

## Rapid Diagnosis of Acute Respiratory Infections by Multiplex Endpoint PCR Technology

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Received: 26 Oct 2021

Accepted: 12 Nov 2021

Published: 17 Nov 2021

J Short Name: ACMCR

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### Citation:

Aurelian Udristioiu, Rapid Diagnosis of Acute Respiratory Infections by Multiplex Endpoint PCR Technology. Ann Clin Med Case Rep. 2021; V7(15): 1-5

### Keywords:

Antibiotic resistance; PCR; Gene; DNA; Resistance markers; Microbiology report

## 1. Abstract

**1.1. Introduction:** The multiplex endpoint PCR technology offers a number of potential advantages, results are available in a matter of hours rather than days, the extreme sensibility facilitates detection of even minutes the amounts of pathogen DNA in clinical samples and the test is not significantly affected by prior administration of antibiotics.

**1.2. Aim:** The aim of this work was to rapidly identify the antibiotic resistance the monitoring of pathogen growth at the patients admitted in Hospitalization Intensive Care Unit, with the diagnosis of Community Acquired Pneumonia, (CAP).

**1.3. Method:** The Analyzer Unyvero™ Pneumonia Application was used in detection of pneumonia associated pathogens and their antibiotic resistance genes using the Pneumonia Unyvero™ System, following PCR pathogen species with sequencing of the amplified microbial DNA.

**1.4. Results:** The main pathogens of community acquired pneumonia from the cohort study, 36 cases, (20 males in mean age 35-66 years and 16 females in mean age 40-55 mean years), were Streptococcus pneumonia, (16 cases), Staphilococcus aureus, (10 cases), Klebsiella pneumonia (5 cases) and other important agents were “atypical”, such as Haemophilus Influenzae, Chlamidophila pneumonie and Moraxela cataralis. A case with Acinetobacter baumani and Proteus Sp. was also widely resistance to mefA gene / ermB gene as all cases of analyzed. The more frequency of genes resistant (29 cases) are ermA gene / ermC / ermB for Staphilococcus aureus and the gene tem+shv / gene / ctx-M with the Chromosomal mutation (7 cases), as gyrA83\_87 Ecoli / Pseu for Klebsiella

pneumonia agents.

Also, most resistance antibiotics were Makrolides, (29 cases and Lincosamides (6 cases) and these cases have had the chromosomal integrates. The most resistance microbe, Pseudomonas aeruginosa (1 case), has been registered as multi drugs resistance [MDR]\*.

**Conclusion** The Unyvero™ results have been available 2 days before the primary microbiology report and 3 days before the final confirmation results, obtained by microbiology culture. The Unyvero Analyzer only provides rapid data to support the therapeutic decision of currant medic.

## 2. Introduction

The high number of severe acute infections from the whole world raised concerns about growing antibiotic resistance as a major burden for today's health care system. While microbiological culture is likely to remain a gold standard for infection diagnosis, there is growing interest at the potential of PCR technology to provide early, time critical information based on detection and recognition of bacterial or fungal pathogen DNA. By this new modern method, the results are chosen in perquisite for giving adequate antibiotics treatment as early as possible in order to improve the standard of care.

Incorporating the new multiplex molecular analysis, these technologies can combine pathogens identification and antibiotics sensitivity, testing in a single automated procedure within hours after collecting the patient sample. The focus of researches now is to move beyond detecting single analyte to multiplex targets and detect more pathogens from a single specimen, ex. Sputum [1].

## 2.1. Principle of the Analysis

The Unyvero™ System Pneumonia Application automates gave us presentations and discussed in latest information on pneumonia diagnosis. The analyzer has integrates in a disposable cartridge, genomic DNA purification, eight parallel multiplex end-point PCR reactions and the qualitative detection of the target amplicons after hybridization onto an array.

