Official Journal of the Asian Pacific Society of Respirology
Respirology 20

REVIEW

Polymicrobial community-acquired pneumonia: An emerging entity

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ABSTRACT

Polymicrobial aetiology in community-acquired pneumonia (CAP) is more common than previously recognized. This growing new entity can influence inflammation, host immunity and disease outcomes in CAP patients. However, the true incidence is complicated to determine and probably underestimated due mainly to many cases going undetected, particularly in the outpatient setting, as the diagnostic yield is restricted by the sensitivity of currently available microbiologic tests and the ability to get certain types of clinical specimens. The observed rate of polymicrobial cases may also lead to new antibiotic therapy considerations. In this review, we discuss the pathogenesis, microbial interactions in pneumonia, epidemiology, biomarkers and antibiotic therapy for polymicrobial CAP.

Key words: community-acquired pneumonia, infection, mixed, pneumonia, polymicrobial.

Abbreviations: CAP, community-acquired pneumonia; CF, cystic fibrosis; COPD, chronic obstructive pulmonary disease; CRP, C-reactive protein; H_2O_2, hydrogen peroxide; HAP, hospital-acquired pneumonia; ICU, intensive care unit; PB1-F2, cytotoxic accessory protein; PCT, procalcitonin; SCV, small colony variants.

INTRODUCTION

Community-acquired pneumonia (CAP) is a critical health problem associated with high morbidity and mortality in all age groups worldwide.[1] CAP is the sixth leading cause of death worldwide and is a major burden on healthcare resources.[1] The incidence of CAP ranges from 3 to 30 cases per 1000 per year in adults in the general population, and increases with age and comorbidities.[2] The pathogens causing CAP may vary according to geographical area and underlying risk factors. *Streptococcus pneumoniae* is the main cause of CAP, accounting for about 30–35% of cases.[1,3,4] Despite the wide spectrum of conventional diagnostic tests for CAP an aetiologic diagnostics is achieved in only 50% of CAP cases. Conventional techniques are slow and labour intensive, have difficulty in differentiating between infection and colonization, are limited to blood and sputum cultures for bacterial causes and are influenced by prior antimicrobial therapy. The majority of CAP patients are treated empirically.[5–7] Development of molecular techniques, especially real-time polymerase chain reaction, has contributed to the recognition of the real incidence of polymicrobial infection in CAP patients. Furthermore, these new techniques can increase the rate of microbiological finding of respiratory pathogens in pneumonia from 49.5% to 76% of the cases.[6] New studies have shown that more than one causative pathogen (polymicrobial infections) are increasingly being diagnosed in a substantial number of cases, which is relevant because cases of polymicrobial pneumonia can influence inflammation and immunity and may be associated with more complex outcomes, and the choice of initial empiric antibiotic treatment may require some modifications. The reported rates for polymicrobial infection vary between 5.7% and 38.4%.[8–12] The clinical relevance of polymicrobial aetiology in CAP patients has not been specifically investigated. We review the prevalence, general characteristics and outcomes of polymicrobial pneumonia cases.

MICROBIOME OF THE LUNG

The human microbiome can be defined as the microbial population living in association with the human body. In particular, there is increased interest in research on the community of viruses (virobiota), bacteria (microbiota) and fungi (mycobiome).[13] Although the lungs were classically believed to be

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Received 15 June 2015; invited to revise 6 and 21 July 2015; revised 20 and 27 July 2015; accepted 28 July 2015 (Associate Editor: James Chalmers).

sterile,[14,15] recently published studies have identified diverse microbial and dynamic communities in the lungs of healthy persons.[16,17]

There is a close association between microbiota and the human immune system. Human microbiota plays an essential part in the pathophysiology of health and diseases,[18,19] as an example, Ichinohe *et al.*[19] showed that the immune response to respiratory influenza virus infection needs commensal bacteria.

The most prevalent genera described in airways are *Streptococcus, Prevotella, Fusobacteria* and *Veillonella*, *Haemophilus* and *Neisseria*.[16,17,20] The study by Chen *et al.*[21] reported the microbiota found in sputum samples from CAP patients and compared it with microbiota in healthy patients and hospital-acquired pneumonia (HAP) patients. Microbiota in healthy controls was characterized by five principal genera: *Streptococcus, Prevotella, Haemophilus, Veillonella* and *Fusobacterium*. The genera reported in CAP patients were *Streptococcus, Rothia, Prevotella, Veillonella* and *Pseudomonas*, and *Streptococcus, Staphylococcus, Pseudomonas, Acinetobacter* and *Rothia* were frequent in HAP patients.

