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Evaluation of Curetis Unyvero, a Multiplex PCR-Based Testing System, for Rapid Detection of Bacteria and Antibiotic Resistance and Impact of the Assay on Management of Severe Nosocomial Pneumonia

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Health care-associated pneumonia due to multidrug-resistant organisms represents a major therapeutic challenge. Unfortunately, treatment is dependent on empirical therapy, which often leads to improper and inadequate antimicrobial therapy. A rapid multiplex PCR-based Unyvero pneumonia application (UPA) assay that assists in timely decision-making has recently become available. In this study, we evaluated the performance of UPA in detecting etiological pathogens and resistance markers in patients with nosocomial pneumonia (NP). The impact of this assay on the management of severe nosocomial pneumonia was also assessed. Appropriate specimens were processed by UPA according to the manufacturer's protocol in parallel with conventional culture methods. Of the 56 patients recruited into the study, 49 (87.5%) were evaluable. Of these, 27 (55.1%) and 4 (8.2%) harbored multiple bacteria by the PCR assay and conventional culture, respectively. A single pathogen was detected in 8 (16.3%) and 4 (8.2%) patients, respectively. Thirteen different genes were detected from 38 patients, including the *ermB* gene (40.8%), the $bla_{\text{OXA-51}}$-like gene (28.6%), the *sul1* (28.6%) and *int1* (20.4%) integrase genes, and the *mecA* and $bla_{\text{CTX-M}}$ genes (12.3% each). The time from sample testing to results was 4 h versus 48 to 96 h by UPA and culture, respectively. Initial empirical treatment was changed within 5 to 6 h in 33 (67.3%) patients based on the availability of UPA results. Thirty (62.2%) of the patients improved clinically. A total of 3 (6.1%) patients died, mainly from their comorbidities. These data demonstrate the potential of a multiplex PCR-based assay for accurate and timely detection of etiological agents of NP, multidrug-resistant (MDR) organisms, and resistance markers, which can guide clinicians in making early antibiotic adjustments.

The respiratory tract is the most common source of infection in acutely ill patients and is one of the leading causes of death in these patients. Pneumonia, a lower respiratory tract infection, is a life-threatening condition which carries high mortality. This infection can be acquired in the community (community-acquired pneumonia [CAP]) or the hospital (hospital-acquired pneumonia [HAP], also known as nosocomial pneumonia [NP]). NP refers to infection that developed while the patient was in an inpatient setting (1). It is further differentiated into ventilator-associated pneumonia (VAP), depending on whether or not the process arose after the patient had been receiving 24 h of mechanical ventilation (2, 3). NP is a frequent and severe infection in the hospital setting, particularly the intensive care unit (ICU), with important morbidity, mortality, and cost implications (3–5). Treating a critically ill patients with severe pneumonia is challenging as it involves making important decisions based on an incomplete clinical picture. Thus, selecting appropriate antimicrobial therapy as quickly as possible is absolutely crucial for a successful outcome, as timely action seems to lead to decreased mortality.

With the current paradigm for treatment of pneumonia, results of conventional microbiology culture of respiratory samples are not available for at least 48 to 72 h. However, it is prudent for the clinician to commence empirical treatment immediately without the benefit of knowing the potential causative pathogen and antimicrobial susceptibility. Providing appropriate and adequate antimicrobial therapy is a vital component of successful treatment for severe NP, as many reports have shown that inadequate antimicrobial therapy increases the mortality rate (6–8). The therapeutic turnaround time (TTAT), that is, the time taken from sending the first specimen for investigation and the results becoming available to initiate appropriate treatment, varies considerably, as the clinical scenario for treating patients with pneumonia is complex. The shorter the TTAT the better the outcome.

Conceivably, availability of robust rapid molecular diagnostic technology in the routine laboratory that can provide accurate and reproducible pathogen detection in hours rather than days might prevent some inappropriate and inadequate therapies. The objectives of this study were to evaluate the performance of a multiplex PCR-based Unyvero pneumonia application (Curetis AG, Holzgerlingen, Germany) assay for the detection of bacteria and resistance markers from respiratory specimens and determine the impact of this assay on the management of severe nosocomial pneumonia.

