The Role of Streptococcus pneumoniae in Community-Acquired Pneumonia

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Abstract

With the notable exceptions of the United States of America (US) and Canada in particular, the global burden of disease in adults due to invasive infection with the dangerous respiratory, bacterial pathogen, *Streptococcus pneumoniae* (pneumococcus) remains. This situation prevails despite the major successes of inclusion of polysaccharide conjugate vaccines (PCVs) in many national childhood immunization programmes and associated herd protection in adults, as well as the availability of effective antimicrobial agents. Accurate assessment of the geographic variations in the prevalence of invasive pneumococcal disease (IPD) has, however, been somewhat impeded by the limitations imposed on the acquisition of reliable epidemiological data due to reliance on often insensitive, laboratory-based, pathogen identification procedures. This, in turn, may result in underestimation of the true burden of IPD and represents a primary focus of this review. Other priority topics include the role of PCVs in the changing epidemiology of IPD in adults worldwide, smoking as a risk factor not only in respect of increasing susceptibility for development of IPD, but also in promoting pneumococcal antibiotic resistance. The theme of pneumococcal antibiotic resistance has been expanded to include mechanisms of resistance to commonly used

classes of antibiotics, specifically beta-lactams, macrolides and fluoroquinolones, and, perhaps somewhat contentiously, the impact of resistance on treatment outcome. Finally, but no less importantly, the role of persistent antigenemia as a driver of a chronic, sub-clinical, systemic pro-inflammatory/pro-coagulant phenotype that may underpin the long-term sequelae and premature mortality of those adults who have recovered from an episode of IPD, is considered.

Key Words: Antibiotic resistance; beta-lactams; cigarette smoking; fluoroquinolones; inflammation; macrolides; persistent antigenemia; pneumococcal conjugate vaccine; vaping.

Streptococcus pneumoniae as a cause of community-acquired pneumonia

The Global Burden of Disease Study 2016 estimated the global, regional and national morbidity and mortality, as well as the etiologies of lower respiratory tract infections (LRTIs; defined as pneumonia or bronchiolitis) in 195 countries between 1990 and 2016.[1] The study also estimated the number of cases attributable to *Streptococcus pneumoniae* (pneumococcus), *Haemophilus influenzae* type b, influenza and respiratory syncytial virus. In 2016, there were 2,377,697 deaths (2,145,584-2,512,809) from LRTIs in people of all ages in those countries, with the pneumococcus identified as being the most common cause of LRTI morbidity and mortality, causing more deaths than all the other etiologies combined. With the introduction of pneumococcal conjugate vaccines (PCVs) in the childhood national immunization programs (NIPs) of many countries, moderate reductions in the mortality of LRTIs were seen in children under the age of 5 years, while the burden of LRTIs in adults >70 years of age remained particularly high.

Nevertheless, it is clear when reviewing data from different parts of the world, that there are regional differences in the epidemiology (including burden, risk factors, etiology, prevalence of antimicrobial resistance, and outcome) of patients with community-acquired pneumonia (CAP) and there have also been global changes in the epidemiology of CAP over the years.[2-5] One recent literature review evaluating the etiology of CAP in adults as

published in PubMed in English through to December 2015, noted the following trends; i) there was an unexplained decrease in the prevalence of pneumococcal infections, particularly in the US/Canada, ii) the pneumococcus, nevertheless, remained the most common bacterial pathogen identified, especially in critically ill cases, iii) there was a much greater frequency of pneumococcal infections in Europe compared with the United States, iv) respiratory viruses were noted to play a greater role than previously documented, v) more recently, infections with *Mycoplasma pneumoniae* and *Legionella pneumophila* were less frequently reported, and vi) the frequency of pathogen identification remained low, being undetected in more than 50% of cases.[6] The authors indicated that the possible reasons for differences in prevalence of pneumococcal infections when comparing Europe and the US may be related to differences in vaccination practices and in the smoking habit.

