Novel diagnostics of respiratory infection in the intensive care unit

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Abstract: Pneumonia is one the leading causes of a critical care admission and is associated with substantial mortality and morbidity. Despite this, the diagnosis of pneumonia and identifying the causative pathogen is not always straight forward, particularly in the immunocompromised or those already receiving antibiotics. Medical belief today encourages the use of broad spectrum antimicrobials in all patients that may have pneumonia. This approach invariably leads to overtreatment of many patients, with consequent side effects. These side effects may lead to an insidious impact on organ function or overgrowth of harmful organisms (e.g., *Clostridium difficile*) which in turn can adversely affect outcome, reduce institutional efficiency and increase healthcare costs. At a society level, it is this over-use of broad spectrum antimicrobials that is helping drive the spread of anti-microbial resistance. Clinicians, urgently need reliable diagnostic aids to inform them that their patient does or does not have an infection and to reliably and rapidly identify the pathogen with any associated resistance genes. Only then can we confidently withhold or prescribe the appropriate antimicrobial.

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The burden of respiratory infection in the intensive care unit (ICU)

Pneumonia is the leading cause of infection related death in the developed world and one of the commonest reasons for admission to a critical care unit (CCU). Patient management revolves around identifying and eradicating the invading pathogen with antimicrobials whilst offering supportive therapies such as oxygen, fluid and, in the more severe cases, mechanical ventilation and advanced organ supports.

Causative pathogens may differ widely, dependent upon epidemiological and clinical factors. In the immunocompetent, bacterial infection predominates; however, in the immunocompromised the infection may also be caused by a range of opportunistic bacteria, viruses and fungi. In this group the mortality, morbidity and cost of treatment is high. To select the appropriate antibiotic, the organism(s) needs to be identified and antibiotic sensitivities determined. For over a century this identification has, for the most part, depended upon culturing the viable organisms in the laboratory from respiratory tract and blood samples. The technique is slow and highly unreliable, often taking several days to gain a result. Prior exposure to antibiotics, inappropriate culture media and fragile organisms can all reduce the yield. For example, blood cultures obtained from a broad range of critically ill patients with presumed infection, only demonstrated a pathogen in approximately 11% of cases (1). The yield is higher, and often poly-microbial, in respiratory specimens (~40%) (1,2), however, it can be difficult to establish the causative organism over colonising bacteria. The low sensitivity and prolonged time (24–72 hours) to obtain the result means that culture rarely alters patient management.

Pneumonia may be classified in a number of ways; in critical care it is often classified by the environment in which it is diagnosed. Community acquired pneumonia (i.e., outside the healthcare setting) is one of the commonest reasons for admission to hospital and the CCU. Hospital acquired pneumonia (HAP), developing after 48 hours of hospital admission, represents the commonest hospital acquired infection. Patients developing pneumonia after 48 hours of mechanical ventilation are described as having a ventilator associated pneumonia (VAP). This classification, though crude, is useful as it describes an increasing complexity in management, costs and mortality but also the likely pathogens involved.

Pneumonia requires urgent treatment and waiting several days for culture results is inappropriate and potentially associated with an increase in mortality. As such, clinicians prescribe empiric antibiotics taking into account the likely organism and resistance profile. These empiric antibiotics are often broad spectrum aiming to cover a number of potential organisms. The antibiotic(s) could then be tailored later once the culture results are known. The approach of using broad spectrum antimicrobials has significant drawbacks and carries a significant cost (empiric antifungal is one of the largest drug cost to CCUs). To the patient, antimicrobials bring side effects that may range from rash and simple diarrhoea to organ failure and overgrowth of opportunistic organisms such as *Clostridium difficile*. The liberal use of broad spectrum antibiotics is the pre-eminent driver for the development of multi-drug resistant bacteria, which has been identified by the UK Department of Health (DoH) as the biggest challenge to healthcare in the coming years (3). As part of their strategy to reduce antibiotic use, the DoH has recommended that diagnostics need to be improved.

The overuse of antibiotics is a global phenomenon. Albeit in the community, a US study of 86 million consultations for respiratory problems, 40 million patients received an antibiotic. However, 27 million of these were thought to have a non-infective cause (2). In hospital, the CCUs are one of the largest users of antimicrobials and consequently have high incidence multi-drug resistant organisms (4,5). However, as in the community, evidence suggests a marked overuse of antibiotics where infection (clinical or microbiological) is only found in approximately 50% of those receiving antibiotics (6,7).