MATERIALS AND METHODS

Patients and specimens. Immunocompetent and immunocompromised severely ill patients with clinically suspected respiratory tract infections who had been admitted to the ICUs or medical wards of Mubarak Al

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Kabeer Hospital, Kuwait, for ≥48 h during the months of January to April 2013 were recruited into the study. Nonrepetitive respiratory samples, mainly sputum, bronchoalveolar lavage (BAL) fluid, and endotracheal (ET) secretions, were obtained from the patients who met the definition of nosocomial pneumonia and sent directly to the hospital diagnostic microbiology laboratory. Other specimens collected included blood for blood culture and blood gases analysis and hematological and biochemical profiles. Our hospital is an 800-bed tertiary teaching hospital with a 26-bed adult ICU and 9-bed pediatric ICU. The medical ethics committee of our Ministry of Health (no. WMTJ/2186/2013) approved the study, and written informed consent was obtained from the patients or relatives.

Definition of pneumonia. A patient was suspected of pneumonia if there were (1) clinical criteria (i.e., new or progressive radiological pulmonary infiltrate) plus 2 or more of the following: temperature >38°C or <35.5°C, leukocytosis (leukocyte count of ≥12,000 cells/mm^3), or purulent respiratory secretions (as determined by Gram stain) (9, 10) or (2) a simplified Clinical Pulmonary Infectious Score (SCPIS) of >5 points (11). The SCPIS included measurement results for the following variables: temperature in degrees centigrade, blood leukocytes per mm^3, tracheal secretion culture, oxygenation (partial arterial oxygen pressure [PaO_2]/fraction of inspired oxygen [FIO_2]) in mm Hg, and chest radiograph. A diagnosis of VAP was made in patients with previous invasive mechanical ventilation for ≥48 h.

Laboratory methods. (i) PCR-based Unyvero pneumonia assay. The Unyvero pneumonia application (UPA) (Curetis AG) identifies 18 bacteria, based on 23S rRNA sequences, and a fungus (*Pneumocystis jirovecii*) (which represent over 90% of the etiological agents of severe nonviral pneumonia) and simultaneously detects 22 resistance markers. For detection of multidrug-resistant (MDR) organisms, the multiplexed PCR targets 3 classical Ambler class A β-lactamases (TEM, SHV, and CTX-M) and 2 families of plasmid-encoded *ampC* genes (Ambler class C). An integrase gene as a surrogate marker for MDR is also included.

All specimens were processed with the UPA assay according to the manufacturer's protocol. Specimens were processed immediately as they arrived, one or two at a time in sequence with no batching. Briefly, 180 μl of the patient's sample and master mix were loaded into a self-contained cartridge (Curetis AG) and then placed in the analyzer, where sample preparation, DNA extraction and purification, amplification, and specific detection took place, generating complete diagnostic information within 4 h. To detect many analytes, 8 multiplexed PCRs were run in parallel for detection with panel-specific microarrays.

(ii) Conventional culture and susceptibility testing. One-hundred-microliter aliquots of the same sets of samples were inoculated in parallel on a set of selective and nonselective routine agar plates (MacConkey [Oxoid, Basingstoke, United Kingdom], blood agar [Oxoid], chocolate agar, and Sabouraud agar [Oxoid]) and incubated under appropriate atmospheric conditions for 24 h or reincubated for 48 h as necessary. Relevant clinically significant bacterial isolates were identified by the Vitek 2 ID system (bioMérieux, Marcy, L'Étoile, France) and Vitek MS (bioMérieux) (when necessary). Antimicrobial susceptibility testing (AST) was performed using Vitek 2 AST cards and Etest (bioMérieux), as required. Penicillin and carbapenem susceptibility of *Streptococcus pneumoniae* and vancomycin susceptibility of methicillin-resistant *Staphylococcus aureus* (MRSA) were determined using the Etest.

Phenotypic detection of resistance mechanisms was carried out using GeneXpert (Cepheid AB, Röntgenvägen, Solna, Sweden) for detection of the *mecA* gene in MRSA. Detections of extended-spectrum β-lactamase (ESBL) and metallo-β-lactamase (MBL) were carried out with the cefotaxime/cefotaxime-clavulanic acid (CT/CTL) and ceftazidime/ceftazidime-clavulanic acid (TZ/TZL) Etest (bioMérieux) and imipenem-EDTA Etest methods, respectively. The following control strains were included in each run as appropriate: MRSA ATCC 43300 (*mecA* positive [*mecA*$^+$]), MRSA ATCC 25923 (*mecA* negative), *Escherichia coli* ATCC 25922 (ESBL$^+$), MBL-producing *Klebsiella pneumoniae* ATCC BAA-1705 (MBL$^+$), and *K. pneumoniae* ATCC-1706 (MBL$^-$).