Differences in the documented etiology of CAP in the various studies may also be attributed, at least in part, to the laboratory diagnostic techniques used. Standard culture techniques, with blood cultures considered a “gold standard”, have yielded low rates of pathogen detection, because of the low sensitivity of blood cultures.[7] Furthermore, although of good specificity and improved sensitivity, urine antigen tests for *S. pneumoniae* and *L. pneumophila*, are still only 70-80% sensitive. Thus because of the limitations of diagnostic testing for non-bacteremic pneumococcal infections, most studies reporting on the incidence of pneumococcal infections report on the occurrence of invasive, bacteremic infections and underestimate the true pneumococcal burden.[8] One recent systematic literature review of studies, which included information on the diagnostic yield of various assays for pneumococcal infections (urine antigen detection testing, and blood and/or sputum culture), estimated that for every case of bacteremic pneumococcal pneumonia there were at least three non-bacteremic cases, thus significantly underestimating the burden of pneumococcal disease when using the former investigation alone.[8] Two recent studies in the US using standard culture microbiology, urine antigen testing and commercially available polymerase chain reaction (PCR) techniques identified pneumococcal infections in <10% of cases, respiratory viruses in 20-27% of cases and no pathogen in approximately 55%-62% of

cases.[9,10] More recently, Gadsby and colleagues evaluated quantitative multi-pathogen molecular testing of respiratory samples in hospitalized adults with CAP.[11] They collected mucopurulent sputum (96%), and endotracheal aspirates (3%) from 323 patients with radiologically-confirmed CAP and undertook culture and multiplex real-time PCR analyses of the samples. Using PCR, they identified a pathogen in 87% of cases compared with 39% with culture alone, the two most common pathogens being *Haemophilus influenzae* (40%) and *S. pneumoniae* (36%). Viruses were detected in 30% of cases with 82% being co-infected with bacterial pathogens. The authors concluded that comprehensive molecular testing significantly increases detection of CAP pathogens from a single lower respiratory tract specimen.

While earlier studies evaluating the clinical and economic burden of CAP in North America,[12] Latin America,[13] the Asia-Pacific region[14] and Europe[15] all indicated that the pneumococcus was the most common cause of CAP, that antibiotic resistance was an issue, and that the morbidity and mortality was high, more recent studies, largely from the US, have noted a much lower incidence of pneumococcal infections in CAP.[9,10] Interestingly, in the latter study, additional use of a novel serotype-specific urine antigen detection assay, as opposed to the commercially available urine antigen detection test, increased the detection rate of pneumococcal cases from 4.4% to 9.7% overall.[16] Furthermore, an active surveillance study for pneumococcal CAP and invasive pneumococcal disease was undertaken in adults hospitalized across five Canadian provinces from 2010 to 2013.[17] Diagnostic testing for pneumococcal CAP was undertaken using sputum and blood culture, a commercial pneumococcal urine antigen detection test and a serotype specific pneumococcal urine antigen detection test. Of a total of 4769 patients with all-cause CAP, testing for *S. pneumoniae* was undertaken in 3851 of these, identifying 23.2% (144/621) of cases among CAP patients in whom all four tests were performed. Among these latter cases, 14.8% were PCV13 type pneumococcal isolates, indicating that 3 years after introduction of PCV13 immunization programs in Canada, vaccine preventable pneumococcal CAP was still a significant problem. A matched nested case-control study of

two prospectively recruited cohorts of hospitalized patients with CAP in Buenos Aires undertaken during 2001-2002 and 2015-2016, observed a reduction in the number of cases of CAP due to the pneumococcus (23.4% versus 8.3%; p<0.001) and an increase in pneumococcal vaccination (polyvalent pneumococcal vaccine; PPV23) before admission (4.1% versus 22.8%; p<0.001). The authors indicated that routine childhood PCV13 vaccination, which was initiated in 2012, may have also contributed.[18] A systematic review of studies published on CAP etiology in Asia, concluded that while *S. pneumoniae* was the most common cause, it was of relatively less importance than that found in western studies.[19]

