Official journal of the Spanish Society of Chemotherapy

Update in infection diseases 2020

Emilia Cercenado

What happened to microbiological diagnosis in 2020 beyond COVID-19?

Servicio de Microbiología y Enfermedades Infecciosas, Hospital General Universitario Gregorio Marañón, Departamento de Medicina. Facultad de Medicina, Universidad Complutense. Madrid

Revista Española de Quimioterapia
doi:10.37201/req/s01.01.2021

ABSTRACT

The global pandemic of COVID-19 has had negative repercussions on the activities and research in clinical microbiology laboratories other than those related to SARS-CoV-2. Nonetheless, the research activity has also continued in other fields. In this brief review, some of the recent publications related to new diagnostic tests, methods for rapid antimicrobial susceptibility testing and for the detection of resistance genes, new diagnostic technologies, and some aspects related to old and emergent pathogens (*Candida auris, Elizabethkingia* spp. *Streptococcus pyogenes*) are summarized.

Keywords: microbiological diagnosis, new diagnostic technologies, resistance to antimicrobials, *Candida auris*, *Streptococcus pyogenes*, Elizabethkingia spp.

INTRODUCTION

The global pandemic of COVID-19 has had negative repercussions on the entire global activities, and these include the activities and research in clinical microbiology laboratories other than those related to SARS-CoV-2 and COVID-19. However, the activity of clinical microbiologists has continued, among others, with the development of new diagnostic tests, new methods for rapid antimicrobial susceptibility testing and for the detection of resistance genes, new diagnostic technologies, and with the study of old and emergent pathogens. This minireview includes a brief summary of the publications presented as a lecture at the XI Updating Course of Antimicrobials and Infectious Diseases last February 2021 in Madrid (Spain).

Correspondence:
Emilia Cercenado
Servicio de Microbiología y Enfermedades Infecciosas
Hospital General Universitario Gregorio Marañón
Dr Esquerdo 46; 28007 Madrid, Spain
E-mail: emilia.cercenado@salud.madrid.org

DIAGNOSTIC MICROBIOLOGICAL TECHNIQUES

In recent years *Candida auris* has emerged as an opportunistic yeast of clinical importance because it causes infections in at risk populations including critically ill and immunosuppressed patients. In addition, it is resistant to many antifungal treatments and persists in hospital environments causing hospital outbreaks difficult to control and to eradicate. Early and accurate diagnosis of *C. auris* infections is crucial, however this organism is difficult to identify by commonly used identification systems, and misidentifications as other *Candida* species is frequent. Alvarado et al. [1] describe the development of conventional and real-time PCR methods for accurate and rapid identification of *C. auris* and its discrimination from closely related species by exploiting the uniqueness of certain glycosylphosphatidylinositol (GPI)-modified protein-encoding genes. They designed species-specific primers for two unique putative GPI protein-encoding genes per species, including *C. auris, C. haemulonii, C. pseudohaemulonii, C. duobushaemulonii, C. lusitaniae* and *C. albicans*. In addition, the efficiency of the *C. auris* primers was validated using a panel of 155 *C. auris* isolates, including all known genetically diverse clades. All primers combinations showed excellent species specificity and in real-time multiplex PCR, *C. auris* was easily differentiated from other related species. *C. auris* limit of detection was 5 CFU/reaction with a threshold value of 32, and the method was also able to detect *C. auris* in spiked blood and serum. The authors conclude that this PCR identification based on unique GPI protein-encoding genes allows for accurate and rapid species identification of *C. auris* and related species.

Since the availability of molecular methods is limited in many routine diagnostic laboratories, Das et al. [2] developed a selective medium in order to significantly reduce the time and cost associated with the identification of *C. auris* even in low-resource health care settings. By using 18 *C. auris* isolates and 30 non-*C. auris* yeasts they standardized a selective medium with yeast extract-peptone-dextrose (YPD) agar, including

various combinations of sodium chloride and ferrous sulphate, followed by incubation at different temperatures and times. For validation, they used 579 additional yeast isolates and 40 signal-positive blood culture broths. The named Selective Auris Medium (SAM) comprising YPD agar with 12.5% NaCl and 9 mM ferrous sulphate incubated at 42°C for 48 h, allowed selective growth of *C. auris*. A total of 95% (127/133) of *C. auris* isolates tested grew on this media within 48 h, and the remaining 6 isolates grew after 72 h, whereas the growth of 446 non-*C. auris* yeast isolates was completely inhibited. The specificity, sensitivity, positive and negative predictive values of the test medium were 100% after 72 h of incubation. The authors indicate that this medium is inexpensive, can easily be prepared, and can be used as an alternative to molecular methods.

