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Laboratory survey and literature review of anaerobic bacteriology: foundations of a clinically orientated and evidence-based workup for anaerobic cultures

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ABSTRACT

Since the introduction of matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) in routine microbiology laboratories, identification of anaerobic bacteria has become easier. These increased possibilities provide new challenges concerning analytical workup and reporting of anaerobes. In February 2015, an extensive web-based survey on pre-analytical, analytical and post-analytical procedures of anaerobic microbiology was sent to 53 Belgian, university and non-university hospital laboratories. Answers of 34 participating laboratories revealed a huge diversity in all analytical stages of anaerobic microbiology. Whether or not colony types were identified was mainly based on anatomical origin of the sample, colony morphology, and total number of different anaerobic isolates in the sample, while reporting of isolate results and performing anti-microbial susceptibility testing was mainly based on anatomical origin of the sample, number of different anaerobic isolates, and the identification of the anaerobic bacteria. These variety of workup procedures were mainly expert-based and have not been extensively clinically validated. For this reason, a standardized, clinically orientated, and feasible procedure for the workup of anaerobic cultures was developed, using MALDI-TOF MS identification, based upon literature data and existing guidelines.

1. Introduction

During the past decades, there has been a changing interest in anaerobic bacteria based upon the verification of their role as pathogens, efficacy of antibiotic treatment, and the elucidation of their virulence factors.

Extensive identification of anaerobic bacteria used to be time consuming or required expensive equipment such as high performance liquid chromatography, technical skills and experience (Jousimies-Somer et al., 2002). Since the introduction of matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS), reliable, fast, inexpensive and easy identification of anaerobic bacteria suddenly became feasible for routine microbiology laboratories (Barba et al., 2014; Biswas & Rolain, 2013; Croxatto et al., 2012; Hsu & Burnham, 2014; Patel, 2015). Through this, a theoretically unlimited amount of information on anaerobic microorganisms present in clinical samples can be gathered, posing new challenges for the microbiology laboratories. Questions about clinical relevance of different anaerobic bacteria arise. Consequently, the need to perform identification or anti-microbial susceptibility testing (AST) and to report anaerobic bacteria to the clinicians is questioned. Currently there are few guidelines suggesting feasible workup schemes for anaerobic cultures using MALDI-TOF MS identification. In 1992, 1995 and 2008, Goldstein et al. published surveys regarding basic anaerobic culture and susceptibility testing methods in hospitals from the United States (Goldstein et al., 1992, 1995, 2008). They concluded that many laboratories were performing anaerobic cultures (especially blood cultures) and AST (Goldstein et al., 1992, 2008). However, culture and workup procedures were not standardized and in dire need of improvement (Goldstein et al., 1992, 1995, 2008). In order to make anaerobic bacteriology more clinically relevant, Goldstein et al. recommend presumptive identification of important pathogens within 24 hours and AST results within 48 hours (Goldstein et al., 1992).

This article summarized current practices of routine microbiology laboratories in Belgium regarding identification, reporting and AST of anaerobic bacteria by means of a web-based survey. Survey results were compared with recommendations of guidelines and literature. Laboratories were not questioned about basic anaerobic incubation practices like the use of indicators in jars ensuring an anaerobic

Abbreviations: AST, Anti-microbial susceptibility testing; BSAC, British Society for Antimicrobial Chemotherapy; CASFM, Comité de l'Antibiogramme de la Société Française de Microbiologie; CLSI, Clinical and Laboratory Standards Institute; ESCMID, European Society of Clinical Microbiology and Infectious Diseases; EUCAST, European Committee on Antimicrobial Susceptibility Testing; ID, Identification; MALDI-TOF MS, Matrix-assisted laser desorption ionization time-of-flight mass spectrometry; MeSH, Medical subject heading; rRNA, Ribosomal ribonucleic acid.

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Table 1
Acceptance and rejection of different sample types.

N=34 (100%) Generally accepted samples	Anaerobic culture is performed				Refused
	Always	After contacting clinician, if not requested	If requested by clinician	If requested and justified by clinician	
Blood	27 (79)	0 (−)	7 (21)	0 (−)	0 (−)
Ascites fluid	25 (73)	1 (3)	8 (23)	0 (−)	0 (−)
Abdominal fluid	24 (71)	0 (−)	10 (29)	0 (−)	0 (−)
Pleural fluid	21 (62)	2 (6)	10 (29)	1 (3)	0 (−)
Joint fluid	20 (59)	1 (3)	12 (35)	0 (0)	1 (3)
CSF	16 (47)	2 (6)	8 (23)	5 (15)	3 (9)
Deep aspirate/biopsy	17 (50)	5 (15)	12 (35)	0 (−)	0 (−)
Deep wound swab	11 (32)	3 (9)	18 (53)	1 (3)	1 (3)
Sinus aspirate	17 (50)	1 (3)	13 (38)	2 (6)	1 (3)
Generally rejected samples					
Bronchial aspirate	1 (3)	0 (−)	2 (6)	6 (18)	25 (73)
Sputum	0 (−)	0 (−)	0 (−)	5 (15)	29 (85)
NP swab	0 (−)	0 (−)	3 (9)	3 (9)	28 (82)
Urine	0 (−)	0 (−)	0 (0)	7 (21)	27 (79)
Vaginal swab	0 (−)	2 (6)	2 (6)	5 (15)	25 (73)
Cervical swab	1 (3)	1 (3)	5 (15)	4 (12)	23 (68)
Catheter tip	2 (6)	1 (3)	2 (6)	4 (12)	25 (73)
Superficial wound swab	6 (18)	2 (6)	4 (12)	3 (9)	19 (56)

