicrobial resistance in EQAS program. This study carried out by WHO cooperation. In their study most laboratories were able to detect methicillin

(oxacillin) resistance in *Staphylococcus aureus*, high-level vancomycin resistance in *Enterococcus faecium*, and resistance to extended-spectrum cephalosporins in *Klebsiella pneumoniae*. Many laboratories, particularly those using disk diffusion tests, had difficulty in recognizing reduced susceptibility to penicillin in an isolate of *Streptococcus*<sup>16</sup>. In the other study by Edson DC *et al.*, in 2003, a test sample was sent to 355 laboratories enrolled in a proficiency testing program to assess their ability to detect low-level penicillin resistance in a strain of *Streptococcus pneumoniae*. One hundred fifty participants reported results for antimicrobial susceptibility testing. Of the 62 respondents using disk diffusion, 34 (55%) failed to report a result that was acceptable for detecting penicillin resistance and 30 (48%) reported a result for one or more drugs not approved for testing *S. pneumoniae*. Moreover, 12 (14%) of the 88 respondents using minimum inhibitory concentration methods reported results for at least one unapproved drug<sup>17</sup>.

In present study the majority of the Iranian microbiology laboratories were able to identify *S. aureus* to genus level adequately. Of 1509 laboratories 1349 (89.4%) performed correct susceptibility testing for methicillin 88 laboratories (5.8%) could not determined resistance of *S. aureus* to methicillin and 72 laboratories did not performed susceptibility testing of *S. aureus* to methicillin. Accurate performance susceptibility testing to vancomycin proved more difficult, while only Of 1509 laboratories, 594 (55.1%) correctly performed susceptibility testing of *S. aureus* to vancomycin and 594 (39.4%) laboratories reported incorrect results (very major errors) and 26 laboratories (1.7%) did not performed susceptibility testing according to vancomycin.

There are several factors that may affect the performance of susceptibility tests and standardized methods are more likely to be reproducible than unstandardized methods. Quality assurance is the overall process by which the quality of the test results can be guaranteed. A major part of this process is the internal quality control testing which is roundly should be done to monitor the precision and accuracy of the tests<sup>18,19,20</sup>. The performance of reagents used in the test and proficiency of the persons carrying out the test. However, there are additional aspects

of that contribute to quality assurance, including participation in external quality assessment scheme, internal quality assessment and the validation of process, in which atypical or controversial results can be detected. In a study by Kiehlbauch in New York City they, evaluated compliance of 320 microbiology laboratories for NCCLS guidelines. They found that nearly 80 Of 153 laboratory compliance the five important factor including inoculation preparation, medium choice, number of disk per plate incubation condition and length of incubation for *S. aureus*<sup>21</sup>. Quality of antibiotic disks is an important factor for performance of susceptibility testing. More than 90% laboratories in our country use homemade antibiotics disk from the same company and for this reason the results of present EQAS study is comparable between laboratories. Unfortunately our recent studies revealed that some of homemade antibiotics disks have poor quality<sup>22,23</sup>. Education is an important part of the quality assurance process as an understanding of the technique, together with their limitation and pitfalls, contributes significantly to the recognition resolution and avoidance of errors<sup>20,21,22</sup>. Other factors such as specimen testing volume can have an impact on the quality of services of offered by clinical microbiology laboratories<sup>24</sup>.

## CONCLUSION

Our study revealed that the majority microbiology laboratories in our country capable for identification of *S. aureus*. More than 90% of laboratories performed susceptibility testing to methicillin correctly, while 40% of laboratories had difficulty in performance of susceptibility testing of *S. aureus* to vancomycin. We recommend all microbiology laboratories quality control of media, antibiotic disks, using of control strains and finally compliance of guidelines such as CLSI.

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