TABLE 1 Basic characteristics of the patients admitted into the study

Characteristic[a]	Value
Demographic data	
Age (range) (yr)	3–92
Mean (± SD) age (yr)	55.6 ± 21.927
Male (no. [%])	34 (69.4)
Female	15 (30.6)
Location of care (no. [%])	
Adult ICU	27 (55.1)
Pediatric ICU	2 (4.1)
Medical ward	20 (40.8)
Comorbidity (no. [%])	
Diabetes mellitus	12 (24.5)
Chronic respiratory disease	17 (34.7)
Chronic cardiovascular disease	8 (16.3)
Chronic renal disease	4 (8.2)
Chronic hepatic disease	7 (14.3)
Solid cancer	10 (20.4)
Valid sample type (no. [%])	
Endotracheal secretion	30 (61.2)
Sputum	12 (24.5)
Bronchoalveolar lavage fluid	7 (14.3)
Clinical laboratory finding	
Median CRP level (mg/dl)	20.8
Median WBC count (×10^9/liter)	13.4
Median platelet count (×10^9/liter)	127.0
Bacteremia (no. [%])	8 (16.3)
Mechanical ventilation (no. [%])	18 (36.7)
Pleural effusion (no. [%])	11 (22.5)

[a] ICU, intensive care unit; CRP, C-reactive protein; WBC, white blood cell.

Impact on patient management. The patients were classified into 1 of 3 groups (12) (i) mild-to-moderate NP, i.e., no usual risk factors, onset any time or early onset, and severe NP; (ii) mild-to-moderate NP with risk factors and onset any time; and (iii) late-onset severe NP or early-onset NP with risk factors.

Those patients whose empirical antimicrobial therapy remained the same after the results of the etiological agents became known, and those patients in whom antibiotic therapy needed adjustment, were analyzed to determine the direct impact of the test system on the management of patients.

Statistical analysis. EpiCalc 2000, version 1.02 (Brixton Health, Llanidloes, United Kingdom) was used to compare the counts and sample size.

RESULTS

Of the 56 patients recruited into this study, 49 (87.5%) were evaluable and 7 (12.5%) who did not meet the definition of NP were excluded. The biodata of the patients are shown in Table 1. The patients were 3 to 92 years old (mean, 55.6 years). Of these, 27 were hospitalized in adult ICUs, 2 in a pediatric ICU (PICU), and 20 on the medical wards. There were 34 males and 15 females. Endotracheal (ET) specimens were obtained from 30 patients, sputum from 12, and BAL fluid from 7. All of the patients had shifts in peripheral white blood cell (WBC) counts to the left, elevated median C-reactive protein (CRP) levels, and increased

TABLE 2 Distribution of microorganisms according to severity of pneumonia

Microorganism (no. of isolates)	No. of patients[b] infected according to indicated detection type						*P* value[c]
	Group 1 ($n = 8$)		Group 2 ($n = 26$)		Group 3 ($n = 15$)		
	PCR	Culture	PCR	Culture	PCR	Culture	
Acinetobacter baumannii (13)	1	0	4	2	8	1	0.007
Haemophilus influenzae (2)	0	0	1	0	1	0	0.24
Klebsiella pneumoniae (10)	0	0	4	1	6	0	0.0013
Klebsiella oxytoca (2)	0	0	2	0	0	0	0.24
Legionella pneumophila (2)	0	0	2	0	0	0	0.24
Moraxella catarrhalis (1)	0	0	0	0	1	0	0.5
Proteus sp. (1)	0	0	0	0	1	0	0.5
Pseudomonas aeruginosa (12)	1	0	4	1	5	1	0.015
Serratia marcescens (3)	0	0	1	0	2	0	0.12
Staphylococcus aureus (2)	0	0	1	0	1	0	0.24
MRSA[a] (3)	0	0	1	0	2	0	0.12
Stenotrophomonas maltophilia (12)	2	0	3	0	6	1	0.0027
Streptococcus pneumoniae (12)	6	2	2	0	2	0	0.015

[a] MRSA, methicillin-resistant *Staphylococcus aureus*.
[b] Group 1, mild-to-moderate nosocomial pneumonia (NP), no usual risk factors, onset any time, or early-onset and severe NP; group 2, mild-to-moderate NP with risk factors and onset any time; group 3, late-onset severe NP or early-onset NP with risk factors.
[c] A *P* value of <0.05 is statistically significant.

oxygen requirements. Sample-to-result times were ca. 4.3 h for the UPA assay versus 48 to 96 h for conventional cultural methods. Results obtained by the UPA were immediately conveyed in person by either W.J. or E.A.R. to the ward/ICU, and the interpretation and significance of each test result were discussed with the treating clinician.

