

Evaluation of Iranian Microbiology Laboratories for Identification of Etiologic Agents of Bacterial Meningitidis. Survey Results of an External Quality Assessment Scheme (EQAS) Programme

Farinaz Rashed Marandi, Mohammad Rahbar, Roghieh Sabourian, Mahnaz Saremi

Department of Microbiology, Iranian Health Reference Laboratory, Ministry of Health and Medical Education, Tehran, Iran.

Abstract

Objective: To determine the ability of Iranian microbiology laboratories for identification and susceptibility testing of *Streptococcus pneumoniae* and *Haemophilus influenzae* as causative agents of bacterial meningitides.

Methods: Two strains of bacteria including *Haemophilus influenzae* and *Streptococcus pneumoniae* as a common causative agents of meningitides were chosen and coded as strain number 1 and number 2. The strains were distributed among 679 microbiology laboratories. All laboratories were requested for identification of each unknown microorganism and susceptibility testing of *S. pneumoniae* against five commonly used antibiotics.

Results: Of 679 microbiology laboratories 310 (46%) laboratories participated in the survey and among these, 258 laboratories completely identified *S. pneumoniae*. About 85% laboratories produced correct susceptibility testing against oxacillin, erythromycin, tetracycline, and vancomycin. Of 310 received responses only 50 laboratories identified *H. influenzae* correctly. The majority of the laboratories did not have the capacity to identify *H. influenzae*.

Conclusion: Microbiology laboratories in our country are qualified for identification and susceptibility testing of *S. pneumoniae*. However, majority of laboratories are not qualified for identification of *H. influenzae* (JPMA 60:48; 2010).

Introduction

Quality assurance of the analytical process performed at the clinical microbiology laboratory is mandatory and should be carried out by using external and internal quality control activities. External quality assessment scheme (EQAS) allows inter-comparison within laboratories, detection of errors, and evaluation of the suitability of some culture media, reagents, diagnostic kits and antibiotic susceptibility disks for the specific designed purpose. EQAS is also useful for continuous education. EQAS is used in the sense of proficiency testing such as systematic assessment by an external organization administering survey for participating laboratories and the laboratories being evaluated by their responses to survey.¹⁻³

The Iranian national external quality assessment scheme for microbiology laboratories was established in 1994 for evaluation of performance and competency testing of microbiology laboratories in both governmental and private sectors. The EQAS covers a wide range of clinical microbiology laboratories activities including identification and susceptibility testing. Our reference health laboratory performs two or three runs of EQAS programme annually. In recent years, the scheme has been actively promoted throughout the country and which had resulted in an increased number of participants in the EQAS programme.

In spite of regular performance of EQAS by Iranian reference health laboratory, many microbiology laboratories do not have the capacity for correct identification and susceptibility testing of some microorganisms. Our recent studies showed that nearly one third of microbiology laboratories in Tehran and other districts were not able to identify and perform susceptibility testing of some microorganisms such as *Acinetobacter baumannii*, *Enterococcus faecalis* and *Enterobacter agglomerans*.⁴ The aim of this study was to determine the performance of some Iranian microbiology laboratories for detection and performance of susceptibility testing of *Streptococcus pneumoniae* and *Haemophilus influenzae* as two important causative agents of bacterial meningitides.

Material and Methods

Two strains of bacteria including *Haemophilus influenzae* and *Streptococcus pneumoniae* as a common causative agents of meningitides were chosen and coded as strain 1 and 2. These strains were lyophilized and distributed among 679 microbiology laboratories in the country. After confirming the growth and purity of specimens, we performed conventional identification and susceptibility testing randomly.^{5,6}

