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Research Paper

The Contribution of Multiplex PCR in the Diagnosis of Community Respiratory Infections.

Hamdani Yousra[1], Drani Meryem[1], Mustapha Mahmoud[1], Ghita yahyaoui[1], Kouara Sara[1].

1 Bacteriology and virology unit, central medical analysis laboratory, Centre Universitaire Hospitalier Hassan II, Faculty of Medicine, Pharmacy and Dentistry, Sidi Med Ben Abdellah University, Fez, Morocco.

SUMMARY :
The use of multiplex PCR (mPCR) has significantly improved the diagnosis of community-acquired respiratory infections, enabling the simultaneous detection of several pathogens. This technique offers greater precision and speed, essential for optimal patient management. mPCR has demonstrated high sensitivity and specificity for the identification of viral and bacterial pathogens. As well as being fast, it is also cost-effective, making it ideal for routine clinical use. Studies have shown that mPCR can identify pathogens in patients previously treated with antibiotics, a capability that conventional cultures often lack. The aim of this project is to analyze the epidemiological and bacteriological profile of the main bacterial and viral species and their resistance genes isolated in community-acquired respiratory infections at the Hassan II University Hospital in Fez from January 2021 to december 2024.

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I. INTRODUCTION :

Multiplex PCR is a molecular biology technique that enables the simultaneous amplification of several genetic targets in a single sample. It is based on the use of several pairs of specific primers, each targeting a particular gene of one or more pathogens. This approach enables rapid, simultaneous detection of several pathogens, optimizing the diagnosis of respiratory infections [1].

Community-acquired respiratory infections represent a major global health problem due to their high morbidity and mortality[2]. The rapid rise in antimicrobial resistance in these bacteria complicates treatment options, making effective infection management difficult. This is of particular concern in low-resource settings, where healthcare professionals often turn to empirical antibiotic therapy while awaiting the results of laboratory cultures [3,4].

Rapid and accurate diagnostic methods are essential to identify both pathogens and their resistance genes. These tools would enable healthcare professionals to choose appropriate antibiotic treatments, improving patient outcomes while reducing antibiotic misuse[2,3].

The aim of this study is to develop the role of multiplex PCR in the simultaneous detection of major pathogens and their resistance genes from respiratory samples. This approach aims to overcome the limitations of traditional culture methods, which are often time-consuming and delay appropriate treatment.

II. MATERIALS AND METHODS

This is a retrospective descriptive study conducted over a 04-year period, from January 2021 to decembre 2024. Respiratory samples analyzed in the laboratory included nasopharyngeal swabs, sputum, protected distal swabs and bronchoalveolar lavage.

Samples were analyzed using the BioFire® FilmArray® multiplex PCR test, enabling simultaneous detection of respiratory viruses and bacteria.

The BioFire® Respiratory 2.1 plus panel targeted several viruses, including Adenovirus, Coronavirus (229E, HKU1, NL63, OC43), MERS-CoV, SARS-CoV-2, Human Metapneumovirus, Human Rhinovirus/Enterovirus, Influenza A and B viruses, Parainfluenza 1, 2, 3 and 4 viruses, as well as Respiratory Syncytial Virus. In terms of bacteria, the panel detected *Bordetella parapertussis, Bordetella pertussis, Chlamydia pneumoniae* and *Mycoplasma pneumoniae.*