The microbiological diagnosis of infections caused by difficult to grow bacterial organisms is challenging since they require enriched media and long incubation times. Moreover, in many of these cases prompt and accurate diagnosis is important for treatment and control of disease transmission. *Bordetella pertussis* and *Helicobacter pylori* are among these difficult to grow organisms that require rapid alternatives, like PCR or other molecular methods, for their detection and identification. The article by Chow et al. [3] describes the evaluation of a commercialized PCR-based kit for the detection of *Bordetella pertussis* and *Bordetella parapertussis* in nasopharyngeal swab specimens. In this multicenter study, a total of 1,103 fresh and residual frozen specimens from eight clinical sites were tested. Combining the data from individual clinical sites using different comparative assays, the overall positive percent agreement (PPA) and negative percent agreement (NPA) for *B. pertussis* were 98.7% and 97.3%, respectively. The overall PPA and NPA for *B. parapertussis* were 96.7% and 100%, respectively. For prospective fresh specimens, the overall PPA and NPA for both targets were 97.7% and 99.3%, respectively. For retrospective frozen specimens, the overall PPA and NPA for both targets were 92.6% and 93.2%, respectively. The kit was 100% specific, and the limits of detection were 150 CFU/ml or 3 fg/µl of DNA for *B. pertussis* and 1,500 CFU/ml or 10 fg/µl of DNA for *B. parapertussis*. The hands-on time of the kit for one sample was 2 min and the total assay run time per 8 samples was 78 min. In conclusion, this study shows that this kit (Simplexa Bordetella Direct kit) was non-inferior to the molecular assays currently available on the market or developed in-house for the detection and differentiation of *B. pertussis* and *B. parapertussis*, being a rapid and accurate approach for better diagnosis of pertussis.

Concerning *H. pylori,* its non-invasive detection and its resistance to clarithromycin is essential for the rapid management of *H. pylori* infection. Pichon et al. [4] conducted a prospective, multicenter study to evaluate the performance of a commercialized real-time PCR based assay (Amplidiag *H. pylori*+ClariR) on DNA from stools from 1,200 adult patients who were addressed for gastroduodenal endoscopy with gastric biopsies and who were naive for eradication treatment. The results were compared with those of culture/Etest and quadruplex real-time PCRs performed on two gastric biopsy samples (from the antrum and corpus) to detect the *H. pylori glmM* gene and mutations in the 23S rRNA genes conferring clarithromycin resistance. In this cohort, 160 patients (14.7%) were found to be infected (positive by culture and/or PCR). The sensitivity and specificity of the detection of *H. pylori* were 96.3% and 98.7%, respectively. The positive and negative predictive values were 92.2% and 99.3%, respectively. The sensitivity and specificity for detecting resistance to clarithromycin were 100% and 98.4%, respectively. The feasibility of the assay was very good and minimally time-consuming (5 min for extraction and 10 min for amplification, with a complete turnaround time of 3 h 45 min), and the total price per patient was less than €30. The very good performances of this non-invasive test for the detection of *H. pylori* and clarithromycin resistance in stools makes it highly recommended for use in all cases where histological study of the gastric mucosa is not necessary. In addition, patients had very good compliance with auto-sampling.

The microbiological diagnosis of fungal meningitis is difficult since testing accuracy varies with each etiological agent, and delay in diagnosis and treatment leads to poor outcomes. Since (1,3)-beta-D-glucan (BDG) measurement in cerebrospinal fluid (CSF) is not specific for any particular cause of fungal meningitis, the study by Davis et al. [5] starts with the hypothesis that this measurement could have some utility as a marker of fungal disease, particularly in cases of subacute meningitis without clear etiologies. In this line, they performed a systematic review in order to characterize the evidence regarding CSF (1,3)-beta-D-glucan measurement to detect fungal meningitis. Fourteen studies were included and a variety of fungi, including species of *Candida*, *Aspergillus, Exserohilum, Cryptococcus, Histoplasma*, and *Coccidioides*, were studied, although most were case reports. Diagnostic accuracy was examined in 5 studies. The analysis revealed that CSF BDG measurement showed >95% sensitivity in one corticosteroid injection-related outbreak of *Exserohilum rostratum*, one study in *Histoplasma* spp. meningitis found 53% (53/87) sensitivity and 87% (133/153) specificity, while another study of *Cryptococcus* spp. meningitis found 89% (69/78) sensitivity and 85% (33/39) specificity. They conclude that CSF BDG testing may be useful, primarily as a non-specific marker of fungal meningitis, and should be used in conjunction eith organism-specific testing. Although the FDA black box warning states that *Cryptococcus* spp. do not make BDG, this review shows that BDG is detectable in cryptococcal meningitis.