A major problem in defining the lung microbiome is taking samples that reflect lung-derived bacteria. Studies of microbiome in the lower respiratory tract of healthy persons show variation in quantity and type of bacteria, reflecting the use of distinct sampling and identification techniques. Bronchoalveolar lavage and sputum samples and non-protected specimen brush or biopsies are the most frequent microbiological sampling techniques used. A limitation of these sampling techniques is the possibility of contamination with bacteria from the upper airway.

PATHOGENESIS OF POLYMICROBIAL INFECTION

During polymicrobial infection, an interaction between microorganisms occurs and the joint effect of two or more pathogens on the disease is worse than that seen with any of the pathogens alone. The complex interaction between microorganisms involves metabolites, quorum signals and natural antimicrobials with a specific and important role (Fig. 1).

Polymicrobial pneumonia may be caused by diverse combinations of respiratory viruses, bacteria and fungi. In general, the upper airways are constantly colonized by several microorganisms such as *S. pneumoniae, Haemophilus influenzae* and *Staphylococcus aureus*. Approximately 20–50% of healthy individuals are colonized by at least one of these species.[22–24] New studies have shown that colonization by *S. pneumoniae* was associated with increased risk of intensive care unit (ICU) admission or death in the case of influenza infection, whereas colonization by *S. aureus* was associated with enhanced risk of decease in adults and children infected with influenza virus; in particular methicillin-resistant *S. aureus* co-infection was associated with severe disease and death in adults and children.[25–28]

Bacterial respiratory infection is often preceded by a viral infection that favours the establishment of secondary bacterial infection caused by a bacterial pathogen-colonizing respiratory mucosa. When a viral respiratory infection occurs, it damages the respiratory epithelium, thus increasing the adhesion of bacteria to the mucosa. In fact, it generates the expression of molecules, such as glycoproteins, on the infected host cell membrane used by bacteria as specific receptors, thereby contributing to bacterial adherence and the establishment of bacterial infection. The principal association of pathogens in CAP is bacterial/viral co-infection, which accounts for approximately 39% of microbiologically confirmed cases of CAP. Atypical pathogens frequently appear as polymicrobial infections, with *S. pneumoniae* often isolated as the main pathogen.[29] Co-infection with atypical pathogens is important because it makes CAP difficult to diagnose and non-responsive to conventional β-lactam therapy.[30]

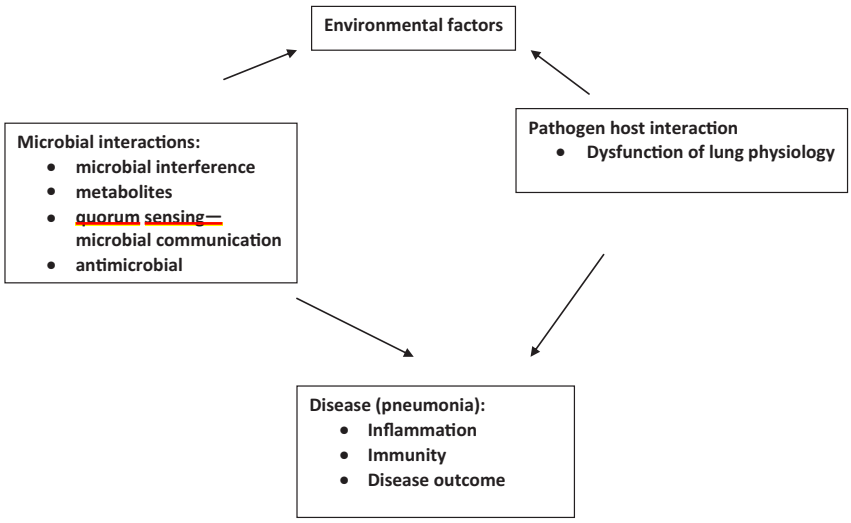

Figure 1 Pathogenesis of polymicrobial infection.