In contrast to this, a literature review evaluating the etiology (and antibiotic management) of CAP in Europe reported on 33 published studies that recorded pathogens and noted that the pneumococcus was the most commonly isolated pathogen and was identified in between 12.0% and 85.0% of patients in the different regions.[20] A meta-analysis of the role of the pneumococcus in adults with CAP in Europe concluded that the observed prevalence varies in the different European regions, that the probability of detecting *S. pneumoniae* was significantly higher if PCR was performed compared to any other diagnostic test and that *S. pneumoniae* was more likely to be isolated in studies with ICU patients, as opposed to those with in-hospital or community-treated patients only.[21] A recent systematic review conducted in the United Kingdom (UK) noted that vaccine-type pneumococcal disease still has a high burden in the UK despite the impact of PCV13 vaccination in children.[22] Furthermore, a prospective study of consecutive hospitalized adults with CAP in Reykjavik, in Iceland, in which PCR analysis of airway samples was included in the diagnostic testing, recorded a potential pathogen in 52% (164/310) of admissions and 74% (43/58) in those with complete data sets.[23] *S. pneumoniae* was the most common pathogen detected (20%; 61/310) and viruses were noted in 15%.

There is no doubt that a significant reason for the changing epidemiology of pneumococcal disease and the disease burden has been the use of PCVs in children, which, when included in childhood routine national immunization programs (NIPs), prevents disease

not only in the targeted group, but also in non-vaccinated children, as well as adults, as a result of herd protection.[24] Recent studies from most regions of the world[25] including North America,[26] Europe[27] and South Africa,[28] have documented the significant direct and indirect effects of childhood vaccination. Furthermore, two recent systematic reviews and meta-analyses of the global literature indicated that rates of IPD and pneumonia in adults in most countries decreased following PCV introduction in the childhood NIPs, that the herd protection is dependent on the PCV coverage rate and the duration of the implementation of the NIPS and that substantial protection for the whole population would be evident within a decade of introduction of childhood PCV programs.[29,30] However, it has been noted that the decline in adult pneumococcal infections in the US was attenuated with increasing age and also in those with comorbidities[26] and that a residual burden of PCV13 vaccine-type CAP still remains in the US population.[31]

Risk Factors for Severe Pneumococcal Disease

These are well recognized and are often associated with immunosuppression, mostly acquired and secondary, as well as with certain types of primary immunodeficiency disorder, particularly antibody and complement deficiency disorders[32] and are summarized in Table 1. Given the increasing realization of the multifactorial involvement of smoking in promoting pneumococcal infection, including antibiotic resistance, this risk factor represents the primary focus of this section of the review.

Table 1: Risk factors for invasive pneumococcal infections

Age
<2 or ≥65 y
Ethnic groups
African descent
Alaskan native

American Indians
Underlying clinical pulmonary diseases
Chronic obstructive pulmonary disease
Asthma
Other chronic clinical conditions
Chronic liver disease
Chronic renal failure
Nephrotic syndrome
Diabetes mellitus
Functional or anatomic asplenia
Sickle cell disease
Splenectomy
Substance abuse
Alcohol abuse
Smoking habit
Crack use
Cocaine use
Immunosuppressive conditions
HIV infection
Congenital immunodeficiency
Malignancy
B-cell defects
Multiple myeloma
Patients undergoing treatment
Alkylating agents
Antimetabolites
Systemic glucocorticoids
Patients with cerebrospinal fluid leaks
Cochlear implant recipients
Solid-organ or hematopoietic cell transplant recipients
Patients with influenza

Source: Reproduced with permission from Wolters Kluwer (Aspa and Rajas).[32]

Smoking

Nuorti *et al.* in their seminal report published in the "New England Journal of Medicine" in 2000, identified active cigarette smoking as being "the strongest independent risk factor for invasive pneumococcal disease (IPD) among immunocompetent, nonelderly adults" (odds ratio [OR] 4.1; 95% confidence interval [CI], 2.4–7.3).[33] In addition to these findings, current smokers who develop pneumococcal CAP have been reported to have a striking 5-fold increase in the risk of 30-day mortality, irrespective of age, co-morbidities and early implementation of guideline-concordant antibiotic therapy.[34] In the case of all-cause CAP, a recent systematic review and meta-analysis, encompassing 27 studies and 460,592 participants, revealed that current smokers have a significantly increased risk for development of CAP relative to never-smokers (OR 2.17; 95% CI, 1.70–2.76, n=13 studies).[35] Passive smoking is associated with a 64% increase in the risk for development of CAP, but only for those aged >65 years (OR 1.64; 95% CI, 1.17–2.30, n=2 studies).[35]