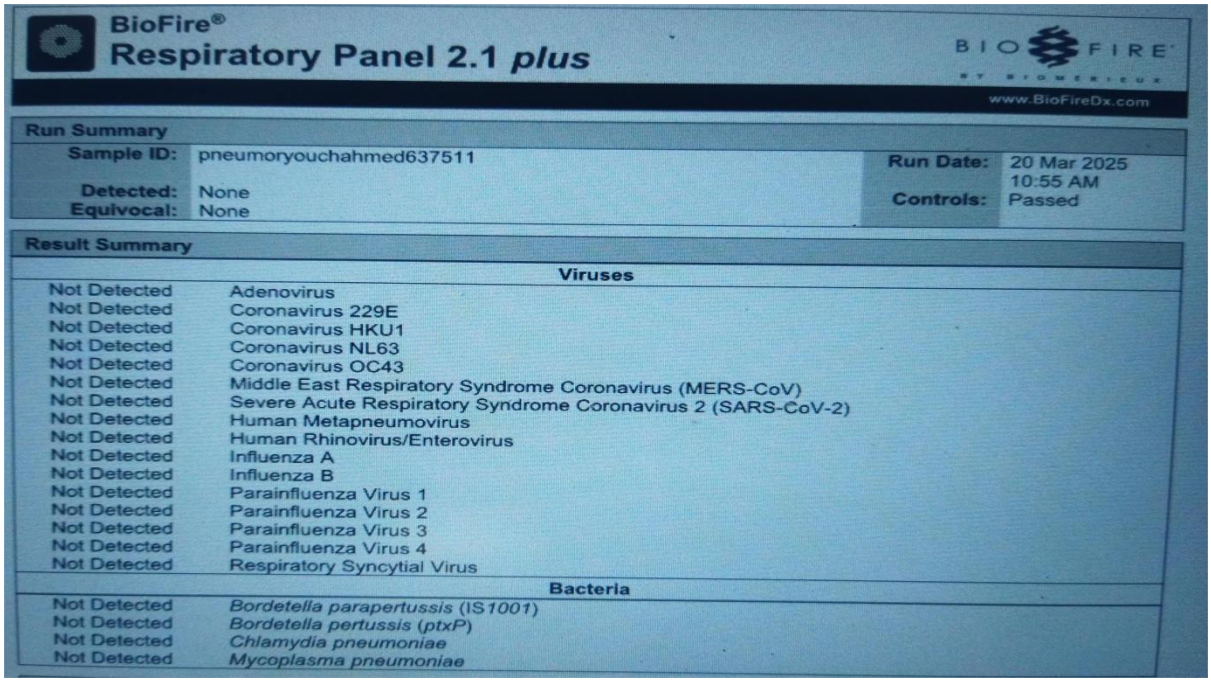
In addition, the FilmArray® Pneumonia plus panel test is a semi-quantitative PCR that facilitates the detection and estimation of microbial load in copies/ml. In addition to identifying respiratory viruses, it also detects additional bacteria such as *Legionella pneumophila*, *Haemophilus influenzae* and *Streptococcus pneumoniae*, as well as resistance genes including: CTX-M, IMP, KPC, mecA/C and MREJ, NDM, OXA-48-like, VIM.

Multiplex PCR is a molecular biology technique that enables the simultaneous amplification of several genetic targets in a single sample. It is based on use of several pairs of specific primers, each targeting a particular gene from one or more pathogens (bacterial or viral).

For deep samples, in particular PDP, sputum and BAL, a cytobacteriological study was carried out in parallel to improve interpretation of results in terms of UFC/ml (table 1).

Table 1: Culture significance limits for deep breath samples

Type of sampling	Significance threshold (CFU/ml)
Sputum	$\geq 10^7$ UFC/ml
Tracheal aspirations	$\geq 10^6$ UFC/ml
BAL (bronchoalveolar lavage)	$\geq 10^4$ UFC/ml
PDP (Protected distal sampling)	$\geq 10^3$ UFC/ml


