Ability of Iranian Microbiology Laboratories for Detection and Susceptibility Testing of Unknown Microorganisms: Survey of 2149 Laboratories

M Rahbar, M Saremi, R Sabourian, S Yazdi

Abstract
The Iranian 18th national external quality assessment scheme (EQAS) for microbiology laboratories were carried out in Feb 2005. In this survey we distributed three unknown microorganism among 2149 microbiology laboratories (each laboratory two microorganism) Of 2149 microbiology laboratory, 1493 laboratory (69.5%) participated in our survey. Of 1493 laboratory, 512 (34.3%) identified Salmonella Para A correctly and obtained the maximum score of point. Of 424 laboratory only 45 (10.6%) identified Stenotrophomonas maltophilia correctly and unfortunately nearly 90% of laboratories were not able for identification of this microorganism or had partially correct answer. Of 1069 laboratory only 343 (32%) identified Listeria monocytogenes correctly. The majority of laboratories performed susceptibility testing correctly and obtained the maximum 5 score of points the mean score of point for susceptibility testing was 3.88.

INTRODUCTION
Blind retesting of previously analyzed specimens can be used an assessment in number of different areas of the laboratory, such as appropriate setup based on the source of the unknown organism, correct identification of unknown organism, appropriate titters of infectious disease of serologies testing and reporting of antimicrobial susceptibility testing results and many more. (Mahon et al 2007, Washington Wet al 2006., Sarp et al 2004 . Isenberg 2004, Vandepitte et al. 1991 ) This processes called external quality assessment scheme (EQAS) or competency assessment Sandle (2005) has described the benefits of participation in the EQAS for laboratories. (i) participating laboratories are able to assess whether their results are comparable with those of other laboratories. (ii) EQAS can provide a valuable educational stimuluses to laboratory staff. (iii) It provides credibility to the participating laboratory by providing evidence that the participating laboratory has a responsible attitude towards quality issues (evidence of participation is required by some acceding agencies); (iv) EQA provides an insight into national performance levels; and (v) EQAS improves national performance levels.

The Iranian national external quality assessment scheme for microbiology laboratories were introduced in 1994 for evaluation of performance and competency testing of microbiology laboratories in both governmental and private sectors. The scheme covers a wide range of clinical microbiology activities including identification and susceptibility testing. We annually perform three run of EQAS programs. In microbiology laboratories various steps have been taken to upgrade the EQAS programs. In recent years, the scheme has been actively promoted throughout country resulting increased participation.

In spite of regular performance of EQAS by reference laboratory of Iran, many microbiology laboratories are not able for identification and performance of correct susceptibility testing of some microorganisms. Our recent studies showed that nearly one third of microbiology laboratories in Tehran were not able to identify three unknown microorganisms such as Acinetobacter baumannii, Enerococcus faecalis and Enterobacter agglomerans. (Abbassi et al 2006 ., Rahbar et al 2007) The aim of this study was to determine ability of Iranian microbiology laboratories for identification and susceptibility testing of three unknown microorganisms.

MATERIAL AND METHODS
In Feb 2005, 18th run of proficiency testing of Iranian
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microbiology laboratories carried out by research center and reference laboratories of Iran. In this survey three unknown microorganisms including Salmonella Para A, Stenotrophomonas maltophilia (ATCC 13637) and Listeria monocytogenes (ATCC 7644) were submitted to 2149, 640 and 1509 laboratories respectively. All laboratories included both hospital and non-hospital microbiology laboratories in governmental and private sectors. S. para A and S. maltophilia were sent to all hospital microbiology laboratories and S. para A and Listeria monocytogenes were sent to non-hospital microbiology laboratories. Bacterial species were cultured in Trypticase Soy Agar (TSA) medium in screw capped tube. They were incubated in 35°C for 24 hours. After confirming the growth and purity of specimens we performed all conventional identification and susceptibility testing. (Isenberg et al., 2004. Washington et al. 2006. NCCLS, 2004) Specimens were placed in specially designed package, containing instructions and other paper works. Post mail shipments were labeled in accordance with carrier regulations.

We asked all laboratories to identify each microorganism and performance of susceptibility testing just for S. para A against ampicillin, trimethoprim-sulfamethoxazole, cefotaxime, ciprofloxacin and chloramphenicol. Scoring of results performed according of WHO criteria. The maximum score of point for identification of each bacterium was 3 score and 5 score for susceptibility testing. (Vandepotte J .1998 ). The results were analyzed by SPSS.

