

Original Article

Nationwide proficiency assessment of bacterial identification and antimicrobial susceptibility testing among 110 laboratories in Lebanon

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Abstract

Introduction: Proficiency testing (PT) is one of the most valuable and important activities for the Clinical Microbiology Laboratories (CML) to enroll in to ensure the accuracy and reliability of results. This first time conducted nationwide study was warranted to assess the PT performance activity among CML in Lebanon.

Methodology: Four training and PT activities were organized for 110 nationwide laboratories involved in providing clinical microbiology services. In each PT activity, five different bacterial species were distributed to each laboratory to provide identification (ID) and antimicrobial susceptibility testing (AMST) according to prior discussions and guidelines.

Results: The percentages of labs that correctly identified the bacterial species and performed the relevant AMST to it, respectively, were as follows: *S. aureus*, (100% and 67.8%); *Enterococcus faecalis* (71% and 82%); *Listeria monocytogenes* (75% and 61%); *Streptococcus agalactiae* (86% and 71%); *Corynebacterium amycolatum* (7% and 33 %); *Pseudomonas aeruginosa*, (93 % and 53.4%); *Klebsiella pneumoniae*, (97% and 67.7%); *Salmonella typhi* ESBL (87 % and 66%); *Enterobacter aerogenes* (89% and 59%) and *Stenotrophomonas maltophilia* (84 % and 65%). The resistant types for the species were specified by labs as carbapenem resistant (CR) *K. pneumoniae* in 78 %, CR *E. aerogenes* in 34 %, MRSA in 83 %, and VRE in 80.5%.

Conclusions: The wide variation as well as the overall humble scoring of accurate results reflects the dire need for the MOPH to establish and maintain a PT activity program, and entrust the reference laboratory to provide continuing education and training sessions.

Key words: Proficiency testing; susceptibility testing; bacterial identification; Lebanon.

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Introduction

Antimicrobial resistance (AMR) has been a surging problem, deleteriously impacting healthcare globally including Lebanon [1-4]. Among efforts to combat such a situation, the Clinical Microbiology Laboratories (CMLs) play a major role especially in providing valid identification and antimicrobial susceptibility results. These are crucial in the treatment of patients suffering from infectious diseases, as well as in public health disease surveillance.

However, like other service providing clinical laboratories the CMLs are faced with great challenges in providing sustainable accurate, reliable, trusted, defensible, and credible test results in the detection and analysis of recovered pathogens [5,6]. To maintain delivering credible results, several governmental authorities and other international recognized professional organizations have established programs towards this endeavor. These include the International

Organization for Standardization (ISO) and College of American Pathologists (CAP), which can also certify/accredit laboratories essentially relying on quality management system (QMS) theme whereby Proficiency Testing (PT) is an integral part [7-8].

The PT program was first mandated for service providing clinical laboratories in the USA as part of the Clinical Laboratory Improvement Amendments (CLIA) act of 1988, entailing a quality management system (QMS) theme [9]. It is one of the most valuable and important activity to be implemented for any laboratory that provides clinical services including the CML. This is to ensure the proficiency, accuracy, and reliability of microbial ID and AMST analysis within the overall quality process of the lab [10-12].

In Lebanon and in the absence of such PT activity in many CMLs, the National Antimicrobial Committee (NAC) at the Ministry of Public Health (MOPH) realized the value of incorporating such warranted

program to the CMLs and urged to initiate it in 2015, in collaboration with the World Health Organization (WHO) Country office in Lebanon. Since then, several training workshops (WSs) and other educational activities took place for governmental and other private CMLs nationwide.

Officialization of the PT training project by the NAC was launched under the patronage of The Minister of Public Health, Dr. Jamil Jabak, at Saint Joseph University of Beirut (USJ), on the 18th of March 2019. Invitations were sent to all healthcare institutions providing CM services in Lebanon. During this meeting, The Minister mentioned that the PT testing should be mandatory and not a voluntary activity for the CMLs.