Broad-spectrum cover could still prove inadequate for patients, particularly in the presence of resistant organisms. Peralta *et al.* showed the risk of inadequate empirical therapy rose from 3% in patients with fully sensitive *E.coli* (in blood), to 35% in those with pathogens resistant to 3 or more antibiotic classes, this was associated with a significant increase in mortality (4). Others have demonstrated similar associations (5,8). This risk is particularly true in institutions providing tertiary care or serving mobile populations with extensive travel to countries with higher resistance rates than the UK (e.g., Middle East or South East Asia). This unfortunately drives many to the empirical use of extended spectrum antibiotics, including carbapenems, even in patients with fully sensitive pathogens, which itself exerts a selection pressure for future opportunistic pathogens including *Clostridium difficile* overgrowth.

Pathogens are likely to acquire resistance to antibiotics at a rate far quicker than new antibiotics can be developed—especially gram-negative organisms. The development of new antibiotics is slow, due to the difficulty in discovering novel molecules, challenges in licensing them, and a low return on investment (9). Even though there are new antibiotics in Phase II/III trials, they are not wholly immune to current resistant patterns, nor are they able to prevent organisms from developing new resistances. Antibiotic stewardship programs (ASPs) conserve existing antibiotics but may run the risk of denying severely unwell patients effective treatment.

CCUs account for a considerable fraction of hospital prescribing for broad-spectrum antibiotics, and pneumonias comprise the largest single group of all hospital-acquired infections (22.8%) (10). Reviewing pathogen prevalence rates in VAP suggests that carbapenems and piperacillin-tazobactams cover 85–86% of pathogens, but that 49% of pathogens could have been covered by amoxicillin-clavulanate and 27% by ampicillin or amoxicillin (11). This means that empirical piperacillin-tazobactam or imipenem results in an undertreatment in 13–15% of cases and over-treatment in 27–49%. It is impossible to identify these patients at risk or under or overtreatment until full culture results are available.

There is therefore a desperate need to be able to balance the competing demands making a prompt and precise diagnosis. This review looks at current and novel diagnostic strategies to diagnose pneumonia and identify the causative pathogen within the CCU.

Imaging: a proxy to detect infection

A chest X-ray (CXR) is often the first imaging technique used to detect a respiratory infection and in fact the

presence of new or progressive infiltrates on chest imaging is often used as one of the criteria in making a diagnosis. Lobar consolidation is usually attributed to a bacterial infection whereas interstitial infiltrates are mostly attributed to viral or fungal chest infections. In reality, it can be difficult to reliably distinguish one from the other on plain CXR. However, the negative predictive value (NPV) of infiltrates may be useful- an absence of a new infiltrate on CXR lowers the likelihood of a significant pneumonia.

Chest computerised tomography (CT) is frequently used in the CCU to support or exclude a respiratory infection. The pattern of pulmonary infiltrates is a useful guide to the aetiology of the infection as well as detecting the presence of other pulmonary pathology such as cavitating lesions, pleural effusions, and by extension, loculated empyemas. CT scans can also detect parenchymal infiltrates not visible on CXR (12). This is particularly true in the immunocompromised patients, including pleural effusions, ground glass opacification, cavitation, cysts, bullae, abscesses and pericardial effusions. There is also reasonable correlation between high resolution CT findings and the causative pathogen, including the detection of tuberculosis (TB), bacterial, fungal pneumonia and pneumocystis pneumonia (PCP). The radiological diagnosis in the hands of an experienced radiologist can correlate well with a microbiological diagnosis (13). A CT scan also more accurately determines the extent of pulmonary infiltrates and its contribution to worsening oxygenation (14).

An ultra-sound scan (USS) of the chest is becoming more common place in CCU due to its ability to give a prompt diagnosis and the machine's portability. USS has been showed to be equal to CXR, if not superior, in detecting lung pathology in the critically ill and can differentiate between pleural effusions, lung consolidation and alveolar interstitial infiltrates. USS has been found to have better sensitivity (0.985 *vs.* 0.735) and specificity (0.649 *vs.* 0.595) comparing to CXR when diagnosing pneumonia (15). USS has better positive and NPVs compared to CXR in identifying patients with who are developing pneumonia (15,16). In a CCU setting, portable chest USS is safe and easy to perform on unstable patients and is at least comparable to CXR without the risk of having to change the patient's position. All this supports the use of bedside thoracic USS as a diagnostic tool in patients with a suspected chest infection.

