Peer Review File

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Reviewer A

Comment 1: It could be useful for the readers to know about strengths and limits of rapid molecular test in real practice.
Reply 1: Thank you for this suggestion. The strengths and limitations discussion has been expanded
Changes in the text: line 179-200

Comment 2: I would ask to the authors to deepen the discussion reporting also what has been previously showed by other authors and in the conclusion which are the future perspectives on this topic.
Reply 2: Additional discussion has been added, including reference to previous papers and addition to future directions for research. To our knowledge, no other centres have described the clinical use of diagnostic arrays in PICU as we have here. There have been some retrospective investigations that have looked at (1) culture yield and antibiotic use pre and post introduction of a diagnostic array and (2) how a diagnostic array performs on batched samples stored in a laboratory. We believe the current case report is helpful as it will improve understanding of how molecular diagnostic arrays work, and are interpreted in real-life practice.

Changes in the text: line 65-66, 213-215

Reviewer B

Comment 1: The role of molecular testing would be better appreciated with additional discussion of its strengths and limitations, which are only hinted at.
Reply 1: Thank you for this suggestion, which has also been highlighted by the other reviewers. The strengths and limitations have now been expanded on as per recommendation.
Changes in the text: line 179-207

Comment 2: For clarity, it should be acknowledged in the main text that this was a custom array, and some discussion of array design/coverage would be useful for the reader who is considering this type of diagnostic testing at their own institution.
Reply 2: Reworded for clarity as per suggestion. Additional detail added and publication cross-referenced regarding array development for readers.
Changes in text: line 74 - 75

Comment 3: In the discussion (line 168), the authors reference potential limitations of molecular syndromic testing, however, these limitations are not discussed in the

manuscript.
Reply 3: The limitations section has been expanded as per recommendation.
Changes in text: line 179-207

Comment 4: In the conclusion (line 183), the authors note "the characteristics of these highly sensitive and pathogen-specific tests must be considered when the results are interpreted." However, there is limited discussion about the characteristics of these tests, or how such characteristics would influence interpretation of results, in the preceding manuscript. Since this was a custom array, it should be acknowledged that role and usefulness of this testing is likely dependent on the array design.
Reply 4: Additional context has been provided both in the introduction and discussion.
Changes in text: line 74 - 75, 179-207

Comment 5: Key findings point – The second sentence seems to suggest that antimicrobial therapy could be stopped completely based on a negative test result. It would be more in line with the manuscript to state that antibiotic coverage could be narrowed.
Reply 5: It is possible to both cease and change antimicrobial therapy based on TAC results. We agree that the overall emphasis of the manuscript is on changing therapy hence has been reworded as per reviewer suggestion.
Changes in text: line 51 (box)

Comment 6: Line 55-56 – though it can be difficult to identify causative pathogens, your previous reference (#2) reported an 81% pathogen detection rate in children with radiographic pneumonia and specimens for testing. For transparency, it would be best to acknowledge this discrepancy, or to be clear about why you are choosing to highlight a detection rate of 16%.
Reply 6: The pathogen detection rate depends heavily on the location from which a sample is obtained and the population in whom the sample is obtained from. In addition, detection of some microorganisms such as rhinovirus may be considered a causative pathogen in some situations and a bystander in others. In addition, how pneumonia is defined will in turn impact the pathogen detection rate. The British audit data (ref #3) was used as the best available local data for comparison. We have attempted to provide additional context in the introduction to address this issue.
Changes in text: line 58-63

Comment 7: Line 65 – it would be helpful to clarify that this was a custom array, and to briefly describe how the array was designed, i.e., how pathogens were selected for testing. The abstract states that this was a custom array, but it is not clear in the manuscript itself.
Reply 7: Additional information has been added regarding the design of the array, with reference provided for readers to manuscript outlining the validation of the array.
Changes in text: line 72-75

Comment 8: Line 68 – why was a Ct result of ≤ 32 chosen?
Reply 8: This threshold was found to correlate with clinically significant growth based on our

national (UKHSA) recommendations. $\geq 10^4$ cfu/mL from Mini-BAL samples, and $\geq 10^5$ cfu/mL from ETT aspirate. More detail is available in the validation paper now referenced as per reviewer recommendation.
Changes in text: line 77-78

Comment 9: Case 1 would benefit from further description of his respiratory course. Was he intubated prior to the increasing oxygen requirement, or after?
Reply 9: Intubation occurred in the local hospital to facilitate emergency laparotomy and the patient remained mechanically ventilated. Deterioration in respiratory status occurred from day 5 which triggered investigations for VAP. Rewritten for clarity.
Changes in text: 84-96