Subsequently, four training and PT activities took place whereby 110 laboratories from all the governates of Lebanon providing CM services were enrolled and provided results. The fourth activity was a repeat PTs from 42 randomly selected laboratories. Hereby, we address and present the results of these PT activities and discuss the performance reflected by the different participants from different regions in the country.

Methodology

Pre Activity Workshops- Imparted Information to the Participating CML Staff

The pre-PT educational workshops were initiated, by CML specialists (GFA and DKS), to train and review diagnostic and antimicrobial clinical microbiology material for CML staff. A total of 185 labs, encompassing all governates in Lebanon, were invited, 170 attended the discussion, among which 152 labs (42 being repeaters in the 4th PT activity) participated in the PT activities and submitted results. These sessions aimed at enlightening the participants about the approaches and tests to use for bacterial identification (ID) and antimicrobial susceptibility testing (AMST) according to standardized quality control essentially based on The Clinical and Laboratory Standards Institute (CLSI) guidelines for disk diffusion (DD) test. Moreover, a summary brochure entitled “Standard Operating Procedures (SOP) for Antimicrobial Susceptibility Testing against Commonly Encountered Bacterial Species in Humans” was produced and distributed to the participants. It provided guidelines for each lab to follow about the category and types of antimicrobial to test against the recovered pathogens from the specimen source.

The European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines and

breakpoints for interpretation of AMST were also accepted to whoever follows them.

PT Isolates Preparation and Distribution to Participating Labs

The PT isolates were clinical ones identified and speciated by the Matrix-Assisted Laser Desorption Ionization Time of Flight (MALDI-TOF) system (Bruker Daltonik, GmbH, Bremen, Germany) at the American University of Beirut Medical Center (AUBMC). Preparation of the PT isolates and coding were coordinated between AUBMC, Saint-Joseph University of Beirut (USJ) and the MOPH. Isolates of five different species were prepared. Each was inoculated into two transport medium (Sterile Transport Swab with Amies Medium, Jiangsu Huida Medical Instruments, China) and distributed to the participant labs.

Antimicrobial Susceptibility Testing (AMST)

The AMST for each of the distributed PT isolates was carried out using the CLSI DD guidelines. The minimal inhibitory concentrations (MICs) were also determined for these isolates by Vitek 2 system (BioMérieux, Marcy L’Etoile, France). In addition, E-test methodology (AB BIODISK, Solna, Sweden) was used for characterizing those isolates with special resistance profiles.

The staff in the participating laboratories were asked to identify and test each pathogen according to the specified instructions provided in the distributed SOP, taking into consideration the source of the specimen. The distributed Gram-positive and Gram-negative bacterial species and the anticipated number of antimicrobial agents to test for each included: Vancomycin resistant enterococci (VRE, 8 antimicrobials for urinary isolates, and 4 for blood isolates), methicillin resistant *Staphylococcus aureus* (MRSA, 13 antimicrobials), *Listeria monocytogenes* (1 antimicrobial), *Streptococcus agalactiae* (5 antimicrobials), *Corynebacterium amycolatum* (6 antimicrobials), *Pseudomonas aeruginosa* (9 antimicrobials), carbapenem resistant *Klebsiella pneumoniae* (20 antimicrobials), *Salmonella typhi* (7 antimicrobials), carbapenem resistant *Enterobacter aerogenes* (17 antimicrobials), *Stenotrophomonas maltophilia* (3 antimicrobials).

Arbitrary Scores

Arbitrary scores were assigned to the performance of each lab based on their bacterial species ID score and AMST results, as well as the overall score for each lab,

as shown below. These scores were not meant or intended to assign ranking for labs, rather to utilize it as a tool for general assessment denominator in reflecting performances in this study.

Identification Score

The ID scores were calculated based on the following criteria: a score of +5 was given to correct species identification, a score of +3 to correct genus identification, and no score was given to wrong identification.

Antimicrobial Susceptibility Testing Score

The AMST scores were calculated based on the following criteria: a score of +5 was given to each of the correctly reported antibiotic, a score of -3 was given to each incorrectly reported antibiotic, and a score of -1 was given to each of the reported misleading/ irrelevant antibiotic.