Newer, but more invasive, techniques seek to directly visualise bacteria and fungi within the alveoli (17). The intrapulmonary delivery of micro-doses of fluorescent smart probes can be used to label gram-negative bacteria, gram-positive bacteria and *Aspergillus sp*. These organisms are then visualised using trans-bronchial, fibre-based endomicroscopy (FE). The intra-pulmonary smart probes and the FE system can be delivered down the working channel of a flexible bronchoscope. As this system requires bronchoscopy, it is likely the main application will be within the CCU. However, the ability to detect bacteria or fungi *in vivo* at the bedside would be paradigm shift in how we visualise pneumonia in critical care, potentially leading to marked improvements in care and antimicrobial stewardship.

Biomarkers: guiding diagnosis and treatment

The ideal biomarker would help clinicians differentiate between the presence or absence of infection. Ideally, it could also differentiate between the various respiratory pathogens (virus *vs.* bacteria *vs.* fungus). Unfortunately, no biomarker today can give all the information required. Currently, most clinicians use a combination of white cell count (WCC), C-reactive protein (CRP) and, in some centres, procalcitonin (PCT), as well as clinical information to guide diagnosis.

CRP is the most commonly used biomarker used to detect bacterial infection. CRP is produced by the liver as an acute phase protein in response to inflammation and tissue injury, however it is highly non-specific. CRP levels increase over time in patients with infection and are stable in patients without. As a marker of general inflammation there is an association between CRP and mortality. In CCU patients with CRP levels of >10 mg/dL on admission, a decrease in CRP after 48 hours corresponded with a mortality rate of 15% and an increase corresponded to a mortality rate of 61% ($P<0.05$) (18). However, as a marker for pneumonia and in particular VAP sensitivity and specificity vary between 70–87% and 65–90% respectively (19). The National Institute of Clinical Excellence (NICE) guideline for community-acquired pneumonia (CAP) has set an arbitrary value of a CRP >100 mg/L to be consistent with CAP and a CRP of 20–100 mg/L would mean there is an intermediate probability of a patient having CAP and antibiotic treatment should be delayed and dependent on clinical progression. A CRP <20 mg/L does not support a bacterial infection.

PCT is precursor of calcitonin that is released by thyroid C cells in healthy patients and from extra-thyroid cells such as neuroendocrine lung cells and adhering monocytes in

cases of infection. Its normal concentration is <0.1 ng/mL in healthy adults. PCT is released within 2–6 hours after bacterial products are present in the bloodstream, its production being driven by exotoxins, TNF-alpha and other cytokines. It is more specific than CRP for detecting bacterial infection and can be downregulated during a viral infection, enabling the potential to distinguish between the two. For instance, PCT was used in the H1N1 influenza pandemic in 2009 to differentiate viral from bacterial pneumonia and can be used to differentiate infective and non-infective exacerbations of asthma and chronic pulmonary obstructive disease (20,21). PCT is also cited as a promising biomarker to differentiate pulmonary TB from bacterial CAP with an increase in serum concentration in bacterial CAP patients compared to little or no increase in pulmonary TB patients (22,23). Similar to CRP, higher and persistently raised values are associated with non-survival (24,25). However, PCT is not completely specific to bacterial infection and can be raised following severe trauma or in para-neoplastic syndromes and auto-immune conditions. Numerous trials and meta-analyses have examined its potential as a diagnostic for bacterial infection and although it is helpful and perhaps superior to CRP, the area under the curve of approximately 0.75 is not sufficient for it to be relied upon as a marker of infection driven inflammation (26-28). Nevertheless, a number of trials since have gone on to use a PCT guided algorithm to stop antibiotics with some success. The PRORATA study (29) demonstrated using a PCT algorithm to stop antibiotic therapy resulted in an absolute 2.7-day reduction in antibiotic use when compared to standard of care approach. However, the antibiotic courses were long, resulting in a decrease from 14.3 to 11.6 days, the trial also had a number of protocol deviations with the algorithm not being adhered to in approximately 50% in both groups. It can be argued that these long courses are not reflective of current practice in many UK hospitals where a 5–7 days' course is more typical. More recently, a trial of over 1,500 patients in The Netherlands (30) showed a PCT guided algorithm could reduce a median duration of antibiotic treatment from 7 to 5 days. Interestingly, they showed this decrease was also associated with a 5% improvement in mortality. A Danish study, at the other end of the duration spectrum, recruited 1,200 patients (the majority with respiratory infections) to also have their antimicrobials managed by a PCT algorithm or standard of care. In this trial, PCT guided therapy increased the median duration of antibiotics from 4 to 6 days. This increase was associated with increased time on a ventilator and longer stays in the ICU (31). These and other trials (32) would suggest PCT can reduce antibiotic duration in places where longer courses are common but also hint at the potential patient harm excessive antibiotics might be causing. The burden of antibiotic related harm is unclear; obvious consequences such as anaphylaxis and promotion of *Clostridium difficile* infections are well recognized however there is literature suggesting a more insidious impact on organ dysfunction that is hard to tease apart from other causes (33,34).