Diagnosis of *Mycoplasma pneumoniae* infection in patients with community-acquired pneumonia (CAP) is challenging. Current diagnostic tests include *M. pneumoniae* specific IgM serology and PCR of respiratory specimens, but these tests are also positive in asymptomatic carriers of *M. pneumoniae* in the upper respiratory tract. In addition, IgM serology may lead to false-negative results early in disease course and after reinfection. The measurement of *M. pneumoniae*-specific IgM antibody secreting cells (ASC) by enzyme-linked immunospot (ELISpot) assay differentiates between *M. pneumoniae* infection and carriage, but this assay cannot be performed at bedside

and no point-of-care (POC) test is available for direct *M. pneumoniae* detection. Meyer-Sauteur et al. [6] assessed a new immunochromatographic POC IgM lateral flow assay (LFA) as an on-site screening tool for the detection of *M. pneumoniae* CAP in children. A set of 239 pediatric serum samples from 94 CAP patients and 145 healthy controls was used from a previous study. CAP patient samples were collected median 7.0 days after symptom onset. The IgM-LFA was performed, and results were visually read after 10 min. The results were identical for both fingertip blood and serum samples. Compared to IgM-ELISA, IgM-LFA-negative results were true negative in 97.8% (n=178/182), and IgM-LFA-positive results were true positive in 87.7% (n=50/57). The IgM-LFA also was positive for all individuals who tested positive with IgM-ELISA+PCR (n=41) and IgM-ASC-ELISpot (n=29). In summary, IgM-LFA results are predictive for *M. pneumoniae* infection, despite the possibility of false-positive results, however, this test cannot currently replace other diagnostic tests and results need to be confirmed with *M. pneumoniae*-specific PCR, IgM-ELISA, and/or IgM-ASC-ELISpot assay.

The microbiological diagnosis of *Pneumocystis jirovecii* pneumonia (PCP) is difficult since the *P. jirovecii* load in the lungs is, in general, low in non-HIV-positive patients. Currently, the laboratory gold standard for the detection of *P. jirovecii* is a real-time quantitative PCR (qPCR) assay, however, this is laborious, require skilled personnel, and execution outside regular working hours of the molecular biology laboratory is limited. The commercialized eazyplex *P. jirovecii* assay (PJA) uses loop-mediated isothermal amplification (LAMP) for detection of *P. jirovecii*. This assay is performed directly with respiratory specimens, without the need for special skills, and delivers a result within 3 to 25 min, requiring a hands-on time of 2 min 45 s. The study of Scharmann et al. [7] compared the performance of this assay with that of *P. jirovecii* qPCR assays. Forty-nine patients with proven PCP and 126 patients without PCP were included. The sensitivity and specificity of the assay (95.7% and 96.5%, respectively) were comparable to those for three different *P. jirovecii* qPCR assays. The detection limit was 10 to 20 *P. jirovecii* cells and the eazyplex PJA reliably discriminated patients with PCP from patients with *P. jirovecii* colonization. This study demonstrates identical performance of the LAMP assay for the diagnosis of PCP, compared to qPCR assays, with the advantages of its practicability, allowing for around-the-clock molecular testing.