Comment 10: Was the addition of amphotericin-B influenced by the previous TAC result, or was this recommended as empiric coverage? Was the antimicrobial treatment narrowed after culture results were available? Did the TAC result play a role in any antimicrobial changes?
Reply 10: Amphotericin-B was recommended as a 'rescue' plan by the microbiology team based on the TAC result. That is, the intensive care team were advised to commence this antimicrobial treatment if the patient were to deteriorate. No antibiotic/antifungal changes were made once treatment for VAP commenced. The TAC findings were important as it would have been unlikely the team would have commenced antifungal therapy until day 8 when *C albicans* was identified on blood culture.
Changes in text: line 100-107

Comment 11: Line 97 – would use clearer language to describe her respiratory support (FiO2)
Reply 11: Reworded as per recommendation
Changes in text: line 93

Comment 12: In Case 2, why was MIS-C the leading diagnosis? This is not clear, particularly given the negative SARS-CoV-2 testing. Was there a recent history of SARS-CoV-2 infection?
Reply 12: Additional information has been provided. Given MIS-C is a latent response to SARS-CoV-2, a patient with the condition would not be expected to have a positive antigen/PCR test at the time – however often serology demonstrating past SARS-CoV-2 infection will be positive. The SARS-CoV-2 serology would certainly have been interesting information in this case, however this test was not available at the time given available resources. There was no history of recent SARS-CoV-2 infection.
Changes in text: line 114-117

Comment 13: Line 106 – would clarify whether the mini-BAL was performed on admission to the PICU or admission to the hospital
Reply 13: Added for clarity – on admission to PICU
Changes in text: line 131

Comment 14: Line 111 – What sample was the C. psittaci/abortus PCR performed on?
Reply 14: This line has been corrected and should have stated serology rather than PCR, this has now been corrected.
Changes in text: line 119

Comment 15: Case 3 would benefit from further context, e.g., description of any lung exam findings or imaging, to help the reader understand why mini-BAL was performed.
Reply 15: Additional context provided
Changes in text: line 131-132, 135-138

Comment 16: Line 132 – The discussion states that these cases show that TAC can diagnose infections "that would not be identified using routine investigations." However, this does not appear true for case 1, in which TAC identified an organism that was also identified by culture.
Reply 16: Reworded to improve transparency
Changes in text: 145-147

Comment 17: Line 162-163 states, "…TAC allowed antimicrobial cover for presumed CNS infection to be ceased early." Are the authors referring specifically to empiric coverage or CNS infection by organisms other than H. influenzae? This is not clear.
Reply 17: Reworded for clarity
Changes in text: line 172-175

Comment 18: Typo on line 86 – "not necessarily contributing to current state" should likely end in a period.
Reply 18: Corrected as suggested
Changes in text: line 99

Comment 19: Use of "however" at the beginning of a clause requires a comma.
Reply 19: Corrected as suggested
Changes in text: Each use.

Comment 20: The figure very nicely highlights the takeaway point from each case. Per author guidelines, the figure legend should include definitions of all abbreviations used in the figure.
Reply 20: Thank you for this suggestion, abbreviations now added
Changes in text: 330-337

Reviewer C

Comment 1: One of the major limitations of this work is the notable age difference of the 3 patients. How the authors consider two almost adult patients (17 and 16 years old) like

children and consider them in the same group of patients in which there is a 2 week old newborn? it would be more interesting to report case reports of truly pediatric patients. Newborns are very different clinically from adult patients.

Reply 1: We agree there is a wide age range of patients selected for this case report which focuses on the identification of a pathogen-specific diagnosis using a new approach in the PICU. In the UK, where this research was completed these are all considered paediatric patients – that is, patients were aged up to 18 years. These cases were selected to demonstrate the range of ways in which clinical decision making may be impacted by the availability of a rapid diagnostic array. Firstly, identification a fungal VAP infection, secondly a rare, atypical respiratory diagnosis and thirdly identification a more common bacterial pathogen. We feel that this approach allows readers to better understand the scope of the test. Given this is a case study, the manuscript is descriptive hence age distribution is not problematic as it would be for a quantitative study involving statistical analysis.

Comment 2: The paper seems rather descriptive: the discussion should be rewritten in a more systematic way, rather than a discussion it seems more like a list of results. The authors should better explain the indications for use and the real limitations of the TaqMan array card technique in children in the PICU.

Reply 2: Thank you for the recommendation. As per this suggestion the discussion has been substantially expanded to discuss the use, benefits and disadvantages of TAC.

Changes in text: line 179-207

Comment 3: The conclusions are very unclear and should be rewritten in a more scientific way: which is the real role of the TAC technique? (for example, the statement "Rapid syndromic diagnostic tests offer a potential solution to this problem": in which way?).

Reply 3: The conclusion has been rewritten to be more specific regarding TAC and future directions as per recommendation.

Changes in text: line 210-215