BioFire®
Respiratory Panel 2.1 *plus*
BIOFIRE BY BIOMÉRIEUX
www.BioFireDx.com

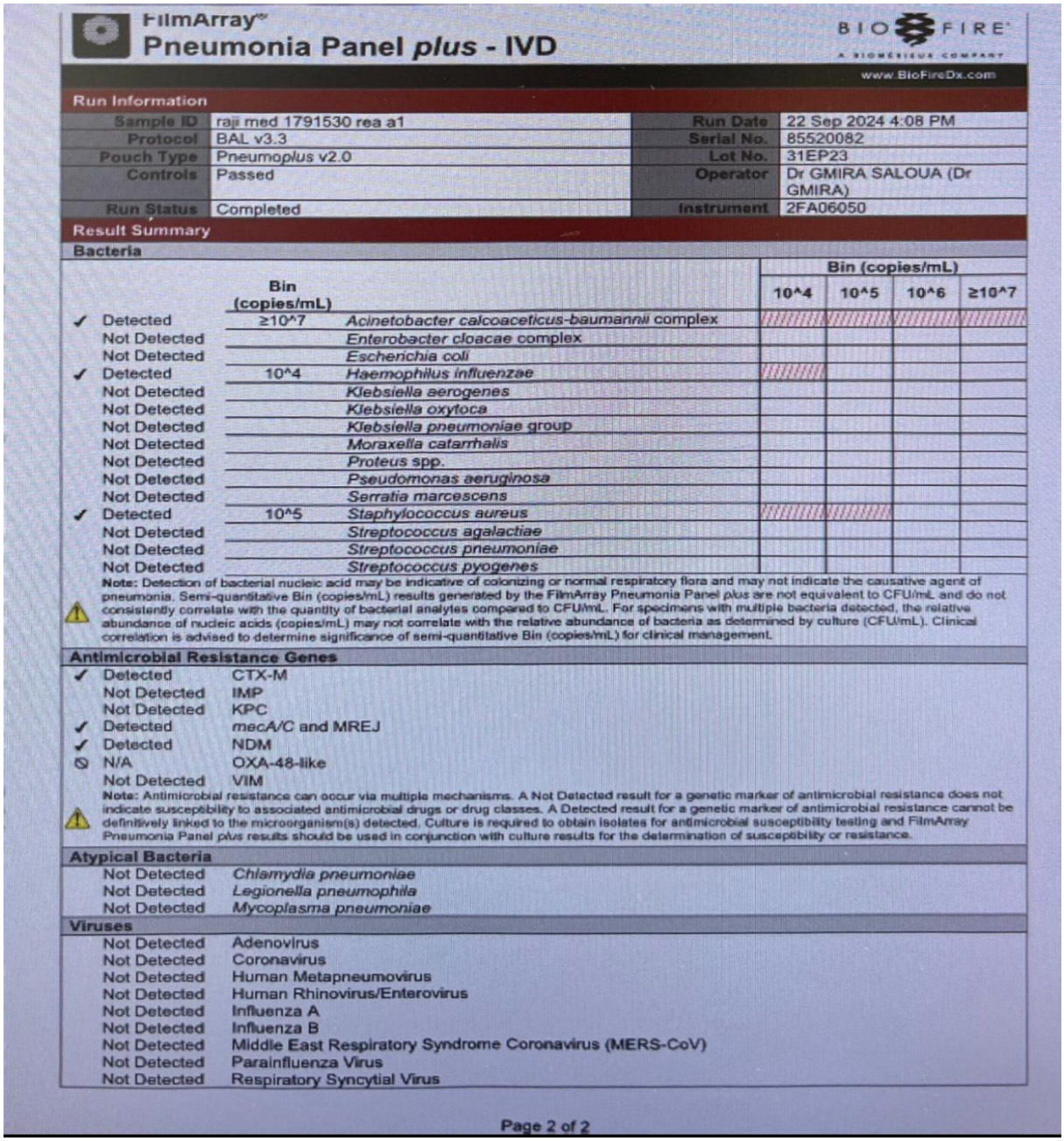
Run Summary

Sample ID:	pneumoryouchahmed637511	Run Date:	20 Mar 2025 10:55 AM
Detected:	None	Controls:	Passed
Equivocal:	None		

Result Summary

	Viruses
Not Detected	Adenovirus
Not Detected	Coronavirus 229E
Not Detected	Coronavirus HKU1
Not Detected	Coronavirus NL63
Not Detected	Coronavirus OC43
Not Detected	Middle East Respiratory Syndrome Coronavirus (MERS-CoV)
Not Detected	Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2)
Not Detected	Human Metapneumovirus
Not Detected	Human Rhinovirus/Enterovirus
Not Detected	Influenza A
Not Detected	Influenza B
Not Detected	Parainfluenza Virus 1
Not Detected	Parainfluenza Virus 2
Not Detected	Parainfluenza Virus 3
Not Detected	Parainfluenza Virus 4
Not Detected	Respiratory Syncytial Virus
	Bacteria
Not Detected	*Bordetella parapertussis* (IS*1001*)
Not Detected	*Bordetella pertussis* (*ptxP*)
Not Detected	*Chlamydia pneumoniae*
Not Detected	*Mycoplasma pneumoniae*

Figure 1: BioFire® Respiratory 2.1 plus panel



FilmArray®
Pneumonia Panel *plus* - IVD
BIOFIRE
A BIOMÉRIEUX COMPANY
www.BioFireDx.com

Run Information

Sample ID	raji med 1791530 rea a1	Run Date	22 Sep 2024 4:08 PM
Protocol	BAL v3.3	Serial No.	85520082
Pouch Type	Pneumo*plus* v2.0	Lot No.	31EP23
Controls	Passed	Operator	Dr GMIRA SALOUA (Dr GMIRA)
Run Status	Completed	Instrument	2FA06050