BACTERIA AND ATYPICAL BACTERIA CO-INFECTION

Atypical pathogens *(Mycoplasma pneumoniae, Chlamydophila pneumoniae and Legionella pneumophila)* are a frequent cause of CAP.[3,29] It has been reported that the combination of atypical bacteria and *S. pneumoniae* comprises the most frequent polymicrobial infection in outpatients with CAP and is also responsible for hospitalized cases.[3] Due to the fact that these bacteria are naturally resistant to ß-lactams, they should be promptly identified and treated. Macrolides and quinolones remain the best empirical treatment for intracellular pathogens because of their good antimicrobial activity and high intracellular concentration. Although antibiotic resistance in these intracellular pathogens does not represent a clinical problem in the present day, recent reports from Asia and France regarding isolation of strains of *M. pneumoniae* resistant to macrolides mean that monitoring these pathogens is recommended to evaluate the clinical impact in CAP. The study by Gutierrez *et al.*[8] reported mixed aetiology in 5.7% of CAP patients; the most frequent combination was bacterial plus atypical bacteria. International guidelines[31,32] on the treatment of CAP recommend initial antibiotic therapy with combinations of penicillins and macrolides, or single-drug therapy with quinolones for patients hospitalized with CAP. Although atypical bacteria are covered by the recommended therapies, there is concern regarding the excessive use of macrolides and quinolones. There is also a risk of treatment failure in outpatients with the use of single-drug therapy because a large proportion of *S. pneumoniae* is resistant to macrolides in some countries.[33,34] There is a need for improved microbiological diagnostic techniques for CAP in order to optimize future treatment choices.

HOW RESPIRATORY VIRUSES PREDISPOSE PATIENTS TO BACTERIAL INFECTION

In order to establish respiratory tract infection, bacteria have to initially adhere to the epithelial surfaces, establishing colonization of the nasopharynx before invading and spreading to the lungs. Bacterial colonization and invasion are facilitated by prior viral infection.

Because of their tropism for epithelial cells, respiratory viruses cause multiple structural modifications in the cells of the respiratory epithelium, thus facilitating bacterial invasion. Bacterial growth is promoted by the rich source of nutrients caused by epithelial damage, the disruption of surfactants and the sloughing of cells into the airways. Additionally, influenza virus reduces human nasal and tracheal epithelial ciliary function: the ciliary beat frequency is reduced and ciliary motion becomes uncoordinated resulting in decreased mechanical clearance of bacteria.[35]

Influenza virus can induce epithelial cells death, compromising the barrier function of the airway and promoting bacteria adherence due to the exposure of sites for adherence. There are other mechanisms that may increase receptor availability for bacteria induced by influenza virus: (i) neuraminidase of the influenza virus cleaves sialic acid, which exposes cryptic receptors for pneumococcal adherence on host cells and disrupts sialylated mucin that can function as decoy receptor for the bacteria. Bacteria express several virulence factors that can be used for attachment to the basement membrane or elements of the extracellular matrix (fibrin, fibrinogen and collagen); an example of this is *S. pneumoniae*, which expresses pneumococcal surface protein A, choline-binding protein A or pneumococcal serine-rich repeat protein. Virulence factors expressed by *S. aureus* include members of the family of microbial surface components recognizing adhesive matrix molecules and members of the serine–aspartate dipeptide repeat-containing family. (ii) The inflammatory response to infections with respiratory viruses can modify the regulatory state and surface display of several proteins, such as the platelet-activating factor receptor, which helps in pneumococcal invasion. (iii) Structural changes in the airway during their regeneration and remodelling after viral infection may provide adherence sites during recovery. Damaged cells that are in an intermediate state of differentiation express apical receptors (asialylated glycans or integrins) where bacteria such as *S. aureus* or *Pseudomonas aeruginosa* can attach. (iv) Some respiratory viruses (influenza viruses, respiratory syncytial virus and human metapneumovirus) induce suppression of phagocytic cells and play a main role in controlling susceptibility to secondary bacterial infection.[36] For example, the non-structural 1 protein of influenza virus interferes with lung immune responses to bacterial infection. Many respiratory viruses produce interferon antagonists that blind the host response during infection of the respiratory tract, and probably function by suppressing the cellular responses that normally assist the clearance of bacteria from the lungs.[36–41]

The dysregulated inflammation process caused by viral and bacterial factors produced in pneumonia in the lungs contribute to the pathogenesis of polymicrobial infection and to the predisposition of the host to a secondary bacterial infection. Viral proteins such as cytotoxic accessory protein (PB1-F2), pneumolysin and Panton–Valentine leucocidin can drive inflammatory responses (Fig. 2).