Bacterial etiology and resistance genes detected by UPA assay versus culture. A summary of the performances of the test assay and culture shows that clinically significant multiple bacteria were detected in 27 (55.1%) cases by UPA and 4 (8.2%) by conventional bacteriological culture. Eight (16.3%) and 4 (8.2%) cases yielded single pathogens by UPA and culture, respectively, a statistically significant finding with a *P* value of <0.0001 (confidence interval [CI], 29.00 to 64.88). In 11 (22.5%) and 37 (75.5%) cases there were no significant pathogens detected by both UPA and culture, respectively. The difference in lack of detection power by UPA and culture reached a statistically significant level ($P < 0.0001$ [CI, 34.24 to 71.88]); 3 of the 11 negative specimens by UPA were acid-fast bacilli (AFB)-positive by Ziehl-Neelsen (ZN) stain and grew *Mycobacterium tuberculosis* by culture. Three (6.1%) specimens yielded "error/not valid run" by UPA, meaning a number of criteria were not fulfilled, such as the cartridge not being properly processed, control within the system failing, or the presence of holes/cracks in the array membrane, dust on the array membrane, and difficulties during array detection by the optic module.

The microbial etiology according to UPA assay and culture is shown in Table 2. Overall, statistically significant pathogens detected by UPA versus culture were *Acinetobacter baumannii*, 13 and 3, respectively ($P < 0.007$ [CI, 4.30 to 36.52]); *Klebsiella pneumoniae*, 10 and 0 ($P < 0.0013$ [CI, 7.08 to 33.73]); *Pseudomonas aeruginosa*, 10 and 2 ($P < 0.015$ [CI, 1.71 to 30.94]); *Stenotrophomonas maltophilia*, 11 and 1 ($P < 0.0027$ [CI, 6.03 to 34.78]); and *Streptococcus pneumoniae*, 10 and 2 ($P < 0.015$ [CI, 1.71 to 30.94]). The etiological agents were further analyzed according to the severity of the pneumonia into groups 1, 2, and 3. In group 1 (8 patients), the causative agents detected by UPA versus culture were *S. pneumoniae* in 6 (75%) cases versus 2 (25%), *S. maltophilia* in 2 (25%) versus 0, *A. baumannii* in 1 (12.5%) versus 0, and *P. aeruginosa* in 1 (12.5%) versus 0, respectively. In group 2 NP (26 patients), detections by UPA versus culture were *A. baumannii* in 4 (15.4%) and 2 (7.7%) patients, *K. pneumoniae* in 4 (15.4%) and 1 (3.9%), *P. aeruginosa* in 4 (15.4%) and 1 (3.9%), and *S. maltophilia* in 3 (11.5%) and 0, respectively. Analysis of microbial agents from group 3 NP (15 patients) indicated that the majority of the etiological agents were detected by the UPA assay. *A. baumannii*, *K. pneumoniae*, *S. maltophilia*, and *P. aeruginosa* were detected by UPA assay versus culture in 8 (53.3) and 1 (6.7%), 6 (40%) and 0, 6 (40%) and 1 (6.7%), and 5 (33.3%) and 1 (6.7%) patients, respectively.

A total of 13 different genes were detected by the UPA assay from 38 infected patients. Analysis of the frequency of genes detected showed that the *ermB* gene (40.8%) was the most commonly detected gene, followed by the $bla_{\text{OXA-51}}$-like gene (28.6%), the *sul1* (28.6%) and *int1* (20.4%) integrase genes, and the *mecA* and $bla_{\text{CTX-M}}$ genes (12.3% each). Other genes, such as bla_{TEM}, bla_{SHV}, and *ermC* were each detected in 8.2% of the cases.

The degree of agreement between UPA and culture. There was no agreement in 18 (36.7%) cases, but the same organisms grew in 3 (6.1%) cases. At least one organism was found by both methods in 14 (28.6%) cases, and both methods yielded no growth in 9 (18.4%) cases.

Treatment changes based on UPA assay. As shown in Table 3, initial empirical treatment was changed within 5 to 6 h after specimen collection in 33 (67.3%) patients based on the results of the UPA assay becoming available soon after 4 h. Thirty (62.2%) of the patients improved clinically and microbiologically compared with 8 (16.3%) in whom there was no improvement. A total of 3 (6.1%) patients died mainly from their comorbidities (1 from chronic myeloid leukemia, 1 from severe myocardial infarction, and 1 from widespread tuberculosis).