Result Summary

Bacteria

		Bin (copies/mL)				
		10^4	10^5	10^6	≥10^7	
✓ Detected	≥10^7	*Acinetobacter calcoaceticus-baumannii* complex				
Not Detected		*Enterobacter cloacae* complex				
Not Detected		*Escherichia coli*				
✓ Detected	10^4	*Haemophilus influenzae*				
Not Detected		*Klebsiella aerogenes*				
Not Detected		*Klebsiella oxytoca*				
Not Detected		*Klebsiella pneumoniae* group				
Not Detected		*Moraxella catarrhalis*				
Not Detected		*Proteus* spp.				
Not Detected		*Pseudomonas aeruginosa*				
Not Detected		*Serratia marcescens*				
✓ Detected	10^5	*Staphylococcus aureus*				
Not Detected		*Streptococcus agalactiae*				
Not Detected		*Streptococcus pneumoniae*				
Not Detected		*Streptococcus pyogenes*				

Note: Detection of bacterial nucleic acid may be indicative of colonizing or normal respiratory flora and may not indicate the causative agent of pneumonia. Semi-quantitative Bin (copies/mL) results generated by the FilmArray Pneumonia Panel *plus* are not equivalent to CFU/mL and do not consistently correlate with the quantity of bacterial analytes compared to CFU/mL. For specimens with multiple bacteria detected, the relative abundance of nucleic acids (copies/mL) may not correlate with the relative abundance of bacteria as determined by culture (CFU/mL). Clinical correlation is advised to determine significance of semi-quantitative Bin (copies/mL) for clinical management.

Antimicrobial Resistance Genes

✓ Detected	CTX-M
Not Detected	IMP
Not Detected	KPC
✓ Detected	*mecA/C* and MREJ
✓ Detected	NDM
⊘ N/A	OXA-48-like
Not Detected	VIM

Note: Antimicrobial resistance can occur via multiple mechanisms. A Not Detected result for a genetic marker of antimicrobial resistance does not indicate susceptibility to associated antimicrobial drugs or drug classes. A Detected result for a genetic marker of antimicrobial resistance cannot be definitively linked to the microorganism(s) detected. Culture is required to obtain isolates for antimicrobial susceptibility testing and FilmArray Pneumonia Panel *plus* results should be used in conjunction with culture results for the determination of susceptibility or resistance.

Atypical Bacteria

Not Detected	*Chlamydia pneumoniae*
Not Detected	*Legionella pneumophila*
Not Detected	*Mycoplasma pneumoniae*

Viruses

Not Detected	Adenovirus
Not Detected	Coronavirus
Not Detected	Human Metapneumovirus
Not Detected	Human Rhinovirus/Enterovirus
Not Detected	Influenza A
Not Detected	Influenza B
Not Detected	Middle East Respiratory Syndrome Coronavirus (MERS-CoV)
Not Detected	Parainfluenza Virus
Not Detected	Respiratory Syncytial Virus

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Figure 2: FilmArray® Pneumonia plus panel

III. RESULTS

A total of 641 samples were received at the bacteriology department of the Hassan II Hospital in Fez, of which 346 (53.9%) were positive. Positive samples were predominantly from children (59%) and adults (41%). In terms of gender, males predominated, accounting for 58% of cases, with a sex ratio of 1.3 and an average age of 15.

The breakdown of positive samples by department shows a predominance of mother and child intensive care units (33.5%), followed by pediatric emergencies (21%), pneumology (12.3%), pediatrics (11%) and adult intensive care units (9.58%).

In children, 81.28% of infections are viral, while 18.72% are bacterial. In adults, viral infections account for 96.29%, versus 3.71% for bacterial infections.

In children, 37.02% of cases were positive for Human Rhinovirus/Enterovirus. Influenza B accounted for 18.08% of infections, followed by respiratory syncytial virus at 10%, *Bordetella pertussis* at 9.40% and *streptococcus pneumoniae* at 7.05%. Coronavirus accounted for 6%, while influenza A infections accounted for 3.50% of cases. Other germs accounted for 8.95% of infections. These data illustrate the distribution of different germs in children, with a notable prevalence of Human Rhinovirus/Enterovirus and Influenza B.