MICROBIAL INTERACTIONS IN PNEUMONIA

Microbial interaction between influenza virus and *S. aureus*

Polymicrobial infection involving influenza virus A and *S. aureus* is one of the main causes of severe CAP[25] (Fig. 3). The emergence of influenza virus H1N1 in 2009 caused the first pandemic in more than 40 years. Several studies found bacterial co-infection in between 10% and 20% of influenza infections; the pathogens most frequently isolated were

Respiratory viruses / Host interaction

Induce alteration in epithelial cells
- reduced ciliary function
- decreased epithelial barrier function
- upregulation of surface receptors for bacterial adhesion

Virus-mediated compromised innate immune cells (lung macrophages, neutrophils)
- suppressed phagocytosis
- impaired microbial killing
- depressed leukocyte migration

Dysfunctional inflammatory response: acute lung injury
- neutrophil influx
- cytokine storm

Figure 2 How respiratory viruses predispose patients to bacterial infection.

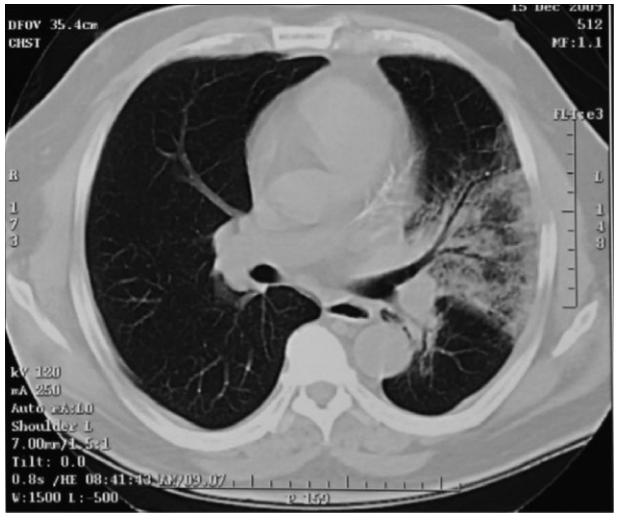

Figure 3 Lobar pneumonia (ground glass with air bronchogram) caused by influenza virus A (H1N1) plus methicillin-resistant *Staphylococcus aureus.*

S. pneumoniae and *S. aureus*[42–47] (Fig. 4). Influenza infection promotes and enhances the nasopharyngeal adherence of *S. aureus.*[48] Moreover, several species of *S. aureus* secreted proteases[49] that cleave influenza haemagglutinin, a step required for the normal cycle of viral replication and for the spread of the virus inside the host.[50]

Microbial interaction between *S. pneumoniae* and *S. aureus*

Streptococcus pneumoniae and *S. aureus* are important pathogens in CAP and cause significant morbidity and mortality globally. The primary ecological niche for *S. aureus* is the anterior squamous epithelium of the upper respiratory tract. About 30% of healthy adults and 10% of healthy children carry *S. aureus*.[51] In the case of *S. pneumoniae*, colonization is most frequent in younger children.

Nasal colonization is a main risk factor for transmission and invasion of respiratory pathogens. Approximately 80% of *S. aureus* infections are caused by the strain that host carried.

Several studies show an adverse association between *S. pneumoniae* and *S. aureus* colonization, especially in the case of vaccine-type strains of pneumococcus.[52–54] Studies in vitro suggest that hydrogen peroxide (H_2O_2), a product of metabolism produced by *S. pneumoniae*, is responsible for this antagonistic relationship because it can kill *S. aureus.*[55] However, the study by Regev-Yochay *et al.*[55] demonstrated that staphylococcus species that secreted higher levels of catalase are resistant to pneumococcus. The presence of pneumococcal pilus is another factor that contributes to this antagonistic relationship due to the interactions of this structure with host immune responses, which is prejudicial to *S. aureus* colonization and gives advantages for pneumococci due to the increased capacity for adherence. However, several factors are implicated in the higher prevalence of *S. aureus* colonization, including pneumococcal vaccination and widespread antibiotic use.