RESULTS

Of 2149 laboratories only 1493(69.5%) laboratories participated in our survey and 656 (30.5%) did not participated in this study. Of 1493 laboratories 512(34.3%) laboratories identified S. para A correctly and obtained maximum 3 score of points and 318 (21.3%) laboratories misidentified this microorganism. Many laboratories had difficulty in identification of S. maltophilia and only 45(10.6%) of laboratories were able in identification of S. maltophilia correctly and 275(64.8%) of laboratories obtained zero score of points. The third organism, (L. monocytogenes) were identified correctly by 343 (32%) of laboratories and obtained the maximum score of points and 491 (46%) of laboratories were not capable in detection of this organism and obtained zero score of points. The other laboratories sent intermediate correctly response. The results of susceptibility testing of S. para A were relatively satisfied and many laboratories obtained maximum five score. 1493 laboratories reported results of susceptibility testing for S para.A The mean of score for susceptibility testing was 3.88.

Figure 1

Table 1: results of 18th survey EQAS in Iranian microbiology laboratorie

<table>
<thead>
<tr>
<th>Organism</th>
<th>Total answer</th>
<th>NP</th>
<th>2</th>
<th>5</th>
<th>1</th>
<th>0</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. para A</td>
<td>2149</td>
<td>193</td>
<td>656</td>
<td>512</td>
<td>541</td>
<td>81</td>
</tr>
<tr>
<td>S. maltophilia</td>
<td>640</td>
<td>424</td>
<td>216</td>
<td>45</td>
<td>29</td>
<td>28</td>
</tr>
<tr>
<td>L. monocytogenes</td>
<td>1509</td>
<td>1069</td>
<td>440</td>
<td>343</td>
<td>180</td>
<td>73</td>
</tr>
</tbody>
</table>

NP= Not participated

DISCUSSION

The main goal of EQAS is to improve the quality and strengthen the capabilities of laboratories. In evaluating the microbiology laboratories in Islamic Republic of Iran it was presumed beforehand 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 different species of microorganisms . In a previous study by Abbassi et al (2006) they evaluated the results of 10th 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 received answers from 437 (89.7%) laboratories. Of 291 laboratories 224 (77%) produced correct answer for S. saprophyticus, Of 146 laboratories 102(69.85) for C. freundii Of 114 laboratories, 34(30%) for Acinetobater baumannii. Of 146 laboratories 37(25.3%) for E faecalis and 0f 177 laboratories 63((35.6%) for E. agglomerance . There are many studies for evaluation external quality assessment in microbiology laboratories worldwide. This study reveals that there is a poor performance testing in our microbiology laboratories for identification unknown micro organisms. There are many studies for evaluation of external quality assessment in microbiology laboratories worldwide. For example the first external quality assessment of clinical microbiology laboratories in Norway in 1982 included 15 country and regional laboratories. The mean number of incorrect identifications was 2.7 (11.3%). Eleven strains were correctly identified by all laboratories, whereas 4 strains were misidentified by 4 to 7 laboratories, accounting for approximately 50% of all misidentifications (Lassen et all ) According to Richardson and his associates in Canada.
(1994) the number of participating microbiology laboratories in EQAS declined from 335 laboratories in 1974 to 190 laboratories in 1994. In the initial evaluation, 21% of laboratories did not have the expected capabilities. In 1989, 50% of laboratories achieved high points (above 80%) for isolating and identifying the microorganisms. However, 25% of laboratories scored less than 50% for bacterial sensitivity testing and only 10% of them had high scores (above 80%). This lack of effectiveness was related to inappropriate selection of chemicals.