Survey was distributed on the basis of the routine

services provided by the participating laboratory. For example, all participating laboratories receive surveys for bacterial meningitides, because they all routinely provide such testing. Shipping of surveys was carried out by post services. The survey packaged samples for shipment according to guidelines of Iranian post office regulations for infectious materials. All laboratories were asked to return their results after three weeks of receipt of the samples. Instructions to participating laboratories for identification of both organisms and susceptibility testing of *S. pneumoniae* to oxacillin, erythromycin, tetracycline, vancomycin and Co-trimoxazole was done. We also asked to report zone of inhibition diameter in mm and interpretation of results as susceptible, intermediate and resistant as recommended by Clinical and Laboratory Standards Institute (CLSI) guidelines. An Excel spreadsheet was created for survey and all responses were initially entered on data sheets and analyzed and given scores according to WHO survey guidelines.^{3,7} Briefly the maximum score of point for complete identification of each bacterium was 3 score of point and score of partial identification was 1-2.5 score of point. Score of point for incorrect identification of each organism was zero. We gave one score of point for susceptibility testing against each antibiotic; otherwise, score of points for performance of correct susceptibility testing against five antibiotics was five.

Results

Of 679 microbiology laboratories, 310 (46%) laboratories participated in our survey. Of 310 laboratories from which we received an answer, 258 laboratories completely identified *S. pneumoniae* and obtained 3 scores and 10 laboratories partially identified this organism and their score was 1-2.5. Forty one laboratories were not able to identify this organism. Of 310 received responses only

50 laboratories produced correct answer for identification of *H. influenzae* and 78 laboratories partially identified this organism. The majority laboratories (182) could not identify *H. influenzae*. We analyzed results of our survey for all microbiology laboratories in the country and separately for Tehran province and other university affiliated microbiology laboratories. In total, microbiology laboratories located in Tehran and other cities were able to identify *S. pneumoniae* correctly but identification of *H. influenzae* was the major error. Our survey reveals that only 16.12 % of laboratories had the capacity for identification of *H. influenzae* completely (Table-1). Regarding susceptibility testing of *S. pneumoniae*, the results of

Table-1: Results of External Quality Assessment Scheme Programme (EQAS) for identification of *S. pneumoniae* and *H. influenzae* in Tehran and other microbiology laboratories.

(n)	<i>S. pneumoniae</i> (n)	<i>H. influenzae</i>
Correct identification (Tehran)	69	24
Partial Correct identification(Tehran)	2	22
Incorrect identification (Tehran)	3	28
Not participate (Tehran)	50	50
Correct identification (other than Tehran)	189	26
Partial Correct identification (other than Tehran)	8	56
Incorrect identification (other than Tehran)	39	154
Not participate (other than Tehran)	319	319
Total	679	679

susceptibility testing were satisfactory. More than 85% of laboratories performed correct susceptibility testing of *S. pneumoniae* to oxacillin, erythromycin, tetracycline, and vancomycin. However, more than 50% of laboratories had

Table-2: Results of Susceptibility testing for *S. pneumoniae* in Tehran.

Antibiotic	Correct answer n (%)	Partial correct answer n (%)	Incorrect answer n (%)	No answer n (%)
Ox	69(89.1)	0(0)	4(5.40)	
Er	63(85.1)	3(4.05)	6(8.10)	2((2.70)
Te	62 (83.8)	6(8.10)	2(2.70)	4(5.40)
Va	65(87.8)	6(8.10)	1(1.35))	2(2.7)
SXT	36(48.6)	16(21.8)	20(27.0)	2(2.70)

Ox= Oxacillin, Er= Erythromycin, Te= Tetracyclin, Va= Vancomycin, SXT= Co-trimoxazole.

Table-3: Results of Susceptibility testing for *S. pneumoniae* in university affiliated laboratories (other than Tehran).

Antibiotics	Correct answer n (%)	Partial correct answer n (%)	Incorrect answer n (%)	No answer n (%)
Ox	161(68.22)	1(0.42)	35(14.83)	39(16.52)
Er	168(71.18)	21(8.89)	16 (6.77)	31(13.13)
Te	159 (67.37)	26(11.01)	208.47)	31(13.13)
Va	176(74.57)	8(3.38)	20 (8.47)	32 (13.55)
SXT	88(37.28)	42(18.26)	78(33.05)	28 (11.86)

Ox= Oxacillin, Er= Erythromycin, Te= Tetracyclin, Va= Vancomycin, SXT= Co-trimoxazole.

difficulty for susceptibility testing of *S. pneumoniae* against co-trimoxazole (Table-2,3).