Thus, a biomarker(s) with a high degree of sensitivity and specificity is still required to enable the clinician to make or refute a diagnosis of a (respiratory tract) infection with relative certainty.

Though not specific to diagnosing respiratory tract infection, the SeptiCyte device is perhaps one of the most commercially advanced having received its 510(k) clearance from the U. S. FDA. The device uses the white cell transcriptome to delineate infection related inflammation from other inflammatory causes (e.g., surgery). In the published study (adult and paediatric) cohorts the device is able to differentiate infection from other forms of inflammation with an AUC of 0.89–0.95 with an improved performance compared to PCT (35,36). There are a multitude of other devices in development some looking at individual markers [e.g., pancreatic stone protein (37), Trem-1 or CD64] or combinations of biomarkers.

Sampling the respiratory tract for markers of infection may allow for improved sensitivity/specificity, especially in those who are ventilated. This is supported by a number of observational studies. Soluble triggering receptor expressed on myeloid cells (sTREM)-1 in bronchoalveolar lavage (BAL) fluid has been shown to be more accurate than other clinical or laboratory factors in detecting bacterial or fungal pneumonia. A value >200 pg/mL conferred a sensitivity of 75% and a specificity of 84% in diagnosing pneumonia (38). In a single centre study, investigators found a score created from the expression of TREM-1 on monocytes (mTREM-1) and neutrophils (nTREM-1) and concentrations of IL-1β, IL-8, and sTREM-1 in lavage fluid were significantly higher in VAP compared with non-VAP and non-ventilated controls (P<0.001). A seven marker BioScore correctly identified 88.9% of VAP cases and 100% of non-VAP cases (39). Elastin fibre (EF), a marker of lung parenchymal destruction, which can be detected in tracheal secretion, was postulated to differentiate colonisation to infection of the lung. Unfortunately, it showed a sensitivity of 32–40% and a specificity of 72% in diagnosing VAP, and thus

inadequate (40). This may be because EF production correlates more with lung destruction more than infection.

Endotoxin measurements in BAL fluid were also studied—as 70% of VAP are caused by gram-negative bacteria. The optimal level was found to be at 6 EU/mL (sensitivity 81%, specificity 87%)—a level equal to or greater than 6 EU/ml distinguished those with gram-negative pneumonia from colonized patients and those with pneumonia from gram-positive cocci (41).

BAL samples were obtained in a multi-centre study to assess the validity of a range of pulmonary biomarkers in 150 patients suspected of having a ventilator associated pneumonia. Thirty-five percent of these samples had the VAP diagnosis confirmed through quantitative microbiology. BAL IL-1β, IL-8, MMP-8, MMP-9 and Hydroxynonenal (HNE) were all studied. Low IL-B levels had a very high NPV for VAP and when combined with IL-8 it had a NPV of 1. It was estimated approximately 30% of the study cohort could have had their antibiotic courses stopped (42).

The field of infection and pneumonia progresses at a pace with increasingly innovative and sophisticated assays. What is unclear, however, is how these new diagnostics may impact on and how the clinician may change behaviour and alter prescribing habits.