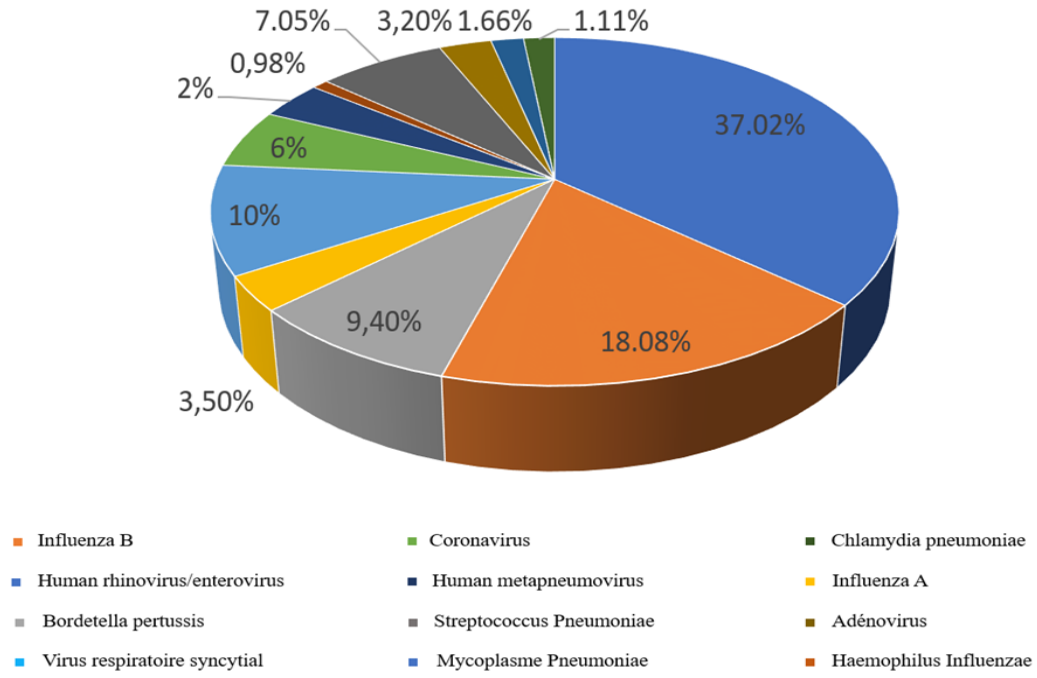

***Figure 3* : Répartition des germes chez l'enfant**

In adults, viruses were dominant, with Influenza A accounting for 27.94% of cases, followed by respiratory syncytial virus at 22.05%, adenovirus at 14.7% and coronavirus at 12.6%. Other viruses included Influenza B (5.8%), human rhinovirus and enterovirus (4.41%), parainfluenza viruses (7.35%) distributed as follows: parainfluenza 1 (0.65%), 2 (2.5%), 3 (2.7%) and 4 (1.5%), and human metapneumovirus (2.94%). In terms of bacteria, *Streptococcus pneumoniae* was present in 1.3% of cases, followed by *Mycoplasma pneumoniae* (0.5%) and *Haemophilus influenzae* (0.41%), while *Chlamydia pneumoniae* was not detected (0%).