Epidemiological studies have shown that children who are colonized with *S. pneumoniae* have a significantly reduced risk of carrying *S. aureus.*[53] The increased incidence of otitis media caused by *S. aureus* in children has been associated with a decline in pneumococcal colonization due to vaccination.

Microbial interaction between *S. pneumoniae* and *H. influenzae*

These two microorganisms share the nasopharynx as the site of colonization; they have a competitive relation due to the overlap in their site and frequency of colonization.

Colonization of the host by these two pathogens involves a synergistic pro-inflammatory response. The H_2O_2 produced by *S. pneumoniae* inhibits the growth of *H. influenzae*. On the other hand, the neuraminidase produced by *S. pneumoniae* desialylates the *H. influenzae* lipopolysaccharide; this effect enhances the bactericidal effect on this pathogen. The study by Lysenko *et al.*[56] shows that virulent pneumococcal serotypes arose during nasopharyngeal competition with *H. influenzae.* This fact influences the outcome of pneumococcal disease progression.[57]

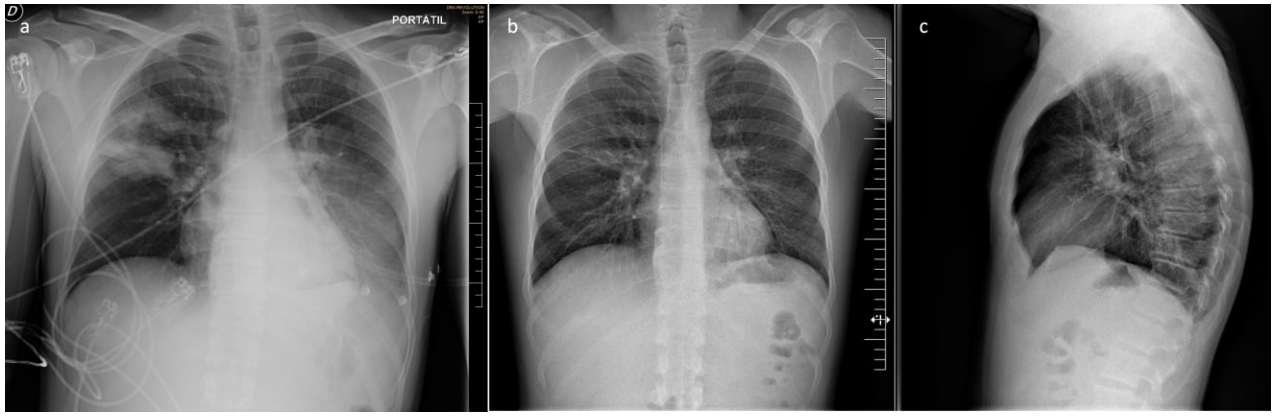

Figure 4 Community-acquired pneumonia caused by influenza virus A (H1N1) plus methicillin-resistant *S. pneumoniae* serotype 12F. (a) Rx at admission; (b,c) Rx 8 days after admission.

Microbial interaction between *S. aureus* and *H. influenzae*

Both species are colonizers of the nasopharynx but *H. influenzae* is in higher density than *S. aureus*. This fact may be attributable to the availability of nutrients that haemolysins (α, ß and γ) of *S. aureus* provides due to lysis of erythrocytes. Nutrients such as haemin and nicotinamide adenine dinucleotide after erythrocytes lysis are available *for H. influenzae*.[50]

Microbial interaction between *S. aureus* and *P. aeruginosa*

The relationship between these two microorganisms is competitive in nature. However, we can find these species as colonizers of the lungs in patients with chronic respiratory diseases (chronic obstructive pulmonary disease (COPD), cystic fibrosis (CF) and non-CF bronchiectasis).[58–60]