Catheter-related bloodstream infections (CRBSI) account for 15%-30% of all nosocomial bloodstream infections (BSI) and several microbiological diagnostic procedures not requiring catheter removal have been devised. Among them, the differential time to positivity (DTP) of ≥2 hours between peripheral-blood and catheter-blood cultures has been described as an excellent test with high sensitivity and specificity values and has been included in different guidelines as a criterium for the definitive diagnosis of CRBSI. The study by Orihuela-Martín et al. [8] assessed the performance of this method over a period of 15 years in their institution. A total of 512 BSI were included, of which 302 (59%) were CRBSI. Discrimination ability of DTP was low for *Staphylococcus aureus* (AUC 0.656 ± 0.06), coagulase-negative staphylococci (AUC 0.618 ± 0.081), enterococci (AUC 0.554 ± 0.117) and non-AmpC-producing *Enterobacteriaceae* (AUC 0.653 ± 0.053); moderate for *Pseudomonas aeruginosa* (AUC 0.841 ± 0.073), and high for AmpC-producing *Enterobacteriaceae* (AUC 0.944 ± 0.039). For the entire sample, DTP had a low-to-moderate discrimination ability (AUC 0.698 ± 0.024). A DTP ≥2 h had a low sensitivity for coagulase-negative staphylococci (60%) and very low for *S. aureus* (34%), enterococci (40%) and non-AmpC-producing *Enterobacteriaceae* (42%). A DTP cut-off of 1 h improved sensitivity (90%) for AmpC-producing *Enterobacteriaceae*. This long experience indicates that DTP used on a routine basis has adequate discrimination ability and performance characteristics for CRBSI diagnosis only when AmpC-producing *Enterobacteriaceae* and *P. aeruginosa* are involved. For other organisms, a negative test should not be used to rule out CRBSI.

DETECTION OF RESISTANCE TO ANTIMICROBIALS

Extended-spectrum beta-lactamase (ESBL)-producing Enterobacterales constitute a global burden for hospital infection, and the identification of carriers by screening patients at risk is recommended by several European guidelines. Blanc et al. [9] evaluated the impact of rapid ESBL tests on the turnaround time (TAT) of screening. Rectal swabs were analysed by culture and synergism tests for identification of non-*Escherichia coli* Enterobacterales that produce ESBLs (NEcESBL). The Rapid ESBL NP (colorimetric test) and NG CTX-M MULTI test (immunoassay) were performed on colonies grown on chromogenic media. PCR and sequencing of ESBL genes were used as the gold standard. Among 473 rectal swabs, 75 (15.9%) grew NEcESBL. ESBL screening using the synergism, Rapid ESBL NP and NG CTX-M MULTI showed sensitivities of 0.96, 0.81, and 0.91; specificities of 1.00, 0.99, and 1.00; positive predictive values of 0.96, 0.94, and 1.00; and negative predictive values of 1.00, 0.98, and 0.99, respectively. When no-NEcESBL were observed, the mean TAT was 30 h. When NEcESBL were detected, the mean TATs were 74.7, 38.0 and 36.7 h for the synergism, Rapid ESBL NP and NG CTX-M MULTI tests, respectively. This study shows that the two rapid ESBL tests evaluated (colorimetric and immunoassay) showed good performances and significantly reduced the TAT of the screening protocol to identify ESBL-producing Enterobacterales. This study underlines the importance and impact of rapid tests to identify emerging antibiotic resistant pathogens.

Carbapenemase-producing Gram-negative bacteria are a public health concern, and methods for rapid detection and characterization of the different carbapenemase subtypes are necessary. Among carbapenem-resistant *Pseudomonas aeruginosa*, phenotypic detection of carbapenemases SPM, IMP and GES is challenging and genotypic tests are not commercially available. In the study of Gill et al. [10] the authors evaluate the performance of the commercially

available Xpert Carba-R (Carba-R) and the research-use-only Xpert Carba-R NxG (Carba-R NxG) in a global collection of 123 *P. aeruginosa* from 12 countries previously categorized via PCR or whole-genome sequencing. Carbapenemase classes tested included VIM, IMP, NDM, SPM, KPC, and GES. Non-carbapenemase (non-CP)-harboring isolates were also tested (negative control). Both assays gave negative results for all non-CP isolates and positive results for all VIM, NDM, and KPC isolates. An improvement in IMP detection among isolates was observed (100% detection by Carba-R NxG versus 58% by Carba-R). All SPM and GES isolates, targets not present in commercially available Carba-R, were positive by Carba-R NxG. Two isolates harbored both VIM and GES, while a third isolate contained VIM and NDM. The Carba-R NxG identified both targets in all 3 isolates, while the Carba-R was negative for both GES-containing isolates. Overall, the Carba-R NxG successfully categorized 100% of isolates tested compared with 68% for its predecessor. The authors conclude that this new Carba-R NxG test expands the detection spectrum of the current Carba-R assay to include SPM, GES, and expanded IMP variants, and increases the global utility of the test. Continuous expansion of genotypic detection assays is very important due to the growing diversity of enzyme subtypes.