Smoking-related increased susceptibility for development of severe pneumococcal disease has generally been attributed to cigarette smoke-mediated suppression of innate and adaptive pulmonary host defenses.[36] Our research findings, some very recent, have, however, revealed additional pathogen-targeted mechanisms that are likely to contribute to smoking-related susceptibility for development of severe pneumococcal disease. In this context, exposure of an antibiotic-susceptible strain of the pneumococcus (strain 172, serotype 23F) to cigarette smoke *in vitro* was found to trigger events at the level of gene expression, which may promote antibiotic resistance. The first of these events involves initiation of biofilm formation, a strategy utilized by microbial pathogens to confer broad protection against penetration of antibiotics.[37] Biofilm is an extensively-hydrated, visco-elastic, extracellular matrix comprised of various types of bacterium-derived polymeric materials, such as cell-wall components and deoxyribonucleic acid (DNA), in which

pathogens are insulated against antibiotics, as well as host defenses.[38] Smoke-mediated enhancement of biofilm formation by the pneumococcus is preceded, within 15–60 minutes of exposure, by increased expression of a number of stress response-related genes.[39] These include the genes encoding a sensor kinase, known as *hk11*, and its cognate response regulator, *rr11*, which, together, comprise the two-component regulatory system 11, TCS11,[39,40] implicated in streptococcal biofilm formation[41,42] and resistance to vancomycin.[43] Other genes upregulated following exposure of the pneumococcus to cigarette smoke include the *SP1857 cat eff* (cation efflux system protein) and *SP2003 abc* (ATP-binding component of an ATP-binding cassette transporter) genes.[39] These are likely to be involved in the expulsion of heavy metal and pro-oxidative, organic chemical toxicants present in cigarette smoke. Interestingly, the *SP 2003 abc* gene, has also been reported to be induced following exposure of the pneumococcus to vancomycin, suggestive of a role for its encoded ABC transporter in promoting antibiotic multidrug resistance.[44]

More recently, we have described a second mechanism by which exposure of the pneumococcus to cigarette smoke promotes antibiotic resistance. This mechanism relates specifically to macrolide/macrolide-like antibiotics, and involves smoke-mediated augmentation of expression of the inducible *erm(B)* macrolide resistance gene.[45] This gene encodes a ribosomal dimethyl transferase enzyme, which abrogates macrolide-mediated inhibition of bacterial protein synthesis. This results from dimethylation of a critical adenine nucleotide (A2058) located in the peptidyl transferase region of domain V of the 23S rRNA component of the 50S subunit of the bacterial ribosome, thereby interfering with the affinity of members of this class of antibiotics for their microbial target.[46,47] Antibiotic resistance mediated by the *erm*(B) gene encompasses all types of macrolides (14-,15- and 16-membered), lincosamides (clindamycin, lincomycin) and streptogramins B.[47]

In this context, our recent studies have revealed that exposure of an *erm*(B)-expressing, macrolide-resistant strain of the pneumococcus (strain 2507, serotype 23F) to cigarette smoke condensate (CSC) *in vitro* in the presence of the macrolide antibiotic,

clarithromycin, resulted in significant upregulation of expression of the *erm*(B) gene relative to that observed in the presence of the antibiotic alone.[45] Unexpectedly, exposure of this strain of the pneumococcus to CSC in the absence of the antibiotic also resulted in significant upregulation of expression of *erm*(B), albeit to a lesser extent than that observed in the presence of clarithromycin alone.[45] These findings raise the possibility that CSC-mediated, spontaneous induction of *erm*(B) by CSC (in the absence of clarithromycin), as well as augmentation of clarithromycin-mediated induction of this macrolide resistance gene, result from a common mechanism activated in response to smoke-related stress.