Impact on outcome of severe NP by Unyvero. Fifteen (30.6%) patients with severe pneumonia (group 3), from whom multiple bacteria were detected, including multidrug-resistant (MDR)

TABLE 3 Treatment changed based on Unyvero results

Treatment or result	Cases (no. [%])	Comment[a]
Treatment changed	33 (67.3)	Within 6 h of specimen collection
Missing	16 (32.7)	No pathogen detected plus invalid readings (errors)
Total	49 (100.0)	
Improved		
Yes	30 (62.2)	Clinical parameters and CXR
No	8 (16.3)	CXR and clinical parameters unchanged
Died	3 (6.1)	Died of comorbidity: 1 CML; 1 MTb; 1 severe MI

[a] CXR, chest X ray; CML, chronic myeloid leukemia; MTb, widespread tuberculosis; MI, myocardial infarction.

strains, were evaluated specifically for the impact of Unyvero on timely intervention and outcome of therapy. All had leukocytosis, elevated C-reactive protein (CRP), and increased oxygen requirements. Detection of resistance genes influenced modification of therapy in all 15 cases with multiple MDR bacteria. In 3 of these patients, Unyvero and culture identified MDR pathogens with good correlations between phenotype and genotype for third-generation cephalosporin, carbapenem, macrolide, and gyrase inhibitor resistance, leading to modification in antibiotic regimes to appropriate therapy. Thirteen (86.7%) of these 15 patients improved clinically and bacteriologically and 2 (13.3%) died, mainly from their comorbidities. Ten (66.7%) would have been inappropriately treated if treatment had been based on results of conventional testing or empirical therapy only.

DISCUSSION

Pneumonia is a medical challenge. NP (that is, hospital-acquired pneumonia and ventilator-associated pneumonia [VAP]) represents one of the most important causes of morbidity and mortality, with high attributable mortality rates of between 25 and 50%. The results of large multicenter studies provide a clearer picture of the severity of the problem on a worldwide scale (8, 9). A confounding issue in the successful management of pneumonia is the long delay in determining the identity of the causative agents and their antimicrobial susceptibility. Current guidelines for the treatment of pneumonia are based on the standard of care, which delays the identification of the etiological agent by 48 to 72 h due to the time it takes to culture the pathogens. One way to improve the TTAT is to implement new diagnostic technologies in microbiology laboratories that would speed up pathogen and resistance identification. These concerns were found to be addressed by the UPA assay. The assay allowed detection and correct identification of clinically relevant etiological agents of NP and important resistance profiles in patients in the ward and ICU settings within 4 h of specimens reaching the laboratory. All specimens were processed immediately upon receipt in the laboratory, thereby eliminating the need for batching (which could have impacted the TTAT), in contrast to cultural methods where specimens were batched. Thus, the limitations in the area of rapid testing for multiple pathogens and the ability to incorporate molecular testing into clinical microbiology laboratories were removed. In addition, not only could the assay be performed in a single day, it also provided valuable relevant results in just a fraction of the time it took by the conventional culture technique. Furthermore, the results of UPA were conveyed directly in person to the wards/ICU by members of our team, who also discussed the interpretation and significance of the results in relation to the clinical condition of the patient. This careful interpretation of the results was undertaken to prevent overtreatment of possible replacement colonizers.

This UPA assay system is akin to a "lab-on-a-chip" or "micro total analysis system (μTAS)" previously described by Yager el al. (13) and Whitesides (14), as it utilizes the highly sensitive and specific technique of nested multiplex PCR in an enclosed disposable cartridge equipped with integrated reagent containers, a DNA purification column, eight PCR chambers, and a corresponding number of detection arrays. As a result, this system permits the enormous benefits of this form of PCR to be feasible in settings where even moderate contamination risks from pathogens or of amplicons are unacceptable. Ultimately, this type of system could allow complex molecular methods to be adopted in point-of-care settings where even community-acquired pneumonia patients present initially to the doctor. Another advantage of the system is that it is automated, which reduces operator workload and error; the process is rapid and reactions from one step to the other are measured in seconds. Pathogen identification and antibiotic susceptibility were simultaneously measured and provided real-time, relevant information about the presence or absence of pathogens and their antibiotic resistance genes.

The UPA assay system is also an efficient solution to the "sample-to-assay" problem, as it uses small volumes of materials without losing its sensitivity. Typically, for a few pathogens, sensitivity is correlated with testing of large sample volumes. The UPA assay system uses all the nucleic acid recovered from the small amount of input material in the first step of the multiplex PCR, and the second stage of amplification then allows specific detection of the analytes in very small-volume PCRs without the loss of sensitivity common in small-volume PCRs (15).