Pathogen detection

If pneumonia is suspected or confirmed the next concern is to identify the causative organism(s). Pathogens may differ widely, dependent upon epidemiological and clinical factors. This is particularly so if the patient has been rendered immune-compromised by treatments for cancer, auto-immune disease, transplant etc., where opportunistic infections can be very rapidly fatal. To select the appropriate antibiotic, the organism(s) needs to be identified and its sensitivity to antibiotics determined. For over a century this identification has, for the most part, depended upon culturing the viable organisms in the laboratory from respiratory tract and blood samples

Blood culture is seen as the cornerstone in the investigation of any patient with severe infection or sepsis (respiratory or otherwise). In a prospective cohort of critically ill patients with suspected infection blood culture was only able to identify an organism in 11% of cases. Whilst an audit of microbiology labs in four European countries demonstrated blood cultures were positive in 9–13% of cases. Blood cultures obtained from patients presenting to the Emergency Department with a community acquired pneumonia, blood cultures were only able to identify the organism in <10% of cases (43-45). Many reasons are cited for this; inappropriate culture technique, prior antibiotic use, fragile organism (e.g., pneumococcus), viral aetiology or no organism present.

Obtaining respiratory samples [e.g., sputum, endotracheal aspirates, bronchoalveolar lavage (BAL)] is also eminently possible, however culture of respiratory tract organisms is still subject to the limitations described. A recent (unpublished) audit from several London hospitals looking at the timing of respiratory samples, found only 50% of samples were taken prior to administration of antibiotics, perhaps explaining the chance of identifying a pathogen was only 40%. Identifying a potential pathogen raises the possibility of an active pneumonia, however the upper respiratory tract is rapidly, within hours of intubation, colonized by potential pulmonary pathogens, even when pneumonia is not present. This makes it difficult to determine whether the isolated microbe is playing an active part or just colonising. Isolating the pathogen from the blood is highly supportive of infection though clearly does not necessarily confirm site of infection. In reality, paired blood and respiratory samples are often non-concordant (due to the absence of growth in the blood culture) (1).

The type of respiratory sample has also been examined for the utility in obtaining appropriate microbiological samples. Traditionally, bronchoscopic samples are taken via BAL or protected specimen brushing (PSB), where a brush at the tip of a catheter comes into contact with the bronchial wall. Alternatively, a mini-BAL is performed without bronchoscopic guidance or blind endotracheal aspirates can be obtained. Theory would suggest that guided sampling, deeper from the respiratory tract and from the affected lobe/segment should improve the diagnostic yield, however the preferred method is still unclear. Some early data pointed to the use of bronchoscopic guided sampling. Fagon *et al.* (45) demonstrated a reduction in 14-day mortality in patients with presumed VAP that were managed using BAL/PBS to guide therapy compared to a clinical-based strategy using endotracheal aspirates (16.2% *vs.* 25.8%, P=0.02). However, yield from the BAL was reduced by the prior use of antibiotics and the clinical advantage was essentially lost by day 28. BAL and PSB sampling did significantly increase the number of antibiotic-free days (11.5±9.0 *vs.* 7.5±7.6, P<0.0001).

However, in 2007 The Canadian Critical Care Trials Group (46) published the result of a 740 patients trial (in

28 centres) comparing the clinical outcome of patients, suspected of having a VAP, where treatment was guided by samples obtained by either endotracheal aspirate or BAL. They were unable to demonstrate any difference in clinical outcomes or overall use of antibiotics. A Cochrane meta-analysis (47), examining these 2 studies and 3 others (1,240 patients in total), suggested that there was no difference in how the cultures were obtained or whether culture was quantitative *vs.* qualitative cultures in terms of mortality (26.6% *vs.* 24.7%), or in antibiotic use. The American Thoracic Society HAP/VAP guidelines (48) incorporate these findings suggesting the use of endotracheal aspirate coupled with semi-quantitative analysis. On the other hand, despite these findings the European Societies of Respiratory and Intensive Care Medicine continue to support the use of distal sampling and quantitative analysis (weak evidence) (49). Their rationale for this guidance surrounds antimicrobial stewardship.

As BAL requires time, a skilled operator and is not without risk in the unstable patient, most UK units continue using non/semi-quantitative microbiology from endotracheal aspirates, reserving BAL for the more complex patient.

To further complicate the discussion, pneumonia reflects a deep infection at the alveolar level, where samples are not directly obtainable. Histological appearances obtained at autopsy are often discordant to microbiological findings from routine respiratory tract samples (50,51) and even those found from trans-bronchial biopsy (52).