## 2.2. Technique

1. The patient sample is pipetted into the Unyvero™ Sample Tube using the Unyvero™ Sample.
2. Transfer Tool, closed with the Unyvero™ Sample Tube Cap, and lysed with the Unyvero™ Lysator.
3. Subsequently, the Unyvero™ Sample Tube and the Unyvero™ Master Mix Tube are inserted into the Unyvero™ Pneumonia Cartridge.
4. The Unyvero™ Pneumonia Cartridge is then inserted into the Unyvero™ Analyzer, which processes it automatically.

The supplied software guides the user through the entire work flow. A bar code reader allows the entry patients data, checks the shelf-life of consumables and stores their lot numbers. The full analysis should take approximately 30 minutes. The analysis of the patient samples is shown by grey test bars on the overview screen. To view the results, tap on the corresponding blue test bar.

A screen opens and shows the following buttons: Summary, Microorganisms, Resistance Markers, information. In the middle of the screen, the respective antibiotic classes for which a therapeutic failure must be considered if they were administered are displayed. On the right side of the screen, the common microbial source of the resistance markers is displayed.

## 2.3. Detection Limits

Following PCR, pathogen species presented can be identified sequencing of the amplified DNA. Detection limits for each pathogen were determined with pathogen dilutions in buffer. At the concentration of 10<sup>6</sup> pathogens / mL all analytes are detected with the Unyvero™ P50 Pneumonia Cartridge. In addition, the majority of the analytes are positively at a concentration of 10<sup>4</sup> pathogens / ML (*S. marcescens*, *S. maltophilia*, *A. baumannii*, *L. pneumophila*, *S. aureus*, *M. organii*, *K. pneumoniae*, *K. oxytoca*, *P. aeruginosa*).

## 2.4. Interfering Substances

Interferences were tested in suitable pools with respiratory drugs, common antibiotics and sample media or individually for example for lyse buffer, blood, human DNA, and common respiratory pathogens, which might be present in respiratory samples. Worst case concentrations were used according to CSLI guideline “EP7-A2. No interference was observed.

## 2.5. Sensitivity & Specificity

The Unyvero™ Pneumonia Application achieved an overall sen-

sitivity of 75.5 % (sensitivity per analyte between 50% and 100%, depending on the microorganism) at an overall specificity of 95.2% (72.3% to 100%, depending on the microorganism). For rare pathogens, the number of cases was insufficient to establish sensitivity and specificity data. For detected resistance markers (genes like *mefA*, *ermA*, *ermB*, *ermC*, *tem*, *shv*, *dha*, *oxa 51* like, *ctx M*, *mecA*, *ebc*, quinolone resistance in *E. coli* and *P. aeruginosa*) in 36 cases out of 32 antibiotic resistant pathogens a correlation between Unyvero P50™ results with the antibiogram was demonstrated. Actually, the firm Curetis is currently conducting a prospective European multicenter clinical trial to generate more clinical performance data.

## 3. Method

Sample type, aspirates sputum from the cohort study, 36 cases, (20 males in mean age 35-66 years and 16 females in mean age 40-55 mean years), patents admitted in Hospitalization Intensive Care Unit with the diagnosis of Community Acquired Pneumonia, (CAP), were performed on the Analyzer Unyvero™ Pneumonia Application, in the day after specimen collection, in the department of Microbiology, from Clinical Laboratory Analyses of Emergency County Hospital. The selection of the samples at the patients admitted in Intensive Care Unit (ICU) with community acquired pneumonia were based on typical clinical signs of severe infection, in evidences of clinician doctors which were included the symptoms such as increased fever, positive X-ray, presence of purulent sputum and on the results of laboratory blood samples with increased white blood cell count, ( $>25000/\text{mm}^3$ ), VSH  $>40$  mm/h, Fibrinogen ( $>550$  mg/dl) and Protein C Reactive ( $>12$  mg/dl). These signs of severe acute infection were primordially for faster results in pneumonia testing. Such quick results from laboratory are requisite for giving adequate antibiotic treatment as early as possible in order to improve the standard of care.