The testing of the UPA assay with clinical samples demonstrates a successful real-world application of this technology. In comparison to the culture technique, the UPA system showed a high percentage of cumulative agreement (ca. 70%). The most common discordance was the detection of pathogens by the UPA assay system in culture-negative samples, apparently due to superior sensitivity of the PCR assay. Furthermore, as shown in this study, the UPA assay system tests for a large panel of pathogens, which leads to fewer negative samples than with the conventional culture method and more multiple-pathogen detection in the same sample, as a result of the limited numbers and types of selective and nonselective culture media used in the routine clinical microbiology laboratory for the culture of respiratory specimens or analysis of previous antibiotic use.

In this study, the UPA assay system demonstrated the importance of providing appropriate and adequate therapy as a vital component of successful treatment of severe nosocomial pneumonia, as many published reports have shown that inadequate antibiotic therapy increases the mortality rate as well as the mean duration of hospital stay (8, 16–18). The UPA assay is an accurate and rapid test system which enabled the treating doctor to perform an adequate decision-making process to select the appropriate antimicrobial therapy and thus improve medical outcomes.

This PCR-based rapid assay detected many pathogens that were not detectable by conventional culture and provided resistance markers in a timely manner.

It is worthy of note that while UPA detected *S. pneumoniae* (a known respiratory pathogen), in specimens from 12 patients, only 2 of these yielded the same organism in the conventional culture method. Failure to isolate *S. pneumoniae* in culture has been observed previously by other workers and may be attributable to prior antibiotic therapy (which may impair the diagnostic validity of respiratory culture) in addition to delays of sample processing (which may reduce the isolation rates and increase indigenous flora) (19). Several studies have demonstrated that sputum and other respiratory cultures became rapidly negative for *S. pneumoniae* during antibiotic treatment, unlike PCR, which remained positive in spite of ongoing therapy (20, 21). Perhaps, in general, another reason for PCR positivity in culture-negative samples is that it has higher sensitivity than culture (22). The inclusion of *Pneumocystis jirovecii* and *L. pneumophila* in the UPA panel offers valuable information. UPA was positive for *L. pneumophila* in 2 patients with moderate-to-severe NP, with supportive clinical and laboratory findings, but negative by culture. These patients improved dramatically on appropriate antibiotic therapy.

In this study, the UPA assay was used to assess the presence in 76 isolates of multidrug-resistance (MDR), defined as resistance to 3 classes of antibiotics. The majority of the patients in the group 2 and group 3 category harbored MDR Gram-negative bacterial pathogens, mainly *A. baumannii*, *K. pneumoniae*, *P. aeruginosa*, and *S. maltophilia*. Detection of MDR was achieved in this assay by 3 classical Ambler class A beta-lactamases (TEM, SHV, and CTX-M), 2 Ambler class C (plasmid-mediated *ampC*) genes, and an integrase gene (*int1*), and MDR was indicated in ca. 55% of pathogens. Based on these results, treatment regimens were changed in 33 (67%) patients within 6 h of specimen collection and laboratory processing (sample-to-result time frame) without waiting for results of conventional culture, which became available approximately 48 to 96 h later. Sixty-two percent of patients improved clinically and microbiologically; 3 patients died mainly because of a comorbidity.

The direct impact of the UPA assay compared with conventional culture was further analyzed in detail in the 15 patients in group 3. There was no doubt that the rapid molecular diagnostic platform (UPA assay) was instrumental to accurate pathogen identification and the selection of appropriate targeted antibiotic therapy, with benefits for the patients. In addition, by decreasing the turnaround time to hours, instead of days, the UPA assay provided clinicians the opportunity to change the empirical therapy to definitive therapy within the shortest possible time frame, thus impacting positively patient management and outcome. In our hospital, guidelines for empirical treatment of NP entail administering ceftazidime plus ciprofloxacin or meropenem. In our patients with moderate-to-severe NP, treatment was changed from the broad-spectrum regimen to targeted antibiotic therapy involving the use of intravenous colistin and/or tigecycline or vancomycin when MRSA was involved, with satisfactory outcomes. This decision was influenced by the results of UPA showing pathogens with multiple resistance to the empirical broad-spectrum antibiotics. The patient who died of widespread tuberculosis (TB) had pulmonary TB that was missed by UPA, which also did not detect any other microorganisms in the specimen from this patient. In addition, routine bacteriological culture of specimens from this patient also did not yield any growth. Based on the demonstration of AFB by ZN stain and radiological findings, the patient was treated with standard quadruple anti-TB drugs but succumbed to his infection.