Pseudomonas aeruginosa produces toxins such as pyocyanin, hydrogen cyanide and quinoline N-oxides, which can obstruct the electron transport pathway, interfering with the growth of *S. aureus* and other pathogenic staphylococci. Also, the production of endopeptidase by *P. aeruginosa* cleaves *S. aureus* peptidoglycan and induces lysis, and the nutrients released from lysis serve as source of iron for *P. aeruginosa*. *Staphylococcus aureus* forms small colony variants (SCV) that are resistant to antimicrobials, especially aminoglycosides and trimethoprim-sulfamethoxazol.[61,62] SCV is a survival strategy of *S. aureus* due to its principal property of strong reduction in growth rate, atypical colony morphology and unusual biochemical properties, which are frequently undetected using standard clinical microbiology procedures.[50]

Microbial interaction between *Candida albicans, P. aeruginosa* and *S. aureus*

Candida albicans is a microorganism not often associated with CAP. Some studies have demonstrated that the associations of *C. albicans* with *P. aeruginosa* or *S. aureus* enhance disease severity in several ways.[63–67] In vivo studies in rats have shown that colonization of the lung by *C. albicans* increases rates of pneumonia due to *P. aeruginosa*.[63] This important microbial interaction should be taken into account in patients frequently colonized with *Pseudomonas* and *S. aureus*, such as COPD and non-CF bronchiectasis patients.

In the host, *C. albicans* exists in both forms (yeast and filamentous cells), and the formation of hyphae is important for its virulence. *Candida albicans* secretes a quorum-sensing signal called farnesol, which inhibits its hyphal growth,[68,69] and, interestingly, 3-oxo-C_{12}–homoserine lactone, a quorum-sensing signal produced by *P. aeruginosa*, has a similar effect on *C. albicans*. *Pseudomonas aeruginosa* secretes bacterial phenazine, which inhibits candidal germination; furthermore, *P. aeruginosa* can bind to localized areas of the hyphal and induce lysis. By means of this action on killed hyphae, *P. aeruginosa* can form a biofilm. Farnesol produced by *C. albicans* alters the regulation of quorum-sensing in *P. aeruginosa*, also *C. albicans* secretes factors that inhibit swarming motility with the effect of increasing biofilm formation and phenazine production, which is a virulence factor in *P. aeruginosa*.[70]

In infections with *C. albicans*, the physical damage caused by this microorganism on organ walls allows *S. aureus* to penetrate the internal organs easily, whereas *S. aureus* secretes proteases that facilitate *C. albicans* to enhance its adhesion to the mucosal layer.[50] During systemic infection, each microorganism helps the other microorganism to evade phagocytosis mediated by polymorphonuclear leukocytes. The proteinase secreted by *C. albicans* degrades the Fc portion of immunoglobulin G and reduces the opsonizing activity against *S. aureus*.[50]

It is known that the base of the biofilm is formed by *C. albicans* and helps the biofilm development of *S. aureus*. The protein agglutinin-like sequence 3 of *C. albicans* mediates the attachment of *S. aureus* to *C. albicans* hyphae.[50,71,72] Farnesol, a product of *C. albicans,* is known to reduce the viability and biofilm capabilities of *S. aureus* because farnesol causes damage to cell membrane integrity.[50]

Interestingly, the susceptibility of *S. aureus* to antimicrobial increases with the presence of farnesol, probably because of cell membrane damage allowing greater penetration of antimicrobials to target sites.[70,73]

EPIDEMIOLOGY

Gutierrez *et al.*[8] conducted a study on 493 adult patients with CAP. Polymicrobial infection was found in 5.7% of patients with microbiologically confirmed diagnosis. Polymicrobial infections were presented in all age groups and in outpatients and inpatients. The most common polymicrobial infections were the combination of *pneumococcus* with *L. pneumophila* and *pneumococcus* with *Pseudomonas* spp. Individuals with polymicrobial pneumonia are more likely to have underlying comorbidities and they may have a more severe disease. de Roux *et al.*,[9] in a study of 1511 CAP cases, found that 13% of patients with microbiological diagnosis presented with polymicrobial pneumonia. *Streptococcus pneumoniae* was the most frequent pathogen (54%); the most prevalent combination was *pneumococcus* plus *H. influenzae*. van der Eerden *et al.*,[74] in a study involving 262 cases of hospitalized CAP patients in the Netherlands, found polymicrobial infection in 6% of the patients; the most frequent combination of microorganisms was bacterial plus an atypical bacterial or a respiratory virus. In a study of 3523 patients with CAP, we found that 14% of cases with microbiologic diagnosis were polymicrobial; *S. pneumoniae* was the most frequent pathogen involved in polymicrobial infections (65%).[3] The most prevalent combinations among polymicrobial pneumonia were two bacteria in 32% of the cases, a bacterium plus a respiratory virus in 29% and a bacterium plus an atypical microorganism in 18%.[3] An interesting study carried out in Japan[75] on 1032 patients with CAP analyzed the aetiology in two groups: severe CAP patients and non-survivors. They found that polymicrobial infection was confirmed in 9.2% of all cases, 18% in severe CAP and 12.5% in the group of non-survivors; polymicrobial infection was a risk factor for severity of CAP in the multivariate analysis. A study addressing polymicrobial infection in 362 ICU patients with CAP found that 11% of cases were polymicrobial and the presence of chronic respiratory disease and acute respiratory distress syndrome criteria on admission to hospital were predictors of polymicrobial aetiology in the multivariate analysis.[11] A recent study in 568 outpatients with CAP found polymicrobial infections in 9% of patients with defined aetiology; the most frequent combination (23%) of pathogens were *S. pneumoniae* plus respiratory viruses[76] (Table 1).