## 4. Results

The main pathogens of community acquired pneumonia from the cohort study were *Streptococcus pneumoniae* (16 cases), *Staphylococcus aureus*, (10 cases), *Klebsiella pneumoniae* (5 cases), and other important agents were “atypical”, such as *Haemophilus Influenzae*, *Chlamidophila pneumonie* and *Moraxela cataralis*. A case with *Acinetobacter baumani* and *Proteus Sp.* was also widely resistance to *mefA* gene / *ermB* gene as all cases of analyzed. The more frequency of genes resistant (29 cases) are *ermA* gene / *ermC* / *ermB* for *Staphylococcus aureus* and the gene *tem+shv* / gene / *ctx-M* with the Chromosomal mutation (7 cases), as *gyrA83\_87 Ecoli* / *Pseu* for *Klebsiella pneumoniae* agents.

Also, most resistance antibiotics were Makrolides, (29 cases and Lincosamides (6 cases) and these cases have had the chromosomal integrates. The most resistance microbe, *Pseudomonas aeruginosa*, (1 case), has been registered as multi drugs resistance [MDR]\*, (Table 1).

**Table 1.** Resistance markers of the Pneumonia panel and the resulting antibiotic resistance.

| No. IDL | Microorganisms detected  | Antibiotic resistance   | Gene resistance  |
|---------|--|---|--|
| 1310_1  | Klebsiella pneumonia   | Makrolides /, [ermB],].Lincosamides   | ermB gene  |
| 1310_2  | Streptococcus Sp.  | Makrolides.   | ermB gene/tem gene   |
| 1310_3  | Staphilococcus aureus<br>Other/Fungi: Chlamidophila pneumonie            | Penicilins (tem)  | ermB gene/tem gene Chromosomal mutation; Pseud. aeruginosa, (gyrA83-Ecoli)                       |
| 1310_4  | Klebsiella pneumonia   | Makrolides / Lincosamides   | ermB gene  |
| 1310_5  | Proteus Sp. Other/Fungi: Haemophilus Influenzae, Chlamidophila pneumonie | Makrolides<br>Oxacilin  | ermB gene/oxa51<br>Chromosomal mutation;<br>Escherichia Coli (gyrA83-87 Ecoli ).                 |
| 1311_1  | Streptococcus pneumonia Moraxela catarrhalis                             | Makrolides,<br>Oxacillin Lincosamides,  | ermB gene/tem gene/ mecA gene  |
| 1311_2  | Streptococcus pneumonia  | Makrolides / [mefA], [ermB],].Lincosamides<br>Penicilins (tem)  | mefA gene / ermB gene / tem  |
| 1311_3  | Streptococcus pneumonia<br>Pseudomonas aeruginosa                        | Makrolides / [mefA],<br>[ermB],].Lincosamides<br>[MDR]* [int1].   | mefA gene / ermB gene<br>[int1 gene].  |
| 1311_4  | Klebsiella pneumonia   | Penicilins (shv)  | mefA gene / shv gene [int1 gene] /sul 1 gene<br>Chromosomal mutation; (gyrA83_3Pseu).            |
| 1311_5  | Streptococcus pneumonia<br>Acinetobacter baumani                         | Makrolides / [mefA],<br>[ermB],].Lincosamides   | mefA gene / ermB gene  |
| 1312_1  | Staphilococcus aureus<br>Klebsiella pneumonia                            | Makrolides / [mefA],<br>[ermB],].Lincosamides<br>3 <sup>rd</sup> Gen Cephalosporins [tem+shv], [ctx-M]. | ermA gene / ermC / ermB<br>tem+shv / gene / ctx-M g Chromosomal mutation; gyrA83_87 Ecoli / Pseu |

#### 4. Interpret Results

The green boxes on the Analyzer of the Unyvero™ Pneumonia Application and values do loosely correlate with the amount of detected DNA and therefore with the number of pathogens in a given patient sample, however the number of pathogens obtained by culture does not always correlate with the number of pathogens in a sample due to limitations of growth. These numbers are reflecting a threshold value depending on the species and serve to give an aid to quantification. (< 250 no green box; 250 – 499 one green box, 500 – 999 two green boxes, >= 1000 three green boxes). It was a specific customer demand to have some form of number. The clinician may still take this data into consideration e.g. in an immune compromised patient and with certain pathogens. Still, a clinician may take this data into consideration e.g. in an immune compromised patient and with certain pathogens. These results were con-

firmed by microbiology culture; however, the final microbiology results were available 3 days after the Unyvero™ results [2].