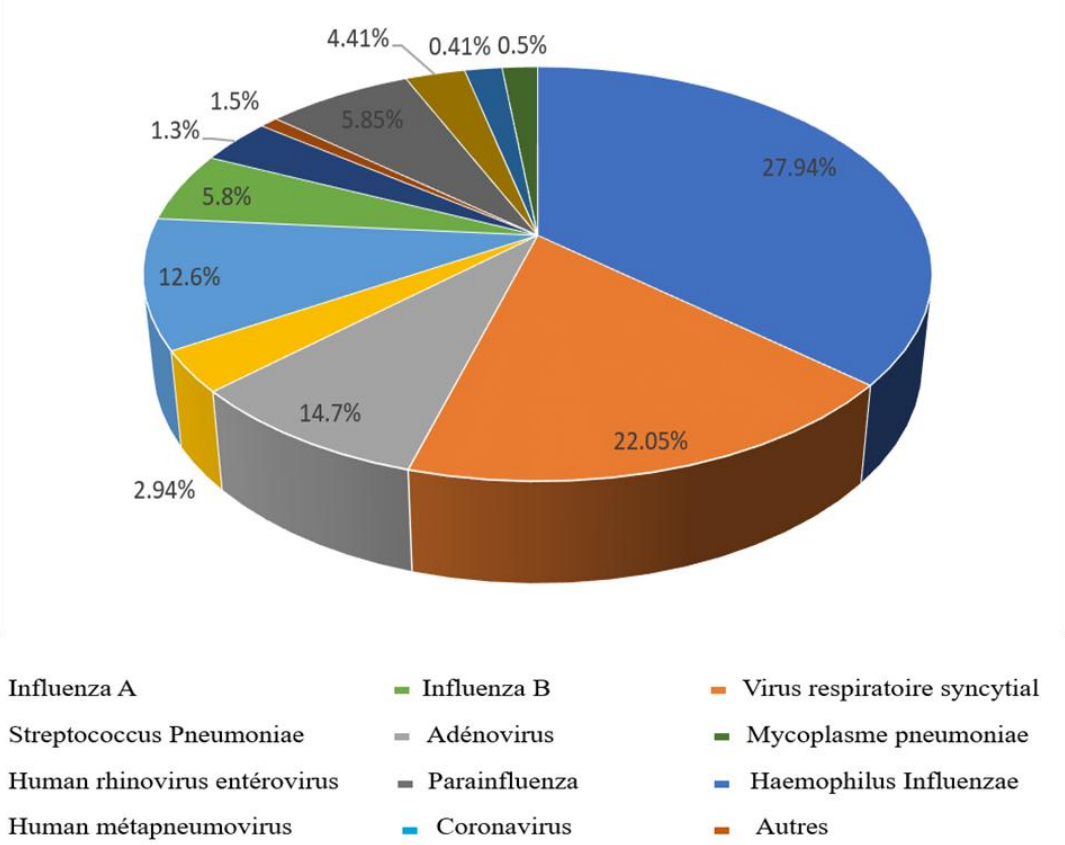

Figure 4 **: Répartition des germes chez l'adulte**

Infection frequency varies with the season. Figure 3 shows a noticeable drop in infections in June and July, with peaks in March and November, indicating a seasonal variation in infections, with periods of high and low frequency throughout the year.

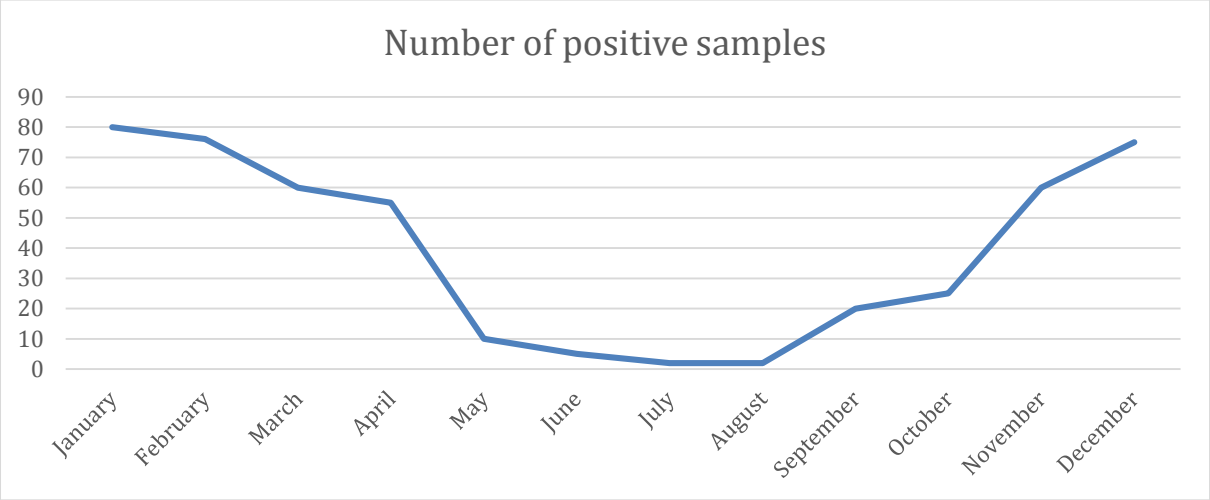

Figure 5: Overall frequency of infections by season

Respiratory samples that tested positive for *Streptococcus pneumoniae* and *Haemophilus influenzae* in multiplex PCR were subjected to concomitant culture. After excluding samples that had not been cultured, three were positive for *Streptococcus pneumoniae* and five for *Haemophilus influenzae* (table 2).