Table 1 Epidemiology of polymicrobial community-acquired pneumonia

Study	Country/year of publication	Study period	Site	Number patients/ incidence	Most frequent pathogens
File[5]	Spain/2005	1999–2001	Outpatients/ inpatients	493/5.7%	Bacterial + atypical *S. pneumoniae* + *L. pneumophila* *S. pneumoniae* + *Pseudomonas* spp.
Templeton *et al.*[6]	Spain/2006	1996–2001	Outpatients/ inpatients	1511/5.4%	*S. pneumoniae* + *H. influenzae*
Lieberman and Lieberman[58]	Netherlands/ 2005	1998–2000	Outpatients/ inpatients	262/6%	Bacteria + atypical bacteria Bacteria + respiratory viruses
Cilloniz *et al.*[3]	Spain/2011	1996–2008	Outpatients/ inpatients	3523/14%	*S. pneumoniae* involved in 65% of mixed cases Bacteria + bacteria (32%) Bacteria + respiratory viruses (29%) Bacteria + atypical bacteria (18%)
Purcell *et al.*[59]	Japan/2013	2002–2011	Outpatients/ inpatients	1032/9.2%	*S. pneumoniae* + influenza virus
Gutierrez *et al.*[8]	Spain/2011	2003–2010	ICU patients	362/11%	*S. pneumoniae* + respiratory viruses
de Roux *et al.*[9]	Norway/2015	2008–2011	Hospitalized	267/26%	*S. pneumoniae* + influenza virus
Rogers *et al.*[60]	Spain/2014	2000–2010	Outpatients	568/9%	*S. pneumoniae* + respiratory viruses (23%)

BIOMARKERS IN POLYMICROBIAL CAP

Biomarkers provide information about the host response to pathogens (bacteria, virus or fungi) causing pulmonary infection. There is rising evidence that multiple causal microorganisms may promote different inflammatory responses, and levels of some biomarkers such as C-reactive protein (CRP) and procalcitonin (PCT) are associated with distinct aetiological patron. These biomarkers (CRP and PCT) show higher levels in bacterial pneumonia than pneumonia caused by respiratory viruses.[77,78]

Several studies suggested that CRP and PCT may be used as a tool for differentiating polymicrobial CAP from viral CAP caused by influenza virus A (H1N1).[79–82]

A recently published study[83] evaluated the relationship between levels of CRP, PCT and WBC in 171 CAP cases with defined microbial aetiology; those authors found that CRP levels of <26 mg/dL were indicative of an aetiology other than polymicrobial in 83% of pneumonia cases, but the positive predictive value was 45%. In that study, CRP was independently associated with polymicrobial CAP. Table 2 shows the studies with biomarkers and aetiology of pneumonia.