In this context, it is noteworthy that like strain 172, exposure of strain 2507 of the pneumococcus to CSC also resulted in upregulated expression of the genes encoding TCS11.[48] Although unproven, it is plausible that induction of both ribosomal methylation and biofilm formation by CSC may converge on TCS11 as a coordinated stress response to smoke exposure. This contention is supported by the findings, albeit in bacterial pathogens other than the pneumococcus, that methylation of ribosomal RNA, like biofilm formation, is associated with protection against environmental/oxidative stressors in *Escherichia coli* and *Staphylococcus aureus*.[49,50] In addition, it is also noteworthy that ribosomal methylation as a mechanism of antibiotic resistance is not restricted to macrolides, lincosamides and streptogramins B. This type of mechanism is broadly operative in mediating resistance to other categories of ribosome-targeted antibiotics, implying that induction of ribosomal methyltransferases by cigarette smoke exposure may pose the threat of multidrug resistance.[51]

The pneumococcus possesses a second major gene-based mechanism of macrolide resistance, which is mediated via induction of the macrolide efflux protein A-encoding gene, *mef*(A).[47] However, unlike its *erm*(B)-expressing counterpart, exposure of a *mef*(A)-expressing strain (strain 521, serotype 23F) of the pneumococcus to CSC failed to cause either spontaneous induction or augmentative induction of the *mef*(A) gene in the absence or presence of clarithromycin respectively.[45]

In addition to induction of biofilm formation and expression of the *erm*(B) gene, the *SP2003 abc* gene, which is significantly upregulated following exposure of the pneumococcus to CSC, has also been implicated in antibiotic resistance as alluded to above. Although the role, if any, of the ABC transporter encoded by this gene in mediating macrolide resistance remains to be established, its potential involvement in promoting resistance to vancomycin has been implied in an earlier study.[43] The authors of this study reported that exposure of two different strains of the pneumococcus, one vancomycin-susceptible (T4, serotype 4) and the other–resistant (Tupelo, serotype 14) to vancomycin (5 µg/ml) for 10 and 20 minutes resulted in altered expression (up- or down-regulated) of 175 genes.[43] Of these genes, 19 encoded ABC transporters (of which more than 60 are encoded by the genome of the pneumococcus).[52] However, only two of the ABC transporter-encoding genes, *viz. SP1715* and *SP2003,* demonstrated prominent upregulation of expression following exposure to vancomycin in both strains of the pneumococcus at both time intervals tested.[43]

Not surprisingly, the aforementioned putative mechanisms of smoke-mediated antibiotic resistance described for the pneumococcus may be of broader relevance, encompassing various types of respiratory bacterial pathogens. This contention is supported by reports that exposure of *S. aureus* to cigarette smoke is also associated with increased biofilm formation and virulence, as well as antibiotic resistance.[53-55]