Over the last years, identification of staphylococci other than *Staphylococcus aureus* (SOSA) has become more frequent, due to a better understanding of their role as opportunistic pathogens and to the availability of MALDI-TOF MS in clinical laboratories. However, testing of SOSA for *mecA*-mediated resistance is challenging. In the study of Humphries et al. [11] isolates of *Staphylococcus capitis* (n=50), *Staphylococcus haemolyticus* (n=50), *Staphylococcus hominis* (n=50), and *Staphylococcus warneri* (n=48), were evaluated by cefoxitin and oxacillin broth microdilution (BMD), disk diffusion (DD), and PBP2a immunoassay, and the results were compared to *mecA* PCR results. No phenotypic susceptibility test correlated well with PCR results across all species, although the PBP2a immunoassay yielded 100% correlation. Oxacillin BMD testing by current Clinical and Laboratory Standards Institute (CLSI) SOSA breakpoints led to 2.1% very major errors (VMEs) and 7.1% major errors (ME). Oxacillin DD yielded high ME rates (20.7 to 21.7%) using CLSI or European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints with VMEs ranging from 0 to 5.3%. Cefoxitin BMD led to 4.9% VMEs and 1.6% MEs, and cefoxitin DD led to 1.0% VMEs and 2.9% MEs. The results of this study indicate that laboratories should be aware that no individual phenotypic test correlates well across all species of SOSA with *mecA* PCR results. Molecular testing for *mecA* or evaluation for PBP2a is the preferred approach for the adequate detection of methicillin-resistance among these staphylococcal species.

The need for rapid antimicrobial susceptibility testing (RAST) in bloodstream infections is important for adjustment of therapy and many attempts have been made to shorten the time required for reporting antimicrobial susceptibility testing results. Recently, the EUCAST developed a disc diffusion RAST method directly from positive blood cultures delivering reliable AST results within 4-8 h of positivity of blood culture bottles. Akerlund et al. [12] validate this method in 55 European laboratories including clinical blood cultures positive for *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Staphylococcus aureus* or *Streptococcus pneumoniae*. Categorical results at 4, 6 and 8 h of incubation were compared with results for EUCAST standard 16-20 h disc diffusion. After analysing 1151 isolates, the number of zone diameters that could be read (88%, 96% and 99%) and interpreted (70%, 81% and 85%) increased with incubation time (4, 6 and 8 h). The categorical agreement was acceptable, with total error rates of 3% at 4 h, 2.1% at 6 h and 2.2% at 8 h. The percentages of false susceptibility ranged from <0.3% to 1.1% and the corresponding percentages for false resistance ranged from <1.9% to 2.8%.

These results support that the EUCAST RAST method can be implemented in routine laboratories providing reliable AST results. This method is easy to use, cheap, flexible, and can be adapted to new antimicrobials without major investments.

NEW DIAGNOSTIC TECHNOLOGIES

The development and use of rapid tests for bacterial detection and AST is one of the priorities for the adequate management of patients and the appropriate use of antimicrobials. Recent phenotypic assays (imaging, microfluidic culture) and molecular methods (PCR, nanoparticle-based assays, microfluidic-based capture and enrichment, electrochemical sensors, CRISPR, sequencing, etc) can reduce assay time to hours but are often not sensitive enough to detect bacteria at low concentrations (<1 to 100 CFU/ml) and require expensive equipment and lengthy, complex sample processing. Abram et al. [13] developed a rapid bacterial detection and AST method in whole blood using one-step, high throughput blood digital PCR. This technology prototype provides a high sensitivity (10 CFU/ml) and a rapid assay time (one hour) and is applicable for the detection of a wide range of antimicrobial resistance genes without requiring blood culture or sample processing. This new diagnostic technology holds great potential for the rapid diagnosis of BSI directly in blood samples.