Overall Score

The overall score for each lab was calculated as cumulative of ID and AMST scores, reflecting a percentage of the total correct anticipated scores.

Provided Feedback to labs

Feedback to labs was essentially based on sharing the anticipated accurate results of ID and AMST for each of the PT pathogen, an opportunity for each lab to assess its own performance anonymously. Also, group discussion took place about the findings, through personnel presence or group e-mail correspondence. Moreover, a wrap-up webinar for all four PT activities was organized on the 9th of December 2020.

Results

Numbers of Labs Invited, Participating in Discussion and Submitting Results

The total number of labs invited (n= 185 labs) / those attended the discussion (n = 170 labs) / and those participated in the PT activities and submitted results (n=152 labs, including the 42 repeaters in activity 4), were as follows: Activity 1 (Date 9-4-2019): 35/34/31; Activity 2 (Date 17-7-2019): 50/44/41; Activity 3 (Date 1-10-2019): 50/42/38 and Activity 4 (Date 26-11-2019): 50/50/42.

The distribution of the 152 labs submitting PT results according to the different Governates were: Greater Beirut, 19 Labs (12.5%); Mount Lebanon, 51 labs (33.5%); Bekaa/ Baalback/ Hermel, 21 labs (14%); North/ Akkar, 32 labs (21%); South/ Nabatieh, 29 labs (19%).

The 42 repeaters labs from PT activity 4, and their results will be noted separately, while the analysis that follows will address the results of the 110 labs.

Performance of Labs in Bacterial Identification

Table 1 summarizes the performance of 110 labs in providing ID for the species level, genus level, the specified resistance types and the incorrect results.

For the Gram-positive bacteria, the percentages of correct identification to the species level, genus level, and incorrect results, respectively, provided by the labs were as follows for: *S. aureus*, 100 %, 0, and 0; *E. faecalis*, 71%, 26%, 3%; *L. monocytogenes*, 75%, 12.5%, and 12.5; *S. agalactiae*, 86%, 1%, and 13%; *C. amycolatum*, 7%, 54%, and 39%. The resistance types for the species were specified as MRSA for the *S. aureus* by 83 %, and as VRE for the *E. faecalis* by

Table 1. Summary of PT bacterial identification results provided by the 162 participating labs.

PT Bacterial Species	No of Labs Performing PT	No (%) Providing Identification			Specified Resistance Type
		Species Level	Genus Level	Incorrect	
Gram Positive					
<i>Enterococcus faecalis</i>	72	51 (71)	19 (26)	2 (3)	
Specified VRE	72				58 (80.5)
<i>S. aureus</i>	72	100 (100)	0	0	
Specified MRSA	72				60 (83)
<i>Listeria Monocytogenes</i>	72	54 (75)	9 (12.5)	9 (12.5)	
<i>Streptococcus agalactiae</i>	80	69 (86)	1 (1)	10 (13)	
<i>Corynebacterium amycolatum</i>	80	6 (7)	43 (54)	31 (39)	
Gram Negative					
<i>Pseudomonas aeruginosa</i>	72	67 (93)	5 (7)	0	
<i>Klebsiella pneumoniae</i>	72	70 (97)	2 (3)		
Specified CRE	72				56 (78)
<i>Salmonella typhi</i>	80	70 (87)	6 (7)	5 (6)	
<i>Enterobacter aerogenes</i>	80	71 (89)	3 (4)	6 (7)	
Specified CRE	80				27 (34)
<i>Stenotrophomonas maltophilia</i>	80	67 (84)	0	13 (16)	

80.5% of the labs. The rates for the Gram-negative bacteria of correct identification to the species level, genus level, and incorrect results, respectively, were as follows for: *P. aeruginosa*, 93%, 7%, 0; *K. pneumoniae*, 97%, 3%, and 0; *S. typhi*, 87%, 6.5%, and 6.5%; *E. aerogenes*, 89%, 4%, and 7%; *S. maltophilia*, 84%, 0, and 16%. The resistance types for the species were specified as CRE for the *K. pneumoniae* by 56 of 72 (78 %) labs, and as CRE for the *E. aerogenes* by 34% of the labs.