Table 2: Germ distribution by M-PCR and culture

germs	Pcr mltiplex	culture
Streptococcus pneumonia	8	3
Haemophilus influenzae	14	5

IV. DISCUSSION

Benefits of PCR :

Multiplex PCR improves the diagnosis of community-acquired respiratory infections by simultaneously identifying several pathogens, while improving detection rates:

Rapid diagnosis:Community-acquired respiratory infections (CARI) are a major health burden worldwide, and a leading cause of morbidity and mortality. Although traditional culture remains the "gold standard" for pathogen detection, culture results can take up to 48 to 96 hours, forcing providers to treat patients empirically. Advances in PCR-based diagnostic tests have dramatically improved the speed and accuracy of detecting respiratory tract infections in patient samples. Accurate and rapid diagnosis of respiratory tract infections is not only crucial for targeted treatment and patient management, but also for antimicrobial stewardship [3,4]. However, the main challenge in the adoption of PCR-based tests remains the interpretation of PCR versus culture results. A study by Singh et al. compared and correlated colony-forming unit per milliliter (CFU/mL) of known culture samples to cycle threshold (Ct) values obtained from a multiplex PCR assay to provide accurate and actionable results to healthcare providers.The results of this study showed a correlation between Ct values obtained by PCR and UFC/ml values obtained by the respiratory culture method. At 10^5 CFU/ml, mean Ct was 19.9, with a range of 17.67-21.7 (± 1.1 SD) for Gram-negative bacteria. For Gram-positive bacteria, *Streptococcus pneumoniae* and *Staphylococcus aureus*, the mean Ct value was 23.9, range 19.8-28 (± 4.1 SD). The study also demonstrated a direct correlation between qRT-PCR and traditional respiratory microbial cultures. Its results also demonstrate the feasibility of quantitative interpretation of molecular results, helping providers to make treatment decisions based on PCR results. Given the faster turnaround times and superior sensitivity and specificity of PCR compared with microbial culture[4].

In our study, the average processing time for the respiratory panel for respiratory samples was around 3 hours, from receipt in the laboratory to transmission of results. In contrast, culture of bacteria from respiratory samples took 72 hours. These results underline the diagnostic advantage of multiplex PCR, enabling rapid identification of bacterial pathogens compared with conventional culture methods.

In our study, the dominant pathogens in community-acquired respiratory infections (CARI) varied according to age group. In children, Rhinovirus/Enterovirus (37.02%) and Influenza B (18.08%) predominated, followed by Bordetella pertussis (9.4%) and Streptococcus pneumoniae (7.05%). These results correspond well with general epidemiological data, Rhinovirus/Enterovirus being recognized as the main agent of pediatric CKD, while Respiratory Syncytial Virus (RSV), although less frequent here, is traditionally associated with winter peaks (November/March). The presence of Bordetella pertussis underlines its continuing importance in areas with insufficient vaccination coverage. In adults, Influenza A (27.94%) and RSV (22.05%) dominate, followed by Adenovirus (14.7%) and Coronavirus (12.6%). These observations are in line with recent studies (Bogdan et al., 2023) [5] on severe CKD, although this low rate is explained by the use of real-time PCR techniques for the detection of SARS-CoV. These results reinforce the importance of these key pathogens, while highlighting gaps requiring further investigation.

Our study reveals a marked seasonal distribution of respiratory infections, with peaks in March and November, accompanied by a significant drop in summer (June/July). These observations align perfectly with existing epidemiological data: winter peaks (November to March) are typical for Respiratory Syncytial Virus (RSV), Influenza and coronaviruses (Bogdan et al., 2023) [5], while the summer decline reflects a common phenomenon for most respiratory pathogens. A notable exception is Rhinovirus/Enterovirus, which circulates year-round, a feature consistent with the pediatric data from this study and the general trends described in the literature. These results reinforce the relevance of seasonal factors in the epidemic dynamics of respiratory infections.

-Detection of difficult pathogens: multiplex PCR is particularly useful for diagnosing infections caused by pathogens that are difficult to identify by conventional methods, such as Legionella species. This capability is crucial in clinical settings, where rapid diagnosis is essential for effective treatment [5,6].

-Sensitivity and specificity: Studies have shown that PCR methods have a sensitivity of 100% and a specificity of 90% [5]. In our study, the respiratory panel demonstrated 100% sensitivity and high specificity. These data are in line with the literature and indicate that PCR methods are highly effective in detecting infections (Table 1).