An important limitation of this study is the relatively small number of patients and the time constraints for carrying out the study. A larger number of patients is required to demonstrate more conclusively the impact of the UPA assay on patient management. This will also permit consistent performance at a high level of technical proficiency, determination of high positive and negative predictive values that can discriminate between a true infection and mere colonization, and importantly, determination of the proper place of this method in an algorithm of clinical laboratory diagnosis of respiratory pathogens. It must be said, however, that because the UPA assay consists of a finite panel, as do all multiplexed PCRs, it may miss some organisms which might grow on culture. Correct interpretation of these UPA results must be carefully made and weighed against the clinical condition of the patient in order to avoid overtreating mere colonizers. *M. tuberculosis* is not included in the UPA panel and hence the UPA failed to detect the 3 cases of tuberculosis which were positive on ZN stain and later grew on culture. The explanation for this omission might be that *M. tuberculosis* is an unusual etiological agent of NP. Another important cautionary note when making comparisons between UPA and culture is that PCR-based identification will also detect dead or treated organisms that may not grow on culture, particularly if the patient has been on medication with previous antibiotics.

In conclusion, the UPA assay detected etiological agents of mild through moderate to severe nosocomial pneumonia and their resistance markers in respiratory samples, with results that were consistent with standard clinical microbiology within a time period of approximately 4.3 h versus 48 to 96 h by conventional culture. This assay holds promise for the future, where rapid detection of pathogens and resistance mechanisms may be determined in standardized assays that will allow clinicians to diagnose pneumonia in real time and initiate appropriate antimicrobial therapy as early as possible. The study also showed that antibiotic resistance with a complex genetic background can be successfully predicted by the careful selection of markers on the assay panel. However, as promising as the assay looks, care must be taken in the interpretation of test results, which should tally with the clinical presentation to avoid treating colonizing organisms instead of the pathogens infecting the patients.

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REFERENCES

1. **Garner JS, Jarvis WR, Emori TG, Horan TC, Hughes JM.** 1988. CDC definitions for nosocomial infections, 1988. Am. J. Infect. Control **16:** 128–140. http://dx.doi.org/10.1016/0196-6553(88)90053-3.
2. **Rello J, Ollendorf DA, Oster G, Vera-Llonch M, Bellm L, Redman R, Kollef MH, VAP Outcome Scientific Advisory Group.** 2002. Epidemiology and outcomes of ventilator-associated pneumonia in a large US database. Chest **122:**2115–2121. http://dx.doi.org/10.1378/chest.122.6.2115.
3. **Chastre J, Fagon JY.** 2002. Ventilator-associated pneumonia. Am. J. Respir. Crit. Care. Med. **165:**867–903. http://dx.doi.org/10.1164/ajrccm.165.7.2105078.