The Classical Method Kirby Bauer Disc Diffusion Method

As an example, in the Culture 1310\_1 raised Klebsiella pneumoniae (+++) and sensible antibiotics were Amoxicillin, Sulfonamide Cotrimoxazol, Quinolone Moxifloxacin, Monobactame Aztreonam, Cephalosporin Cefotaxim and Ampicillin resistance, (R), Amoxic./Clavulanacid(R), Piperacillin (R) , Piperac/Tazobactam (R), resistance spread especially on our intensive care unit.

#### 5. Discussions

The Unyvero™ Pneumonia Cartridge can detect the following microorganisms: Acinetobacter baumannii, Chlamydomphila pneumoniae, Enterobacter sp., Escherichia coli, Haemophilus influenzae, Klebsiella pneumoniae, Klebsiella oxytoca, Legio-

nella pneumophila, *Moraxella catarrhalis*, *Morganella morganii*, *Pneumocystis jirovecii*, *Proteus* sp., *Pseudomonas aeruginosa*, *Serratia marcescens*, *Staphylococcus aureus*, *Stenotrophomonas maltophilia* und *Streptococcus pneumoniae*. Simultaneously, the following genes associated with antibiotic resistance are detected in the same cartridge: *ctx-M*, (Cephalosporins, Penicillins), *dha* (Cephalosporins), *ebc* (3rd Gen. Cephalosporins), *ermA*(Macrolides / Lincosamides), *ermB* (Makrolides / Lincosamides) , *ermC* (Macrolides / Lincosamides) , *gyrA 83*, (*E. coli* ), *gyrA87*, *int1*, *kpc*, *mecA*, *mefA/E*, *msrA*, *oxa51* like, *parC*, *shv*, *sul1*, and *tem* (Fluoroquinolones, *E. coli*) [3]. In our cases from the study, the main pathogens of community acquired pneumonia are *Streptococcus pneumoniae*, and *Haemophilus influenzae* but inappropriate therapy is linked to the presence of resistance organism such as *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Acinetobacter*. In *Streptococcus pneumoniae* resistance to macrolides and tetracycline depends largely on acquired genes, as does tetracycline and trimethoprim resistance in *Haemophilus influenzae* whereas, in both species, quinolone resistance is a mutation. In case of *S.aureus* methicillin resistance, (MRSA), is determined by the acquired *mecA* gene which is easily detected by multiplex endpoint PCR technology, respectively Unyvero™ Pneumonia Analyze. A few resistances in Enterobacteriaceae are largely or entirely mutational, the best example being quinolone resistance, which mostly occurs by mutation of *gyrA* genes, like in the present study *Acinetobacter baumannii* acquires antibiotic resistance by a number of mechanisms. Resistance is often associated with acquired genes, with these various compromising penicillins, aminoglycosides, tetracyclines, antifolates and chloramphenicol. In some cases these genes are carried on plasmids, as in Enterobacteriaceae, in others they are chromosomally integrated, often in large "resistance islands". Resistance to carbapenems may involve acquired carbapenem genes, but can also arise by activating "bla gene" which occurs in all *A. baumannii* isolates but is not ordinarily expressed. In *Pseudomonas aeruginosa* (1 case), the detection of multi drug resistance [MDR]\*, was achieved by two family of plasmid encoded *ampC* genes and by three classical Amber class A beta-lactamase (*tem*, *shy*, *ctx-M*).

### 5.1. Transferable Antibiotics Resistance (Resistance Genes)

Most resistance markers that are detected by the Unyvero™ Pneumonia Application are genes, which are transferred by mobile genetic elements like plasmids or integrons [6]. Most resistance in Enterobacteriaceae is associated with acquired resistance genes often carried by plasmids, variously encoding  $\beta$ -lactamases, aminoglycosides, modifying enzymes, r-RNA methylases or efflux pump. Presence of such a gene correlates with a resistance against a particular antibiotic class [4].