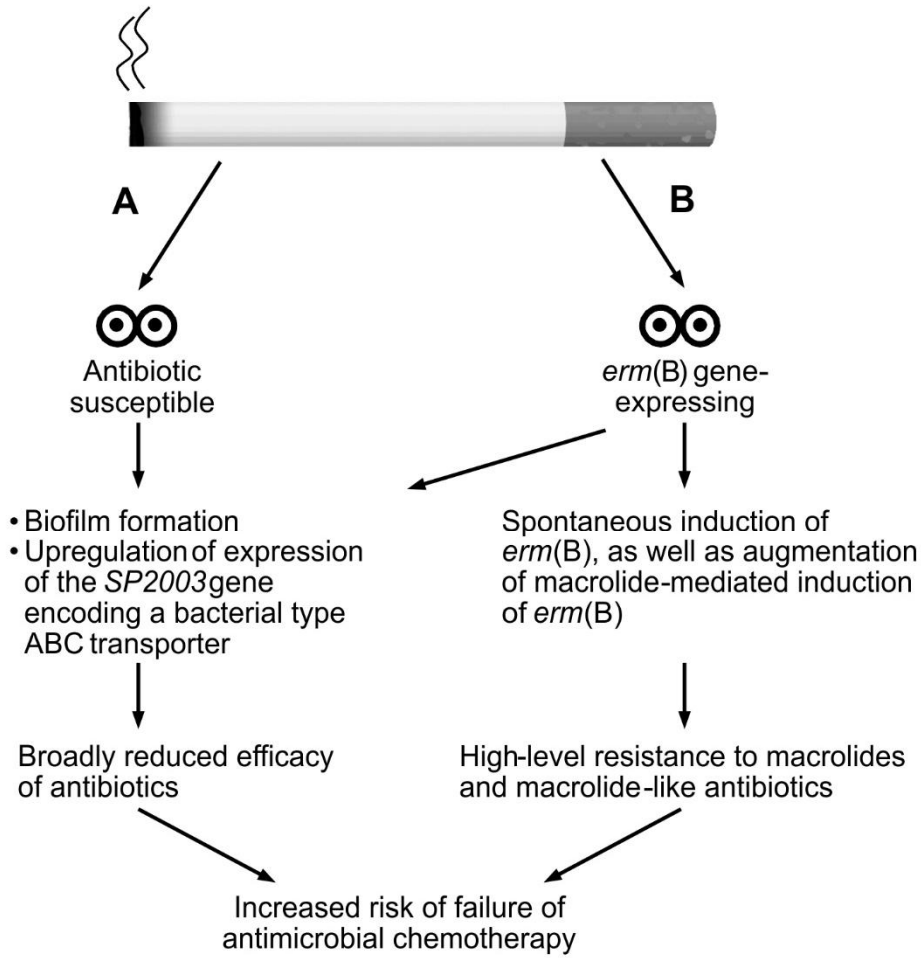

Figure 1

Exposure of both antibiotic-susceptible (A) and *erm*(B) macrolide resistance gene-expressing (B) strains of the pneumococcus (depicted as ᑕᑐ) to cigarette smoke results in induction of genes which trigger biofilm formation and expression of an ABC transporter, seemingly involved in efflux of antibiotics. These mechanisms may attenuate the therapeutic efficacy of a broad range of antibiotics. In addition, exposure to cigarette smoke also results in spontaneous induction of the *erm*(B) gene, as well as augmentation of expression of this gene following exposure of the macrolide-resistant strain of the pneumococcus to macrolides and macrolide-like antibiotics, conferring high-level resistance to these agents.

The proposed mechanisms of antibiotic resistance associated with exposure of the pneumococcus to cigarette smoke are summarized in Figure 1. These are, distinct from the point mutations described in the genes encoding DNA-gyrase and RNA polymerase

following exposure of *Pseudomonas aeruginosa* to mutagens found in cigarette smoke, conferring resistance to ciprofloxacin and rifampicin, respectively.[56]

E-cigarettes/vaping and pneumococcal infection

Studies focused on the direct effects of e-cigarette vapors on the pneumococcus are sparse. However, two studies have reported that exposure of airway alveolar macrophages and epithelial cells to nicotine-containing vapors promotes changes in these cells, which increase susceptibility to pneumococcal infection. In the case of alveolar macrophages, exposure to these vapors results in cytotoxicity,[57] while exposure of airway epithelial cells facilitates attachment of the pneumococcus via upregulation of the platelet-activating factor (PAF) receptor, the receptor for pneumococcal surface phosphorylcholine.[58] In addition, and of possible relevance to the pneumococcus, exposure of methicillin-resistant *S. aureus* (MRSA) to nicotine *per se*, as well as to e-cigarette vapors, has been reported to augment biofilm formation and resistance to host-derived antimicrobial peptides such as cathelicidin LL-37.[59]

Causes and Mechanisms of Antibiotic Resistance

Factors such as immunosuppression, clonal spread of resistant strains of bacterial pathogens due to excessive use of antibiotics, as well as smoking in the case of the pneumococcus and possibly other respiratory bacterial pathogens, represent major contributors to the development of antibiotic resistance. On the other hand, and as mentioned above, the widespread practice of immunization of the very young in particular, as well as the elderly, with serotype-restricted pneumococcal polysaccharide conjugate vaccines, most commonly PCV13 and its predecessor,PCV7, has been associated with substantial reductions in both the use of antibiotics and development of resistance in some settings.[60,61] These benefits of PCV-based immunization strategies are, however, threatened by the emergence of antibiotic resistance among non-vaccine serotypes of the pneumococcus.[61] Although incompletely understood, the association of serotype

replacement with antibiotic resistance has been attributed to elimination of competition by antibiotic-susceptible vaccine serotypes in the nasopharynx, enabling emergence of previously suppressed, resistant non-vaccine serotypes.[62] In this setting, antibiotic resistance comes at the expense of reduced fitness of these non-vaccine serotypes.[63] This may be overcome, however, via genetic transfer of metabolic and virulence components from vaccine to non-vaccine serotypes of the pathogen, conferring both fitness and persistence on the latter serotypes.[63]