The CRISPR-Cas9 (clustered regularly interspaced short palindromic repeats-CRISPR-associated protein-9 nuclease) system, has been engineered to create site-specific double-strand breaks for genome editing and provides a new tool and approach to eradicate carbapenem-resistant (CR) genes and plasmids. Hao et al. [14] conducted a proof-of-concept study (pCasCure) to demonstrate that CRISPR-Cas9-mediated resistance gene and plasmid curing can effectively resensitize CR *Enterobacteriaceae* to carbapenems. The results showed that pCasCure effectively cured bla_{KPC}, bla_{NDM}, and bla_{OXA-48} in various clinical isolates of *Enterobacteriaceae* species with a >94% curing efficiency. In addition, the pCasCure efficiently eliminated, with a few exceptions, several epidemic CR plasmids, successfully restoring the susceptibility to carbapenems, with a >8-fold reduction of MIC values in all tested isolates. In the next future, the integration of pCasCure

in an optimal deliver system will make it applicable for clinical intervention and may serve as a potential tool to control the dissemination of carbapenem resistance in clinical pathogens.

OLD AND EMERGENT BACTERIAL PATHOGENS

Emerging Gram-negative bacterial pathogens have gained global attention in recent years as a cause of nosocomial infections. Among them, the intrinsically multidrug resistant *Elizabethkingia* genus is one example of a worldwide pathogen, primarily infecting immunocompromised individuals and associated with high mortality (20%-40%). Burnard et al. [15] describe a series of 22 clinical and 6 hospital environmental *Elizabethkingia* spp. isolates obtained in a hospital in Australia over a 16-year period. They performed whole-genome sequencing and identified 22 *E. anophelis*, 3 *E. miricola*, 2 *E. meningoseptica*, and 1 *E. bruuniana* isolates, most of which branched as unique lineages. Global analysis revealed that some Australian *E. anophelis* isolates were genetically closely related to strains from the United States, England, and Asia. They also demonstrated evidence of nosocomial transmission in patients. Furthermore, AST against 39 antimicrobials revealed almost ubiquitous resistance to aminoglycosides, carbapenems, cephalosporins, and penicillins, and susceptibility to minocycline and levofloxacin, and less commonly to trimethoprim-sulfamethoxazole. This study demonstrates important new insights into the antimicrobial resistance, genetic diversity, environmental persistence, and transmission of this emerging pathogen.

For many years, we have been taught that *Streptococcus pyogenes* was universally susceptible to beta-lactams. However, in 2019 two related *S. pyogenes* strains with reduced susceptibility to ampicillin, amoxicillin, and cefotaxime were reported. The two strains had the same mutation in the *pbp2x* gene, encoding penicillin binding protein 2X. Musser et al. [16] investigated a library of 7,025 genome sequences of *S. pyogenes* clinical strains recovered from intercontinental sources for mutations in *pbp2x*, and they identified 137 strains that had mutations in this gene, observing that these strains had decreased susceptibility *in vitro* to multiple beta-lactam antibiotics.

Phylogenetic analysis showed that, with one exception, strains of the same *emm* type with the same amino acid replacement were clonally related. These results indicate that clinical isolates of *S. pyogenes* with *pbp2x* mutations associated with small decreases in beta-lactam susceptibility are more widespread worldwide than appreciated. Probably, clinical microbiology laboratories not routinely performing beta-lactam susceptibility testing of *S. pyogenes*, reasonably must consider to do it.