In another study (Tenover et al., 2001) to evaluate bacterial resistance, the Centers for Disease Control and Prevention (CDC) and WHO distributed 6 different strains of bacteria among 130 laboratories in the United States and other countries. Most of the laboratories were able to identify S. aureus, Enterococcus faecalis, and Klebsiella pneumoniae against methicillin, vancomycin, and cephalosporin respectively. However, the rest, especially those that used the disk diffusion method for evaluating the sensitivity of S. pneumoniae against penicillin, had problems. In addition, the majority of laboratories had problems for evaluating reduced sensitivity of S. epidermidis to vancomycin. Other study by Engler et al. (2000) showed only 3 of 23 reference laboratories were able to identify correctly 6 lyophilized Corynebacterium diphtheriae strains and to detect the C. diphtheriae toxigenicity. A study by Kumasaka in Tokyo (2000) revealed that poor performance in the EQAS survey was closely related to poor laboratory management, the type of training, experience of the medical technicians, and the supervisory ability of the consultant physicians in independent laboratories. In a study in the United Kingdom, Pitt et al. (2000) concluded that the physiological concepts of job satisfaction and climate are factors that might affect external and internal quality control. In another study by Matynia et al. (2005) to five consecutive isolates of S. aureus, the corresponding susceptibility was part of Polish external quality assurance scheme. Clinical laboratories were by the national centre of quality control in microbiology. Of 1376 isolates submitted as S. aureus from 276 medical centers, 13% (< 1%) had been misidentified by local laboratories. Of 181 (13.5%) MRSA isolates, most were identified correctly (98% of laboratories).

The microbiology laboratory serves as the first step in identification and performing of susceptibility testing of microorganisms isolated from patients specimens. The main goal of EQAS is to improve the quality and strengthen the capabilities of laboratories for correct identification and susceptibility testing. The experiences of error as reported for the various groups of laboratories in our programs in the different microbiology EQAS programs was relative and may not represent the exact rate of error experienced in actual practice. Because of following (i) the generally accepted opinion that external proficiency testing results represent the best effort of some laboratories, and it has been reported that the proficiency of laboratories as measured in blinded studies, in which laboratories did not know they were being tested, was lower than their proficiency testing under condition when the laboratories knew they were being tested, (ii) The differences among the laboratories in the extent of identification reported for certain types of samples, (iii) the variation in occurrence of microbial species in different patients population, (iv) the differences in frequency with which various microbial species encountered by individual laboratories and the difference in the types and quality of patients specimens tested by individual laboratories.

Many laboratories were restructured so that they no longer had experienced medical technologist or pathologist, or medical microbiologist dedicated to the performance of microbiology testing. However, they still chose to perform all levels of laboratory testing for diagnosis of infectious disease. The laboratories that were not restructured and that maintained testing done by experienced, dedicated personnel continued to show improvement in performance on the proficiency tests samples, by the end of the observation period, they made errors in bacterial identification and susceptibility testing <5% of the time. Those laboratories that were restructured and staffed with generalists as well as increased the variety of what they offered continued to make many serious errors in identification and susceptibility testing. This finding is likely because they down-graded their technical expertise by employing less-experienced personnel, in contrast to the laboratories that maintained staff with focused expertise. In doing some the restructured laboratories double the number of errors made in bacterial identification. (Peterson et al., 2001)

There are other factors that may affect the identification and susceptibility tests and standardized methods are more likely to be reproducible than unstandardized methods. Quality assurance is the overall process by which the quality results can be guaranteed. A major part of this process is the internal quality control testing which is routinely undertaken.
to monitor the precision and accuracy of the test procedures, the performance of regents, and the performance of the person carry out the tests. However, there are additional aspects that contribute to quality assurance, including regular participation in external quality assessment schemes, internal quality assessment and the validation process, in which atypical or contradictory results can be detected. Education is an important part of the quality assurance process as an understanding of the techniques, together with their limitations and pitfalls, contributes significantly to the recognition, resolution and avoidance of errors (Sharp et al., 2004; Brown et al. et al., 2001) . Unfortunately many of laboratories in our county do not have material and reagents for performance these tests and internal quality controls are very poor. For this reason the majority of laboratories have problems for identification of unusual microorganisms.

Conclusion .The results of this and other EQAS shows that many microbiology laboratories very poor proficiency testing results for identification unknown microorganisms. We are planning to establish a proper policy for manufacturers (or importers) to produce the necessary and important media and reagents. In addition, adding special postgraduate training courses and distribution of scientific guidelines will be helpful. With these new policies, we hope in future to upgrade the capabilities of the microbiology laboratories in our country.

References


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Author Information

Mohammad Rahbar
Department of Microbiology, Reference laboratories of Iran

Mahnaz Saremi
Department of Microbiology, Reference laboratories of Iran

Rogaieh Sabourian
Department of Microbiology, Reference laboratories of Iran

Soheilla Hekmat Yazdi
Department of Microbiology, Reference laboratories of Iran