As recently reported in a study originating from Canada, other potential mechanisms of PCV vaccine-related antibiotic resistance include differential induction of herd protection by vaccine serotypes.[64] In this context, herd protection conferred by the highly-invasive serotype 3 of the pneumococcus (represented in PCV13, but not PCV7) has been disappointing, possibly due to poor, post-immunization opsonophagocytic activity of antibodies produced in response to the capsular polysaccharides of this strain.[65] Poor immunogenicity appears to be associated with the emergence of the predominant global clonal complex of serotype 3 of the pneumococcus, CC180, within which the emerging Clade II exhibits increased virulence and possibly antibiotic resistance.[65]

Irrespective of the mechanisms that may be operative in the setting of pneumococcal, non-vaccine serotype antibiotic resistance in particular, the findings of a very recently reported international whole-genome sequencing study are noteworthy.[66] This study was focused on pneumococcal lineages associated with serotype replacement and antibiotic resistance based on whole genome sequencing of strains of the pathogen isolated from children aged <3 years hospitalized with IPD in the pre- and post-immunization periods.[66] The authors reported a significant increase in the prevalence of resistance to penicillin in non-vaccine serotypes in the post-PCV period relative to the pre-PCV13 period (29% vs 21%, $P = 0.0016$), as well as a corresponding increase in erythromycin resistance (11% vs 1%, $P = 0.0031$).[66] Although indicative of an emerging threat, these findings should, however, be viewed in the context of a recently reported point-of-prevalence study, which

reported low, global rates of antibiotic resistance in adult patients with proven pneumococcal pneumonia, diagnosed within 24 hours of admission to 222 hospitals spanning 54 countries.[67] Continental prevalence rates of *S. pneumoniae* drug resistance were 7.0% and 1.2% for Africa and Asia, respectively, with a corresponding rate of 1% for Europe, South America and North America, most commonly macrolide (0.6%) and penicillin resistance (0.5%).[67]

Genetic Determinants of Pneumococcal Antibiotic Resistance

Genetically determined antibiotic resistance of the pneumococcus, as well as other types of respiratory bacterial pathogens, is mediated by various mechanisms, most commonly altered target binding and accelerated efflux in the case of the pneumococcus. Exploitation of these mechanisms by the pneumococcus results predominantly from horizontal transfer of antibiotic resistance genes. In this context, bacterial horizontal gene transfer is achieved via several mechanisms, these being conjugation, transduction and transformation, with the pneumococcus being particularly adept at acquiring antibiotic resistance genes via transformation. This may occur either by unidirectional transfer between viable organisms, or by uptake of naked, fragmented DNA released by disintegrating bacteria, most commonly of the same strain and species. During the course of transformation, it has been estimated that fragments of DNA comprising up to ten genes attach to DNA-binding proteins expressed by competent, recipient bacterial cells, enabling entry of genetic material and integration into the bacterial genome via homologous recombination.[68] Efficient transformation is dependent on the recipient microorganisms being primed for both competence and expression of essential DNA-binding proteins.[68]

Competence has been described as a “transient state marked by a shift in both transcriptomic and proteomic profiles”.[69] In the pneumococcus, acquisition of competence is under the control of a transcriptomic initiation complex consisting of: i) the alternative sigma

specificity factor protein, SigX, known as the master regulator of competence; ii) a competence co-regulator (activator of SigX) known as ComW, which is responsive to quorum sensing mechanisms; and iii) RNA polymerase.[69-73] Resultant formation of the RNA polymerase holoenzyme, consisting of the core enzyme and SigX, enables correct transcription by directing the enzyme to specific sites in the promotor regions of target genes.[69] These cooperative interactions between bacterial RNA polymerase and sigma bacterial transcription initiation factors have been identified as attractive targets for development of novel antimicrobial agents, including those with anti-pneumococcal activity.[74]

Mechanisms involved in promoting resistance of the pneumococcus to various classes of antibiotics have been covered extensively in several recent reviews.[11,47,75-77] Accordingly, only those which mediate resistance to classes of antibiotic commonly used in the treatment of pneumococcal infection, specifically β-lactams, macrolides and respiratory fluoroquinolones (levofloxacin, moxifloxacin), are covered here.