Gene acquisitions mutations and gene of mosaic are all important.

The general pattern is that resistance mostly arises by gene acquisition in Enterobacteriaceae [5]. In order to control the spread of resistance it is important to have an understanding of the molecular biology of the different mobile genetic elements and of the ecology of the environments in which spread is likely [6].

Antibiotic resistance occurs by different mechanisms in different pathogens. Mutational changes conferring important resistances can include reduced cytoplasmic membrane transport, resulting in resistance to aminoglycosides, up-regulation of efflux, compromising antibiotics depending on particular pumps affected, DNA mutation resulting in resistance to quinolones [7]. Modification of enzymes is major aminoglycoside resistance mechanism.

According to this modification, the involved proteins are classified into the 3 major classes of acetyltransferases (AACs), nucleotidyltransferases, and adenytransferases (ANTs). Macrolides share a similar mode of antibacterial action with, and have comparable antibacterial spectra as, the antibiotic classes of streptogramins B and lincosamides. As a result, despite the fact that they are chemically distinct, these antibiotics have been described as the single class of macrolide-lincosamide-streptogramin B (MLS) antibiotics [8].

Resistance to  $\beta$ -lactam antibiotics involve a complex mixture of mutation and mosaic gene formation in *Streptococcus pneumoniae*. Also,  $\beta$ -lactam antibiotics inhibit cell-wall synthesis by binding to penicillin-binding proteins (PBPs) within bacteria and by interfering with structural crosslinkage of peptidoglycans. These actions prevent terminal transpeptidation in the cell walls of bacteria.

*Staphylococcus methicillin* (MLS) resistance occurs because of the presence of r-RNA methylases, encoded by ERM genes. Inactivating genes and efflux pumps which constitute the other 2 mechanisms for this result, are encoded by ERE and MSR determinants, respectively [9, 10]. Also, in environments in which bacteria reside closely together and in relatively high density, such as the oral cavity and the gut, gene transfer is more likely. To control the spread of resistance, laboratory professionals must comprehend the molecular biological characteristics of the ecology of the environments in which spread is likely and of the different mobile genetic elements [11].

The Unyvero™ result was available 2 days before the primary microbiology report and 3 days before the final classical culture method, confirmation test. A more adequate and result guided antibiotic therapy regime with the usage of an ESBL active carbapenem would have been made possible much earlier. In addition, appropriate hygiene measures could have been taken earlier decreasing the risk of antibiotic [12]. The carbapenem resistance in emerging in *Klebsiella pneumoniae* involves the genes *Oxa-48*, *Pseu*, detectable by PCR [13]. The isolates were identified by conventional methods [14]. All isolates were non duplicate. *Escherichia coli* ATCC 25922 was used as quality control strain.

In the present studies, the majority of the isolates (95%) from bronchial secretions were carbapenem resistant. High resistance rates to carbapenems have been observed in previous studies, ranging from 75% to 100% for imipenem and from 61% to 77% for meropenem [15]. The emergence of *A. baumannii* strains with increased carbapenem resistance in this area of the world may be due to the extensive misuse of carbapenems [16]. The most prevalent mechanism of carbapenem resistance in *A. baumannii* is the enzymatic degradation by carbapenem-hydrolyzing  $\beta$ -lactamases, MBL, mostly VIM and IMP, has been reported sporadically in some parts of the world [17].

## 6. Conclusions

Rapid molecular diagnosis assays has the potential to speed up pathogen and resistance identification, which may enable clinicians to make an early, informed diagnosis in patients with pneumonia. The Unyvero only provides data to support the therapeutic decision. While microbiological culture is likely to remain a gold standard for infection diagnosis, there is growing interest at the potential of PCR technology to provide early, time critical information based on detection and recognition of bacterial pathogen DNA. The most important feature in our cases of this study, is the correct detection for *Klebsiella pneumoniae* and *A. baumannii*, these results potentially would have had great impact on the antibiotic therapy regime as well as hygiene measures, patient isolation and preventing of nosocomial infections.