Performance of Labs in Antimicrobial Susceptibility Testing

Table 2 summarizes the performance among different labs participating in the first three PT activities in regard to AMST results. For the Gram-positive bacteria, the percentages of correct AMST results are presented in Table 2. They are summarized for the pathogens as follows: *S. aureus* (MRSA) (67.8%); *E. faecalis* (VRE) (82%); *L. monocytogenes* (61%); *S. agalactiae* (53.1%) and *C. amycolatum* (10.1 %). For the Gram-negative bacteria, they are summarized for the pathogens as follows: *P. aeruginosa* (53.4%); *K. pneumoniae* (CRE) (67.7%); *S. typhi* (ESBL) (59.4%); *E. aerogenes* (CRE) (48.8%) and *S. maltophilia* (50%).

Overall Performance score for ID and AMST Results for all the labs

Figure 1 shows the summary of 110 labs performance scores (%) in regard to ID, AMST and the overall performance in the first three PT activities.

Concerning the ID, 28 labs showed a score of ≤ 75% (range ≤ 35- 75%), while 82 labs achieved a higher score, ranging between 76% and ≥ 96%.

In regard to AMST, the majority of labs (n =107) scored ≤ 75% (range ≤ 35- 75%), while only 3 labs achieved a score between 76% and 90%.

The overall scores (ID plus AMST) for labs indicated that 50 labs scored ≤ 45, 26 labs scored between 46% and 55%, 23 labs scored between 56% and 65%, 8 labs scored between 66 % and 75%, while 2 labs scored between 76% and 85%, and only one lab scored between 86% and 95%.

Results of Repeat PT testing in Activity 4

Table 3 presents a summary of results reflecting the performance of 42 labs repeating 5 PTs in the fourth activity. Compared to their first PT scores, the scores for the repeated ID revealed no change in 29%, positive change in 19%, and negative change in 52% of the labs,

Figure 1. Summary of 110 Labs performance in regard to identification, antimicrobial susceptibility testing, and overall performance in the PT activity.

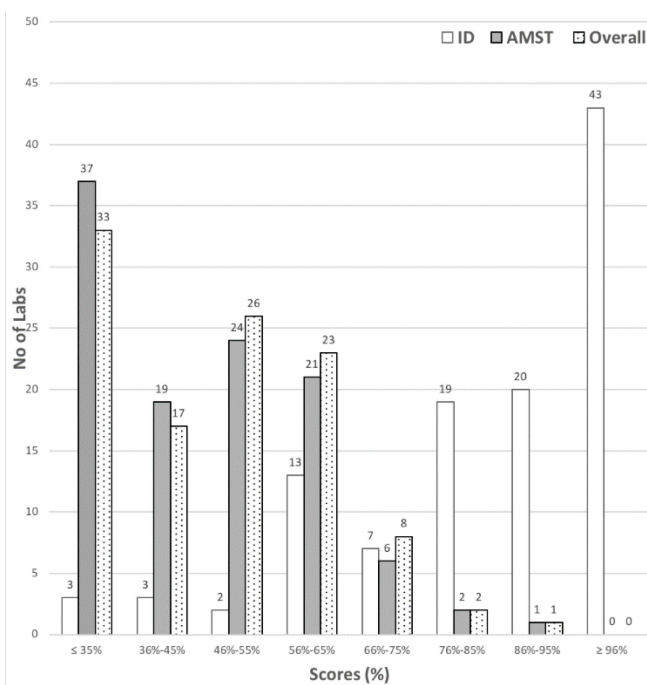


Table 2. Performance of antimicrobial susceptibility testing among different labs participating in the three PT activities.