CSF = cerebrospinal fluid; NP = nasopharyngeal

environment, maximum time of oxygen exposure before incubation, re-opening of jars, and the use of pre-reduced agar plates or culture medium used for primary incubation. This could be the subject of an updated web-based survey.

2. Material and methods

A Web-based survey with 15 multiple-choice questions on the pre-analytical, analytical and post-analytical procedures in anaerobic bacteriology was sent by e-mail to 53 Belgian laboratories, in university and non-university hospitals (mean of 900 beds, range from 200 to 2000 beds). For the composition of the survey questions, CLSI guidelines (M56A; M11-A8; M100-S25), the Wadsworth-KTL Anaerobic Bacteriology Manual and the Manual of Clinical Microbiology were consulted (Clinical and Laboratory Standards Institute, 2012, 2014, 2015; Jousimies-Somer et al., 2002; Versalovic et al., 2011).

Relevant literature was identified using the MeSH Database on the PubMed website. The search terms used were: “Clinical anaerobic microbiology”, “Anaerobic bacteria and clinical relevance”, “Workup anaerobic microbiology”, “Anaerobic infections and clinical relevance”, “Anaerobic bacteria and susceptibility”, “Anaerobic infections and management”, “Anaerobic bacteria and virulence”, “Anaerobic bacteria and anti-microbial susceptibility testing”, “Anaerobic infections and outcome”. Additionally “PubMed Clinical Queries” were used (from 1966; http://www.ncbi. nlm.nih.gov/entrez/query.fcgi: Systematic Reviews; Clinical Queries using Research Methodology Filters). UpToDate Online version 23.3 (2015) was checked for these terms: “Anaerobic infections”, “Anaerobic microbiology”. Following reference works and handbooks were consulted: Clinical Microbiology Procedures Handbook – Section 4 Anaerobic Bacteriology (referred to as ‘Garcia’) (Garcia & Hall, 2010), Wadsworth-KTL Anaerobic Bacteriology Manual (referred to as ‘Wadsworth’) (Jousimies-Somer et al., 2002) and the Manual of Clinical Microbiology (Referred to as ‘Versalovic’) (Versalovic et al., 2011).

3. Results

Overall, 34 laboratories participated in this survey: five university and 29 non-university laboratories. The other 19 laboratories did not answer the invitation e-mail and did not participate. All responding laboratories had facilities (anaerobic jars, cabinets) to culture and isolate anaerobic bacteria. Results of the seven most relevant questions are discussed in detail. Results of the other questions are presented in the appendices section (Figs A.1–A.5 and Tables A.1–A.2).

3.1. Which samples are accepted/rejected for culture of anaerobic bacteria?

All participating laboratories performed anaerobic culture on certain sample types while others were refused (Table 1).

3.2. Does your laboratory use specific anaerobic collection or transport media?

Most participating laboratories (65%) did not use defined collection or transport media for anaerobes. A combination of Amies swab systems, syringes or anaerobic blood culture bottles was used in some laboratories (29%). Only a few laboratories used specific tubes, vials or jars containing a pre-reduced transport medium with reducing agents (6%). A swab in liquid Amies medium was the most used transport medium for anaerobic samples (21%).

3.3. What is the current rationale in your laboratory for identifying, reporting and AST of anaerobic bacteria?

The decision whether a colony on an anaerobic culture medium should be identified, reported or tested for susceptibility was mainly based on anatomical origin of the sample (normally sterile body sites) and the number of anaerobic isolates (up to 2 anaerobic isolates). Colony morphology played an important role in the decision whether anaerobic growth should be identified. The kind of isolated anaerobic bacteria (*Bacteroides fragilis* group, other Gram-negative anaerobic bacteria and histotoxic *Clostridium* spp.) was also decisive for reporting and performing AST (Tables 2–4).

3.4. Which identification method is used for anaerobic bacteria in your laboratory?

In this survey 68% of the laboratories used MALDI-TOF MS technology for routine identification of anaerobic isolates. Only 31% of the laboratories had access to 16S rRNA gene sequencing and none of them used this method for routine identification of anaerobes. Nearly all laboratories (91%) used Gram staining for presumptive identification of anaerobic bacteria and in a single laboratory (3%) this was the only identification method for anaerobes. Most laboratories used selective/differential agars (63%) or biochemical identification techniques (57%) but these methods were rarely used for routine identification (6% and 23%, respectively) (Fig. 1).