4. **American Thoracic Society, Infectious Diseases Society of America.** 2005. Guidelines for the management of adults with hospital-acquired, ventilator-associated, and healthcare-associated pneumonia. Am. J. Respir. Crit. Care. Med. **171:**388–416. http://dx.doi.org/10.1164/rccm.200405-644ST.
5. **Vincent J-L, Bihari DJ, Suter PM, Bruining HA, White J, Nicolas-Chanoin MH, Wolff M, Spencer RC, Hemmer M.** 1995. The prevalence of nosocomial infection in intensive care units in Europe. Results of the European Prevalence of Infection in Intensive Care (EPIC) Study. EPIC International Advisory Committee. JAMA **274:**639–644.
6. **Kollef MH, Ward S.** 1998. The influence of mini-BAL cultures on patient outcomes: implications for the antibiotic management of ventilator-associated pneumonia. Chest **113:**412–420. http://dx.doi.org/10.1378/chest.113.2.412.
7. **Ibrahim EH, Sherman G, Ward S, Fraser VJ, Kollef MH.** 2000. The influence of inadequate antimicrobial treatment of blood stream infections on patient outcomes in the ICU setting. Chest **118:**146–155. http://dx.doi.org/10.1378/chest.118.1.146.
8. **Iregui M, Ward S, Sherman G, Fraser VJ, Kollef MH.** 2002. Clinical importance of delays in initiation of appropriate antibiotic treatment for ventilator-associated pneumonia. Chest **122:**262–268. http://dx.doi.org/10.1378/chest.122.1.262.
9. **Fabregas N, Ewig S, Torres A, El-Ebiary M, Ramirez J, de La Bellacasa JP, Bauer T, Cabello H.** 1999. Clinical diagnosis of ventilator-associated pneumonia revisited: comparative validation using immediate postmortem lung biopsies. Thorax **54:**867–873. http://dx.doi.org/10.1136/thx.54.10.867.
10. **Woodhead MA, Torres A.** 1997. Definition and classification of community-acquired and nosocomial pneumonia, p 1–12. *In* Torres A, Woodhead MA (ed), Pneumonia. European Respiratory Society Journals, Sheffield, United Kingdom.
11. **Luna CM, Blanzaco D, Niederman MS, Matarucco W, Baredes NC, Desmery P, Palizas F, Menga G, Rios F, Apezteguia C.** 2003. Resolution of ventilator-associated pneumonia: prospective evaluation of the clinical pulmonary infection score as an early clinical predictor of outcome. Crit. Care. Med. **31:**676–682. http://dx.doi.org/10.1097/01.CCM.0000055380.86458.1E.
12. **American Thoracic Society.** 1996. Hospital-acquired pneumonia in adults: diagnosis, assessment of severity, initial antimicrobial therapy, and preventative strategies. A consensus statement, American Thoracic Society, November 1995. Am. J. Respir. Crit. Care. Med. **153:**1711–1725. http://dx.doi.org/10.1164/ajrccm.153.5.8630626.
13. **Yager P, Edwards T, Fu E, Helton K, Nelson K, Tam MR, Weigl BH.** 2006. Microfluidic diagnostic technologies for global public health. Nature **442:**412–418. http://dx.doi.org/10.1038/nature05064.
14. **Whitesides GM.** 2006. The origins and the future of microfluidics. Nature **442:**368–373. http://dx.doi.org/10.1038/nature05058.
15. **Poritz MA, Blaschke AJ, Byington CL, Meyers L, Nilsson K, Jones DE, Thatcher SA, Robbins T, Lingenflter B, Amiott E, Herbener A, Daly J, Dobrowoiski SF, Teng DH, Rinie KM.** 2011. FilmArray, an automated nested multiplex PCR system for multipathogen detection: development and application to respiratory tract infection. PLoS One **6:**e26047. http://dx.doi.org/10.1371/journal.pone.0026047.
16. **Rello J, Vidaur L, Sandiumenge A, Rodriguez A, Gualis B, Boque C, Diaz E.** 2004. Deescalation therapy in ventilator-associated pneumonia. Crit. Care. Med. **32:**2183–2190.
17. **Vincent J-L, Sakr Y, Sprung CL, Ranieri VM, Reinhart K, Gerlach H, Moreno R, Carlet J, Le Gall JR, Payen D, Sepsis Occurrence in Acutely Ill Patients Investigators.** 2006. Sepsis in European intensive care units: results of the SOAP study. Crit. Care. Med. **34:**344–353. http://dx.doi.org/10.1097/01.CCM.0000194725.48928.3A.
18. **Vincent J-L, Rello JJ, Marshall J, Silva E, Anzueto A, Martin CD, Moreno R, Lipman J, Gomersall C, Saker Y, Reinhart K, EPIC II Group of Investigators.** 2009. International study of the prevalence and outcomes of infection in intensive care units. JAMA **302:**2323–2329. http://dx.doi.org/10.1001/jama.2009.1754.
19. **Lim WS, Baudouin SV, George RC, Hill AT, Jamieson C, Le Jeune L, Macfarlane JT, Read RC, Roberts HJ, Levy ML, Wani M, Woodhead MA, Pneumonia Guidelines Committee of the BTS Standards of Care Committee.** 2009. BTS guidelines for the management of community acquired pneumonia in adults: update 2009. Thorax **64**(Suppl 3)**:**iii1–iii55. http://dx.doi.org/10.1136/thx.2009.121434.
20. **Stralin K, Backman A, Holmberg H, Fredlund H, Olcen P.** 2005. Design of a multiplex PCR for *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Mycoplasma pneumoniae* and *Chlamydophila pneumoniae* to be used on sputum sample. APMIS **113:**99–111. http://dx.doi.org/10.1111/j.1600-0463.2005.apm1130203.x.
21. **Kee C, Fatovich DM, Palladino S, Kay ID, Pryce TM, Flexman J, Murray R, Waterer GW.** 2010. Specificity of a quantitative real-time polymerase chain reaction assay for the detection of invasive pneumococcal disease: identifying *Streptococcus pneumoniae* using quantitative polymerase chain reaction. Chest **137:**243–244. http://dx.doi.org/10.1378/chest.09-2185.
22. **Johansson N, Kalin M, Tiveljung-Lindell A, Giske CG, Hedlund J.** 2010. Etiology of community–acquired pneumonia: increased microbiological yield with new diagnostic methods. Clin. Infect. Dis. **50:**202–209. http://dx.doi.org/10.1086/648678.