However obtained, microbiology samples should ideally reach the lab within half an hour of collection. Storing samples in the refrigerator or frozen may be an acceptable alternative. However, the low sensitivity and prolonged time (24–72 hours) to obtain the result means that culture rarely alter management.

Therefore, there have been some innovative techniques developed to diagnose VAP/HAP quickly and more reliably than culture. Exhaled breath analysis is one of those techniques.

Electronic nose (E-nose) devices are portable and have the potential for point of care application. Using them to analyse volatile organic compounds (VOC) in exhaled breath would be a non-invasive, easily applied way to reduce diagnostic time. This technology has previously been used to diagnose colorectal cancer, head and neck cancers, TB, cystic fibrosis, chronic obstructive pulmonary disease (COPD) and asthma. By analyzing the air above the bacterial culture medium, *in vitro* studies have indicated that the e-nose is able to distinguish between infected and non-infected states. The e-nose was also able to accurately predict chest CT results in 80% of patients with pneumonia. Interestingly, the ROC curve comparing patients who had a confirmed diagnosis of VAP with positive BAL fluid with a control population showed that the e-nose had a sensitivity of 88% with a specificity of 66%. Changes in VOCs can be the result of either the bacteria and their metabolites or the inflammatory response of the patient—meaning that a change in VOCs *in vitro* would be different from a *in vivo* scenario. That is not to say that the e-nose has no use at all in the diagnosis of VAP. It can be used as an adjunct, alongside other available tests and probability scores, to improve the diagnostic accuracy of VAP. The e-nose itself may benefit from using gas chromatography (mass spectrometry of exhaled gases). Once specific groups of VOCs have been isolated to form biomarkers for certain diseases or micro-organisms, this would increase the sensitivity and specificity of e-noses (53).

This is being taken forward by the multicenter prospective observational study BreathDx (Molecular Analysis of Exhaled Breath as Diagnostic Test for Ventilator-Associated Pneumonia), where exhaled breath is to be analysed using thermal desorption-gas chromatography-mass spectrometry (TD-GC-MS). The primary endpoint of the study is to determine the accuracy of cross-validated prediction for positive respiratory cultures in patients with suspected VAP, with a sensitivity of at least 99%. It would also potentially allow them to put together patterns of VOCs in exhaled breath indicative of specific micro-organisms (54).

Pilot studies using polymerase chain reaction (PCR) analysis of exhaled breath condensate fluid (EBCF) taken from heat and moisture exchanger (HME) filters within ventilator circuits have shown that EBCF has a high concordance with BAL samples taken contemporaneously (55). This could reduce the time required for qualitative and quantitative results from days to hours. In the 51 patients recruited for this study, PCR of EBCF matched 100% of the organisms grown in those with positive BAL fluid cultures. In addition, PCR detected low levels of bacterial DNA in EBCF and BAL fluid in 8 cases where BAL fluid culture did not. This technique could also be used to inform the clinician of the changing lung microbiome in the ventilated patient by obtaining serial samples of EBCF, which may herald the development of VAP, although further studies are required. However, this technique does not allow for antimicrobial-sensitivity testing which would

be important in known extended spectrum beta lactamase (ESBL) and cabapenemase-producing organisms, such as Klebsiella pneumoniae, an important cause of VAP.

A direct E-test is a method of rapidly detecting antibiotic susceptibility via a plastic strip containing a pre-defined gradient of antibiotic which can be directly applied to respiratory samples. Susceptibility data at 18–24 hours correlated to standard culture results at 48–72 hours in 98% of cases. This would lead to the more effective use of antibiotics and reduction in antibiotic duration (56).

Molecular diagnostic platforms

In a bid to improve reliability and decrease time to pathogen identification molecular techniques are being increasingly adopted. One of the commonest methodologies is the use of matrix-assisted laser desorption/ionization-time-of-flight mass spectrometry (MALDI-TOF MS). The process involves laser ionization of molecules which are then separated by charge and mass prior to detection (57). MALDI-TOF MS can rapidly identify bacteria and yeast but must do so from colonies grown on culture plates. The technique can significantly reduce the time to pathogen identification when compared to standard microbiology but still relies on a positive culture. Though some early work suggested clinical and economic benefit from the use of MALDI TOF MS, a more recent prospective, multi-centre trial, randomized patients with positive blood cultures to management with conventional diagnostics (2,810 patients) or by the use of MALDI TOF (2,740 patients). Twenty-eight-day survival was similar in both groups (82%) (58).