Bacterial Spp.	Nb of expected AMA* to test	Number of labs Reported results	Performance of Lab in providing antimicrobial results		
			Correct / anticipated total (%)	No incorrect (lab range)	No misleading (lab range)
MRSA	13	72	635/936 (67.8)	301 (1-11)	18 (0-4)
VRE (U) ⁺ & B ⁺⁺	8 +4	72	338/412 (82)	66 (0-8)	52 (0-15)
<i>Listeria monocytogenes</i>	1	72	44/72 (61)	22 (0-1)	76 (0-10)
<i>Corynebacterium amycolatum</i>	6	42	23/228 (10.1)	25 (0-5)	7 (0-5)
<i>Streptococcus agalactiae</i>	5	42	101/190 (53.1)	49 (0 – 5)	27 (0 – 6)
<i>Klebsiella pneumoniae</i> CRE	20	72	975/1440 (67.7)	464 (0-19)	42 (0-4)
<i>Pseudomonas aeruginosa</i>	9	72	346/648 (53.4)	281 (1-19)	38 (0-8)
<i>Salmonella</i> Typhi	7	42	158/266 (59.4)	74 (0 – 5)	24 (0 – 5)
<i>Enterobacter aerogenes</i> CRE	17	42	315/646 (48.8)	246 (0 –14)	15 (0 – 2)
<i>Stenotrophomonas maltophilia</i>	3	42	57/114 (50)	27 (0 – 3)	120 (0 – 21)

while their AMST scores showed 7%, 62% and 31%, respectively. As for the overall score, it was 5%, 55%, and 40% showing no change, positive change and negative change, respectively.

Discussion

The results in this first nationwide PT activity in Lebanon, though humble, enabled us to identify gaps and explore improvement needs among the participating laboratories, and their resource setting, in the bacterial ID and AMST. Overall, the performance results for bacterial ID of pathogens were generally higher than that of the AMST results.

Concerning the performance of bacterial ID results to the species level among Gram- positive bacteria, all labs were able to identify *S. aureus* (100%). However, it was concerning to detect variable rates (14%-93%) of missed identification to the species level among the other pathogens. Regarding the Gram-negative bacteria, the laboratories correct identification to the species level generally indicated higher rates (84%-97%) than those for Gram-positive pathogens.

In regards to AMST performance, it was concerning to note many deficiencies among many labs. This was reflected in the variable range (33%-82%, mostly less than 75%) of labs that correctly performed relevant AMST against the pathogens. Noteworthy, several labs missed specifying the resistance types for the tested species: 17% as MRSA for *S. aureus*, 20% as VRE for *E. faecium*, 28% as CRE for *K. pneumoniae*, and in 66% as CRE for *E. aerogenes*. These results indicate the need for more training so that labs can improve reporting the resistance type of such MDR pathogens as an alert to physicians and for infection control purposes. In fact, such low results in AMST performance were not anticipated since several educational training workshops were conducted. More stunning is the 1974 AMST results that were interpreted incorrectly and

placed in the report. In a clinical setting, this would result in reporting inaccurate information to the treating physician and possibly misleading patient's management.

What is also concerning is the low overall combined ID and AMST scores. These were essentially based on accounting for the wrong, misleading and irrelevant provided results, and indicated that 33% of the labs scored less than 35%, and only 10% of the labs scored between 66% and 95%. Thus, this unfortunate humble performance by many labs warrants long term follow-up and mentoring, by MOPH and others, to ensure successful improvement of laboratory diagnostic capacity. Interestingly, the impact of such an approach was positively noticed among 62% of the 42 laboratories that repeated the PTs activities. Also, with such an overall modest to low performance among the 110 labs in this PT activity, one would wonder about the earlier data reported in surveillance studies from Lebanon [3,4].