## References

1. Caliendo AM. Multiplex PCR and emerging technologies for the detection of respiratory pathogen. *Clin Infect.* 2011; (4): 5326-5330.
2. Rulls<sup>®</sup> Curetis AG. Unyvero™ P50 Pneumonia Application Guide 00118 V2.0, Hamburg, Germany, 2013.
3. Kugelberg E, Lofmark S, Wretling B, Andersson ID. Reduction of the fitness burden of quinolone resistance in *Pseudomonas aeruginosa*. *J. Antimicrob. Chemother.* 2005; 55 (1): 22-30.
4. Gerzova L, Videnska P, Faldynova M, Sedlar K. Characterization of Microbiota Composition and Presence of Selected Antibiotic Resistance Genes in Carriage Water of Ornamental Fish. *PLOS ONE Journal Information.* 2014; 9(8): e103865.
5. M van Hoek AHA, Mevius D, Guerra B, Mullany P. Acquired Antibiotic Resistance Genes: An Overview. *Front Microbiol.* 2011; 2: 203.
6. Davies J, Davies D. Origins and Evolution of Antibiotic Resistance. *Microbiol Mol Biol Rev.* 2010; 74(3): 417-433.
7. Wright G D. Bacterial resistance to antibiotics: enzymatic degradation and modification. *Adv Drug Deliv Rev* 2005; 57: 1451-1470.
8. Roberts MC. Update on macrolide-lincosamide, treptogramin, ketolide, and oxazolidinone resistance genes, -ermA, ermB, ermC Makrolides / Lincosamides, gyrA83 of *E. coli*. *FEMS Microbiol. Lett.* 2008; 282: 147-159.
9. Jacoby AG. AmpC  $\beta$ -Lactamases. *Clin Microbiol Rev.* 2009; 22(1): 161–182.
10. Bonnet R, Dutour C, Sampaio MLJ, Chanal C. Novel Cefotaximase (CTX-M-16) with Increased Catalytic Efficiency Due to Substitution Asp-240@Gly. *Antimicrob Agents Chemother.* 2001; 45(8): 2269.
11. Breurec S, Guesseend N, Timinouni M, Le TAH. *Klebsiella pneumoniae* resistant to third-generation cephalosporins in five African and two Vietnamese major towns: multiclonal population structure with two major international clonal groups, CG15 and CG258. *Clinical Microbiology and Infection.* 2013; 19(4): 349-355.
12. Oliviera JE, Silva CA, Oliviera GM, Zaneta DM. *Antimicrob Agents Chemoter.* 2009; 53(7): 2887-289.
13. Livermore MD. Pneumonia-causing pathogens and their resistance. *Curetis Symposium, ECCMID.* 2014.
14. Clinical Laboratory Standards Institute. Performance standards for Antimicrobial Susceptibility Testing: Twentieth Informational Supplement. M100-S20. CLSI: Wayne, PA, USA. 2010.
15. Bonnin RA, Rotimi VO, Al Hubail M, Gasiorowski E, Al Sweih N and Nordmann P. Wide dissemination of GES-type carbapenemases in *Acinetobacter baumannii* isolates in Kuwait. *Antimicrob Agents Chemother* 2013; 57: 183-8.
16. Al-Agamy MH, Khalaf MG, Tawfick MM, Shibl AM, and El Kholy A. Molecular characterization of carbapenem-insensitive *Acinetobacter baumannii* in Egypt *International Journal of Infectious Diseases.* 2014; 22: 49-54.
17. Alm El-Din RA, El-Bassat H, El-Bedewy M, El- Mohamady H. Prevalence of metallo- $\beta$ -lactamases producers among carbapenem-resistant *Acinetobacter baumannii* strains isolated from diabetic foot ulcers. *AJMR.* 2014; 8(15): 1580-1585.