Molecular diagnostics involving nucleic acid amplification techniques are increasingly available for pathogen detection (bacteria, fungi or viruses). Commonly the technique involves a polymerase chain reaction (PCR) whereby these devices seek to identify the genetic finger print of the organism along with any associated resistance genes. These devices are generally able to identify the pathogen direct from sample (blood, BAL etc.) without the need for culture in a matter of hours. These systems may detect:

(I) Specific targets such as TB e.g., GeneXpert MTB;
(II) A range of pre-defined organisms, by the use of a number primers (multiplex) e.g., BioFire FilmArray. It is clearly important that this panel includes all the likely organisms that may be encountered;
(III) Broad range assays enabling the assay to detect most microbial DNA comparing the amplicons against known libraries.

These techniques could hold significant advantages over standard culture techniques by the way of speed and that they are unlikely to be affected by prior antibiotic use. However, these devices are expensive to purchase and to run. The throughput is too small (and cost too prohibitive) for them to replace culture today. In hospitals that use them they are often reserved for difficult to treat populations such as the critically ill or the immunocompromised.

One of the most advanced technologies in this area was Abbott's Iridica device which coupled broad range PCR to electro-spray ionisation mass spectrometry (PCR/ESI-MS). It was able to identify over 1,200 pathogens, bacteria, fungi and viruses, direct from sample (with no need for culture) in approximately 6 hours. The potential for this technology has been demonstrated in a number of observational studies. The RADICAL study (1) paired PCR samples with routine microbiology in critically ill patients being investigated for potential infections (mostly respiratory). In blood, PCR/ESI-MS was three-times more likely to identify pathogens compared to standard blood cultures and demonstrated a negative predictive value (NPV) of 97%. A panel of independent experts would have recommended a change in management in 41% of patients, which includes treatment initiation, altering the antibiotic spectrum, or a change in antibiotic duration based on PCR/ESI-MS results. This technique could increase the detection of contaminants and commensals but the organisms most frequently detected were associated with infection. Much has been emphasized regarding the importance of rapid initiation of antibiotic therapy in suspected infection, but this must be balanced against the risks and stewardship issues surrounding over-enthusiastic or inappropriate antibiotic use. The PCR/ESI-MS test would be able to help target antibiotic therapy in patients who have already been commenced on antibiotics and have negative cultures (salvage microbiology). Several studies have also noted that in those patients in whom the PCR was able to detect microbial DNA (in the blood) but were culture negative had a significantly higher mortality than those where both molecular and culture techniques were negative (59,60). Whether this reflects undetected active infection or DNA translocation from the bowel is unclear, but it identifies a sicker cohort.

PCR devices also have the capability to detect a range of antibiotic resistance genes. As an example of potential benefit this technology could bring, within the RADICAL study, PCR/ESI-MS identified the *mecA* resistance gene

in the blood of 10 patients; none of these patients were on antibiotics that could treat organisms expressing mecA, they all died.

In a further study using PCR/ESI-MS, 32 mechanically ventilated patients with presumed HAP/VAP, routine culture was able to identify a pathogen in 12 patients (38%) and PCR/ESI-MS was positive in 21 patients (66%; P=0.045). The vast majority of this discordance was the PCR/ESI-MS identifying DNA in patients who had already received antibiotics, potentially raising this as an important indication for using this technology.

In an Emergency Department population being investigated for presumed infection (61), 273 blood samples were compared using both culture and PCR/ESI-MS, 207 of these samples were negative by both techniques but in the remaining samples, culture and PCR-ESI-MS were both able to detect the same organism in 32 samples though PCR/ESI-MS was able to detect 46 extra cases of pathogenic microbial DNA.

Despite these and many other encouraging studies, including a yet unanalysed multi-centre, international study, Abbott ceased further development and withdrew the technology from the market in 2017, stating commercial reasons. The advantage that broad range PCR has is the ability to detect a large array of microbes compared to multiplexed/real time PCR that use primers for specific organisms.