CONFLICT OF INTEREST

The author declares that there is no conflicts of interest

REFERENCES

1. Alvarado M, Álvarez JB, Lockhart SR, Valentín E, Ruiz-Gaitán AC, Eraso E, de Groot PWJ. Identification of *Candida auris* and related species by multiplex PCR based on unique GPI protein-encoding genes. Mycoses. 2021; 64:194-202. doi: 10.1111/myc.13204
2. Das S, Singh S, Tawde Y, Chakrabarti A, Rudramurthy SM, Kaur H, et al. A selective medium for isolation and detection of *Candida auris*, an emerging pathogen. J Clin Microbiol. 2021; 59:e00326-20. doi: 10.1128/JCM.00326-
3. Chow SK, Arbefeville S, Boyanton Jr, BL, Dault EM, Dunn J, Ferrieri P., et al. Multicenter performance evaluation of the Simplexa Bordetella Direct kit in nasopharyngeal swab specimens. J Clin Microbiol. 2021; 59:e01041-20. doi: 10.1128/JCM.01041-20.
4. Pichon M, Pichard B, Barrioz T, Plouzeau C, Croquet V, Fotsing G, et al. Diagnostic acuracy of a noninvasive test for detection of *Helicobacter pylori* and resistance to clarithromycin in stool by the Amplidiag *H. pylori*+ClariR real-time PCR assay. J Clin Microbiol. 2020; 58:e01787-19. doi: 10.1128/JCM.01787-19.
5. Davis C, Wheat LJ, Myint T, Boulware DR, Bahre NC. Efficacy of cerebrospinal fluid beta-D-glucan diagnostic testing for fungal meningitis: a systematic review. J Clin Microbiol 2020; 58:e02094-19. doi: 10.1128/JCM.02094-19.
6. Meyer Sauteur PM, Pánisová E, Bachmann LM, Ambroggio L, Bergera C. Evaluation of IgM lateral flow assay as a screening tool for *Mycoplasma pneumoniae* infection in childhood pneumonia. J Clin Microbiol. 2020; 58:e01498-20. doi: 10.1128/JCM.01498-20.
7. Scharmann U, Kirchhoff L, Schmidt D, Buer J, Steinmann J, Rath PM. Evaluation of a commercial loop-mediated isothermal amplification (LAMP) assay for rapid detection of *Pneumocystis jirovecii*. Mycoses 2020; 63:1107-1114. doi: 10.1111/myc.13152.
8. Orihuela-Martín O, Rodríguez-Núñez O, Morata L, Cardozo C, Puerta-Alcalde P, Hernández-Meneses M, et al. Performance of differential time to positivity as a routine diagnostic test for catheter-related bloodstream infections: a single-centre experience. Clin Microbiol Infect. 2020; 26:383.e1e383.e7. doi: 10.1016/j.cmi.2019.07.001.
9. Blanc DS, Poncet F, Grandbastien B, Greub G, Senn L, Nordmann P. Evaluation of the performance of rapid tests for screening carriers of acquired ESBL-producing *Enterobacterales* and their impact on turnaround time. J Hosp Infect. 2021;108:19-24. doi: 10.1016/j.jhin.2020.10.013
10. Gill CM, Asempa TE, Tickler IA, de la Cruz C, Tenover FC, Nicolau DP. Evaluation of the Xpert Carba-R NxG assay for detection of carbapenemase genes in a global challenge set of *Pseudomonas aeruginosa* isolates. J Clin Microbiol. 2020; 58:e01098-20. doi: 10.1128/JCM.01098-20.
11. Humphries RM, Magnano P, Burnham CAD, Bard JD, Dingle TC, Callan K, et al. Evaluation of surrogate tests for the presence of mecA-mediated methicillin resistance in *Staphylococcus capitis, Staphylococcus haemolyticus, Staphylococcus hominis*, and *Staphylococcus warneri*. J Clin Microbiol. 2021; 59:e02290-200. doi: 10.1128/JCM.02290-20.

12. Akerlund A, Jonasson E, Matuschek E, Serrander L, Sundqvist M, Kahlmeter G, on behalf of the RAST Study Group. EUCAST rapid antimicrobial susceptibility testing (RAST) in blood cultures: validation in 55 European laboratories. J Antimicrob Chemother. 2020; 75:3230-3238.doi: 10.1093/jac/dkaa333.

13. Abram TJ, Cherukury H, Ou CY, Vu T, Toledano M, Li Y, et al. Rapid bacterial detection and antibiotic susceptibility testing in whole blood using one-step, high throughput blood digital PCR. Lab Chip. 2020; 20:477. doi: 10.1039/c9lc01212e.

14. Hao M, He Y, Zhang H, Liao XP, Liu YH, Sun J. CRISPR-Cas9-mediated carbapenemase gene and plasmid curing in carbapenem-resistant *Enterobacteriaceae*. Antimicrob Agents Chemother. 2020; 64:e00843-20. doi: 10.1128/AAC.00843-20

15. Burnard D, Gore L, Henderson A, Ranasinghe A, Bergh H, Cottrell K, et al. Comparative genomics and antimicrobial resistance profiling of *Elizabethkingia* isolates reveal nosocomial transmission and in vitro suusceptibility to fluoroquinolones, tetracyclines, and trimethoprim-sulfamethoxazole. J Clin Microbiol. 2020; 58:e00730-20. doi: 10.1128/JCM.00730-20.

16. Musser JM, Beres SB, Zhu L, Olsen RJ, Vuopio J, Hyyryläinen HL, et al. Reduced *in vitro* susceptibility of *Streptococcus pyogenes* to beta-lactam antibiotics associated with mutations in the *pbp2x* gene is geographically widespread. J Clin Microbiol. 2020; 58:e01993-19. doi: 10.1128/JCM.01993-19.