Searching for comparable published studies about the PT activities in different countries of our region enabled to find only one comparable study from Turkey involving 118 laboratories [13]. Similar to our study, they reported that mistakes done in bacterial ID were lower than the high error rates detected in AMST results [13]. In Africa, successive External Quality Assessment (EQA) ID and AMST studies carried out in 2002 (39 labs), 2009 (78 labs) and 2011 to 2016 (81 labs) showed comparable ID and lower AMST scores in respect to the current study from Lebanon. Nevertheless, their ID scores rose with continued PT performance over the years: 65%, 69% and 76% in 2002, 2009 and 2011 to 2016, respectively. However, their AMST scores remained low (42%-54%), close to what is generated in the current PT study [14, 15].

All in all, this first national PT activity in Lebanon delineated analysis errors in CMLs and pointed to the

Table 3. Summary of Results for 42 Labs repeating 5 PTs.

PT activity	Changes in Repeat PT scores	No (%) of Labs showing changes	Range (%) of Change in score
Identification	No change	12 (29)	
	Positive change	8 (19)	8-28
	Negative change	22 (52)	8-40
AMST*	No change	3 (7)	
	Positive change	26 (62)	2-31
	Negative change	13 (31)	2-34
Overall	No change	2 (5)	
	Positive change	23 (55)	2-32
	Negative change	17 (40)	2-40

need for improving ID and AMST capacity to avoid providing inaccurate results that can lead to misdiagnosis and inappropriate treatment in patient care. Thus, clinical laboratories should acknowledge and embrace such PT activities as an opportunity to improve the quality of their provided results as well as to support antimicrobial stewardship programs to control resistance and share credible data on international platforms [8, 16, 17].

Lessons learned from this nationwide PT activities pointed to several positive aspects including: 1) Reflection of keen enthusiasm and positive feedback about the educational and PT program by all the lab staff who attended the diagnostic presentations, workshops and discussion, especially in helping them streamline their work. 2) Expression of satisfaction about the provided WSSs, SOPs, and other educational material, as well as QC bacterial strains. 3) Satisfaction about the discussion related to guidance for QC aspects in reagents and tests to use for ID and AMST. 4) Benefit from feedback of results so that each lab learns and improves from deficiencies. 5) Contentment on self-improvement by staff while keeping up-to-date with diagnostic information.

On the other hand, there were different concerns expressed by lab staff that included: 1) Shortages and lack of reagents pertinent to ID & AMST, especially among public/ governmental labs. 2) Lack of dedicated staff assignment to clinical microbiology, covering the service is a rotational part with other clinical division. 3) Shortage or absence of CML specialists in many of the labs. 4) Unfortunately, some labs claimed doing the analysis themselves while in fact they outsourced the analysis to other labs.

Based on all of the above, this first-time nationwide PT activity in Lebanon raises a couple of recommendations/ suggestions that will encourage and assist the MOPH to implement in order to ensure release of reliable clinical microbiology results including: 1) Introducing a PT unit as an integral part of its health units infra structure. 2) Enforcing PT activities on the labs to be implemented in their clinical operations. 3) Implementing PT activities to account labs for their reliable, credible or deficient results as an integral part of the accreditation or certification requirements towards providing quality-controlled results for the welfare of patients' care in this country. 4) Organizing continuous medical education (CME) sessions that emphasize proper laboratory testing methods, the importance of quality control, and the basic concepts of quality assurance.

Conclusions

Providing accurate and validated microbiology laboratory results is critical in patient's care specially to those suffering from infectious diseases, as well as in providing surveillance results. The heterogeneity in clinical microbiology capacity and competence levels among the 110 different participating labs in these PT activities in Lebanon was clearly noticed. Despite providing didactic trainings and workshops, the low performance among many of these laboratories is unfortunate, and thus necessitates long term education, follow-up and mentoring to ensure successful improvement in laboratory proficiency. Moreover, this activity should be enforced as part of accreditation and certification as a requirement for laboratories offering clinical microbiology services to guarantee reliable results reflecting on proper patients' management and safety. Finally, it should be an assumed obligation that any lab who is performing microbiological diagnosis to have a full or part time clinical microbiologist to oversee and assure an adequate performance of the diagnostics. This is a critical issue for correct patient management, liability, surveillance, and public health in regard to lab infectious diagnosis and antimicrobial susceptibility performance aspects.

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