Discussion

Clinical microbiology laboratories through routine identification, susceptibility testing and participating in various surveillance programmes, help monitoring the development and spread of antimicrobial resistance in their communities. The accuracy data generated by both formal and informal surveillance system has been debated for several years, which has led to a call for more careful monitoring of laboratory performance through external quality assurance and proficiency testing programmes.⁸⁻¹⁰ Proficiency testing is an external quality assurance method in which laboratories are sent simulated clinical specimen or bacterial isolates to be tested by routine laboratory methods. Proficiency testing provides data about the accuracy identification, susceptibility testing and can determine if a laboratory's method is sufficiently sensitive to identify and detect novel resistance patterns. This method of quality assurance also allows a clinical laboratory's performance to be assessed in comparison to reference methods and to other peer laboratories. Several reports suggest that providing feedback on proficiency testing results improve the quality of testing among clinical laboratories.^{8,11-13}

Our previous studies on the EQAS for identification and susceptibility testing coordinated by Iranian health reference laboratory, highlighted the types of testing errors that were common among participating laboratories. The main problem in all studies was poor proficiency of laboratories for identification of some microorganisms. However, performance of routine susceptibility testing by disk diffusion method was relatively satisfactory.

The importance of *S. pneumoniae* and *H. influenzae* as a common causative agents of bacterial meningitides is well established. However until recently, the epidemiology of invasive bacterial meningitides in our country was poorly documented. The growing rates of antibiotics resistant strains of *S. pneumoniae* and *H. influenzae* worldwide, combined with the promise of new conjugative vaccines, promoted the Iranian center for disease control in cooperation of reference health laboratory to initiate a national EQAS program for detection and susceptibility testing of *S. pneumoniae* and *H. influenzae*.

Unfortunately only about 50% of laboratories participated in our survey. The majority of laboratories identified *S. pneumoniae* correctly and the results of susceptibility testing were satisfactory. More than 85% of laboratories performed correct susceptibility testing of *S. pneumoniae* to oxacillin, erythromycin, tetracycline, and vancomycin. However more than 50% of laboratories had difficulty for susceptibility testing of *S. pneumoniae* against

co-trimoxazole. Of 310 received responses only 50 laboratories produced the correct answer for identification of *H. influenzae* and 78 laboratories partially identified this organism. The majority of the laboratories, 182 could not identify *H. influenzae* (The major error). Feedback letters were sent to all participants including the results of each laboratory and recommendation to improve isolation and susceptibility testing for the both organisms.

There are many factors that may affect the identification and susceptibility testing results. The standard methods are more likely to be reproducible than non standardized methods. Performance of quality assurance is an important process by which the quality results can be guaranteed. A major part of quality assurance is the internal quality control testing which is routinely undertaken in a laboratory to monitor the precision and accuracy of the test procedures.¹²⁻¹⁵ Performance of personnel and reagents quality control is an important step in quality assurance process. However, there are additional aspects that contribute to quality assurance, including regular participation in EQAS programmes and methods validation. Education is an important part of the quality assurance process as an understanding of the techniques, with their limitations and pitfalls which together, contribute significantly to the recognition, resolution and avoidance of errors.¹⁶ Other reasons for poor results of EQAS in our country is due to lack of some material and reagents and poor quality of home made reagents and susceptibility testing antibiotics disks.^{17,18}

Conclusion

Microbiology laboratories in our country are qualified for identification and susceptibility testing of *S. pneumoniae*. However, majority of laboratories were not able to identify *H. influenzae*. Poor performance for identification of *H. influenzae* might be due to lack of some material such as culture media and reagents.

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