TREATMENT

Polymicrobial pneumonia is present in all ages and it should always be remembered that unless *S. pneumoniae* is the principal pathogen involved in CAP, approximately 10–35% of pneumococcal cases are polymicrobial, usually involving atypical bacteria or respiratory viruses.[3,10,84] A study by Waterer *et al.*[85] showed that mortality associated with bacteremic pneumococcal pneumonia was reduced when patients received empirically a combined antibiotic therapy including a macrolide; this finding might be explained by the existence of polymicrobial infection although anti-inflammatory effects of macrolides could play an important role as well. International guidelines have included the idea that atypical pathogens will be involved in polymicrobial pneumonia in all patient groups.[1,86]

The elevated rate of viral-bacterial co-infection in CAP suggests that new treatment options should be taken into consideration and should also be considered during influenza season. Rapid identification of influenza virus (A, B) may allow physicians to effectively use neuraminidase inhibitors within 36–48 h of onset of symptoms, thereby reducing the complication of secondary bacterial infections. Furthermore, prevention of polymicrobial infection by influenza and pneumococcal vaccine should be addressed. A detailed understanding of the interactions between *S. pneumoniae* and host immune response is important for understanding the pathophysiology of pneumonia[87] and may lead to the development of novel therapeutic and preventive strategies.

We believe that the detection of mixed infection is important, especially in severe CAP. In one of our previous studies (Cilloniz *et al.*[11]), we found that patients with severe CAP and mixed infection had worse

Table 2 Aetiology and biomarkers

Study	Country/year of publication	Study period	Site/population	Biomarker	Results
Roux *et al.*[63]	France/2011	2009–2010	ICU/severe H1N1 influenza infection	PCT	PCT combined with clinical judgment suggest that bacterial infection is unlikely
Azoulay *et al.*[64]	Australia/2011	6 July 2009–2 August 2009	ICU/H1N1 influenza infection	PCT	PCT was neither sensitive nor specific in determining isolated H1N1 infection in this series of patients
Roux *et al.*[65]	Korea/2011	2009 (7 months)	Outpatients/inpatients Viral pneumonia H1N1 influenza virus	PCT/CRP	The sensitivity and specificity for detection of mixed bacterial infection pneumonia was 56% and 84% for PCT > 1.5 ng/mL, and 69% and 63% for CRP > 10 mg/dL
Delisle *et al.*[67]	Spain/2014	2009–2010	Outpatients/inpatients with CAP	WBC/CRP/PCT	High CRP levels may be useful for clinicians to suspect mixed CAP

CAP, community-acquired pneumonia; CRP, C-reactive protein; H1N1, influenza virus A; ICU, intensive care unit; PCT, procalcitonin; WBC, white blood cells.

Table 3 Antibiotic treatment of polymicrobial community-acquired pneumonia for ICU patients

Antibiotic treatment regimen	Evidence	Recommendation
Empirical	To cover mixed pneumonias in severe CAP (we found 22% in our series[11])	We recommend: β-lactam† IV + macrolide‡ + neuraminidase inhibitors§ during influenza pneumonia
Definitive	Microbiological results	Adjust according to microbiological results

†Ceftriaxone, cefotaxime, ceftaroline.
‡Azithromycin, clarithromycin.
§Oseltamivir, zanamivir. CAP, community-acquired pneumonia; IV, intravenous.

outcomes. Early detection of mixed CAP would improve the initial adequacy of antibiotic or antiviral treatments (Table 3).

CONCLUSION

There is a suggestion that polymicrobial CAP is associated with more severe disease.[88] The differential clinical diagnosis between viral and bacterial CAP is not easy,[89] whereas clinical suspicion or diagnosis is extremely difficult: no clinical signs or radiological findings can help the clinician.[90–94] No statistical differences have been reported with regard to age, immunological status, laboratory parameters or severity score CURB-65 between bacterial and polymicrobial CAP.[92–94] Only higher levels of CRP may be a useful tool for clinicians to suspect polymicrobial CAP.[83] Molecular methods, useful for detecting polymicrobial infections,[92,94,95] should be routinely added to conventional pathogen-diagnostic methods.

Recent developments in molecular diagnostics have resulted in increased detection of polymicrobial infection in CAP populations. Some new studies have shown that there is a complex relationship between multiple pathogens, the immune system and the microbiome. The use of culture-independent techniques will help us to understand polymicrobial interactions in health and disease.

Acknowledgements

The authors gratefully acknowledge Dr Nicola Petrosillo (2nd Division of Infectious Diseases, National Institute for Infectious Diseases 'Lazzaro Spallanzani', via Portunse 292, 00149 Rome, Italy) for his editorial assistance.

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