Our study highlights the increased sensitivity of multiplex PCR (mPCR) compared with conventional culture methods, as illustrated in Table 1. While cultures showed low sensitivity rates (37.5% for Streptococcus pneumoniae (3/8 confirmed cases) and 35.7% for Haemophilus influenzae (5/14 confirmed cases)), mPCR

confirms its diagnostic excellence, consistent with literature data. Indeed, validated studies (Tran et al., 2024) [2] report sensitivities in excess of 90% for these agents, with 98% for S. pneumoniae and 93% for H. influenzae.

Interpreting Pneumonia Plus panel results for bacterial agents is tricky, as the results obtained are semi-quantitative (nucleic acid copies/ml), unlike conventional culture methods which provide quantitative data (CFU/ml as illustrated in Table 1.

Panel Pneumonia Plus multiplex PCR is an extremely sensitive technique, but it cannot distinguish between colonization and true respiratory infection. Furthermore, although mPCR is more sensitive, it may not entirely replace culture, as culture remains the gold standard for certain pathogens[4].

-Impact on treatment: The use of multiplex PCR can prevent unnecessary empirical antibiotic treatment. If PCR results are available within 2 hours, empirical antibiotic treatment would be suspended until results are obtained. If results are delayed, and if conventional methods and cultures provide results after 48 to 72 hours, empirical treatment would then be initiated. [7,8]. A study by Paz et al involved the use of multiplex PCR in parallel with standard cultures. This approach enabled some patients to avoid unnecessary antibiotic therapy for 48 to 72 hours. In this study of COVID patients, 61% avoided empirical treatment, and 71% of those already taking antibiotics had their treatment de-escalated thanks to PCR results [7,8]. As for our study, it showed that the initial empirical antibiotic treatment should have been modified in 81.28% of patients with respiratory infections. This indicates that the use of multiplex PCR can significantly influence clinical decision-making regarding antibiotic therapy.

Limits and difficulties Multiplex PCR

Multiplex PCR is a targeted detection method that cannot identify all the pathogens involved in community-acquired respiratory infections. Consequently, a negative multiplex PCR result does not exclude the presence of a respiratory infection caused by a micro-organism not included in the detection panel.

Multiplex PCR (mPCR) is a powerful tool for diagnosing a variety of infections, but it has notable limitations that can affect its effectiveness. Understanding these limitations is essential to optimize its use in the clinical setting.

*** Sensitivity and specificity problems**

mPCR may ignore clinically relevant species due to the inability to design primers that amplify all targets with equal efficiency, particularly in complex samples [9]. Detection limits can vary significantly, leading to potential false negatives, particularly when targeting low-abundance pathogens [10].

*** Key efficiency and optimization challenges**

It is essential to balance primer concentrations; unequal amplification can skew results, making it difficult to compare data between different assays [11]. The complexity of optimizing multiple primers increases the risk of methodological bias, which can compromise diagnostic accuracy [11].

The multiplex PCR (M-PCR) method cannot be used to assess the sensitivity of identified pathogens to available anti-infective treatments.

*** Cost and accessibility**

mPCR is often expensive and requires specialized technical expertise, limiting its use mainly to laboratories in developing countries. In low-incidence areas, routine testing may not be cost-effective, underscoring the need for economic evaluations in high-burden settings. [12]

*** Practical considerations**

The careful planning and optimization required can make mPCR more resource-intensive than traditional methods [13]. In personalized medicine, although mPCR can detect multiple targets, it does not always provide the specificity required for personalized treatments [13]. Consequently, they must be used alongside traditional methods such as microscopy and culture for bacterial agents and PCR for viruses. This combined approach improves diagnostic accuracy, particularly in cases with low pre-test probabilities.

Despite these limitations, mPCR remains a valuable diagnostic tool, but careful consideration of its challenges is necessary for effective application.

V. CONCLUSION:

multiplex PCR (mPCR) is proving to be an indispensable tool for the diagnosis of community-acquired respiratory infections, thanks to its ability to detect multiple pathogens simultaneously from a single sample. Its main advantages include high sensitivity, rapid turnaround and improved therapeutic guidance. However, its limitations, such as high cost and the need for expertise to interpret results, must be taken into account. All in all, mPCR is a major asset for the effective management of respiratory infections, despite certain constraints.

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