Despite the loss of such a device, other PCR technologies continue to show promise. In a cohort of 53 critically ill patients having a BAL for the diagnosis of potential pneumonia, the use of a multiplexed PCR was able to identify a pathogen in 66% of samples, significantly more than by microscopy (23%) or culture (40%). It is again worth note that 74% were receiving antibiotics thus likely to have significantly inhibited the non-molecular techniques (62). Multiple other studies have also demonstrated improved time and an increased likelihood to identifying potential pathogens in septic patients (63-65).

Nevertheless, in the respiratory tract there is constant concern as to whether the pathogen is colonizing or infecting. One advantage of culture over molecular techniques is the ability to quantify the microbes detected. Though not universally agreed, a cut off $>10^4$ colony forming units/ml is often used to identify infection over colonisation. Most molecular techniques are thought to be essentially semi-quantitative. This may be changing as Clavel *et al.* (66) employed quantitative PCR to compare with standard quantitative microbiology. The group prospectively enrolled 120 patients from 4 centres in France. Each patient with a potential VAP underwent. 103 of these patients also had a preceding endo-tracheal aspirate. In this population, 90 (75%) patients had a pathogen identified through culture and there was close association between the culture and molecular technique in both identifying the organisms and being able to quantify them.

Though newer molecular pathogen detection systems may increase the chance of detection, reduce time to detection and possibly quantify the pathogen it is unclear the impact these results would have on patient management. Several studies have done retrospective reviews of patient management. In these studies, a panel of experts have reviewed the PCR results and commented on whether that should have changes patient management. The impact of this 'in-vitro' decision making varies widely; In the RADICAL study the PCR result could have altered antibiotic prescribing in 41% of cases (1), while a study by Dierke *et al.* using multiplex PCR (MPCR) (SeptiFast) in a range of patients (n=101) with severe infections demonstrated the PCR result would impact on prescribing in only 8% (63).

Lodes *et al.* obtained 148 blood samples from 104 consecutive critically ill surgical patients (64). The team analysed these using culture and MPCR. Similar to other studies they found a positive blood culture in 20% of samples and a positive PCR finding in 40%. However, unlike other studies the clinicians were informed of the PCR result, leading to a change in antibiotic prescribing in 17% of cases. The trial was too small to detect any clinical benefit. A trial, randomising patients with respiratory or abdominal sepsis to treatment guided by standard of care microbiology (n=37) *vs.* standard of care plus the use of multiplexed PCR (n=41) was undertaken at two university hospitals in Berlin (67). Blood culture was positive in 18.9% and 12.2% respectively, whilst in the intervention group MCPR was able to identify a pathogen in 24%. Although far too small a trial to show any clinical benefit, the intervention group demonstrated a trend towards a decreased time to appropriately adapt antibiotic therapy (16 *vs.* 38 hours).

Increasingly available in the research arena is the possibility of metagenomic sequencing. A technique that is able to amplify the entire microbial genome and then sequence it. This could be of use not just in understanding the current infection, but for tracking outbreaks and understanding the microbiome. Currently the sample preparation is complex and time consuming and understanding the acquired data makes it difficult to

deploy clinically. As technology progresses this will change, we will have access to more information than we ever had, the size of the technology is also rapidly shrinking. Abbott's IRIDICA system took up most of a laboratory, whilst the newer sequencing devices (e.g., MinIon, Oxford Nanopore) are slightly larger than a smart phone bringing the possibility of bedside pathogen detection.

Much larger prospective trials are now required to demonstrate the potential utility of all these devices. However even if concerns regarding the cost, the relevance of DNA detection are all settled there is still concern as to how these results will be accepted by the front line clinical staff. Will the results alter behaviour in a positive way, reserving antibiotics for those with evidence of true infection and tailoring to the appropriate antibiotic when the pathogen is identified? Or, will clinicians continue to prescribe long courses of broad spectrum antibiotics, just in case the 'machines have missed something'?

Conclusions

The diagnosis and treatment of respiratory infection remains a multifaceted challenge for all physicians in the critical care. However, our response to this has been encouraging—there have been innovative uses of existing technology (USS), as well as new forms of diagnostics in the forms of biomarkers and molecular diagnostic platforms. Continued work and understanding of these techniques will not only help us differentiate patients in infected and non-infected states, but also promote the judicious use of antimicrobials. We can only hope that with these tools in our pockets, we can have a meaningful impact in reducing both patient harm and antimicrobial resistance

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Footnote

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