Resistance of the pneumococcus to β-lactam antibiotics

The anti-bacterial action of β-lactam antibiotics results predominantly from the irreversible binding of these agents to one or more of six enzymes involved in the synthesis of the peptidoglycan backbone of the cell-wall of Gram-positive bacteria.[11,75] Inhibition of these enzymes, known collectively as penicillin-binding proteins (PBPs), results in weakening of the cell-wall and eventual bacteriolysis. Acquisition of resistance results from horizontal transfer of genes encoding PBPs which have reduced affinity for β-lactams. The resultant "mosaic" genes generated via homologous recombination confer mostly low-level β-lactam resistance, which, with the exception of central nervous system infections, may be overcome by administration of high doses of these antibiotics.[11,75] In the pneumococcus, resistance to β-lactams is associated most commonly with structural alterations to three PBPs, *viz*.

PBP1a, 2x and 2b, occurring predominantly in clinical isolates of the pneumococcus, which harbour mosaic genes. [11,47,78]

Although not recognized as a β-lactamase-producing pathogen,[79] one study has, however, described the apparent involvement of a novel metallo-β-lactamase in mediating resistance of strain ATC 49136 of the pneumococcus to ampicillin.[80]

Resistance of the pneumococcus to macrolide antibiotics

As described above, development of genetically determined resistance of the pneumococcus to macrolide and macrolide-like antibiotics occurs via transformation. This, in turn, results in the acquisition of genes, which confer resistance either by enzymatic modification of target ribosomal antibiotic-binding sites, or by driving antibiotic efflux. In the case of the former mechanism, expression of the ribosomal, dimethylase-expressing *erm*(B) gene results in dimethylation of A2058 situated in domain V of the 23S component of the large (50S) ribosomal subunit. The consequence is interference with the binding of macrolides to the inner wall of the lumen peptide exit tunnel, thereby attenuating the inhibitory effects of these antimicrobial agents on peptide chain elongation.[47,77,81] This type of *erm*(B) gene-mediated mechanism results in high-level resistance, which is unlikely to be overcome by high-dose administration of macrolides,[77] despite the propensity of these agents to concentrate intracellularly in eukaryotic cells.[82]

Two macrolide efflux pumps, macrolide efflux protein A and macrolide efflux protein E, encoded by the *mef*(A) and *mef*(E) genes, respectively, are utilized by the pneumococcus to expel these antibiotics.[76,83] However, unlike resistance mediated via the *erm*(B) gene, acquisition of the *mef* genes only confers resistance to 14- and 15-membered macrolides, but not to 16-membered macrolides, lincosamides or streptogramins B.[47] In addition, the level of resistance resulting from macrolide efflux is lower than that conferred by the *erm*(B) gene.[77]

Resistance of the pneumococcus to fluoroquinolone antibiotics

Fluoroquinolones are the only class of antibiotics which target bacterial DNA synthesis, most importantly, moxifloxacin and levofloxacin, which are known as the "respiratory fluoroquinolones" due to their potency against bacterial respiratory pathogens, including the pneumococcus.[11,47,76] With respect to their mechanism of antimicrobial action, fluoroquinolones target the type II class topoisomerase enzymes, DNA gyrase and topoisomerase IV. These enzymes, each of which is comprised of two subunits (gyrA and gyrB; parC and parE), promote unravelling of the coiled structure, as well as breakage and re-ligation, of DNA, which are critical events in bacterial DNA synthesis.[84] However, as mentioned below, acquisition of resistance necessitates stepwise, progressive accumulation of point mutations in the subunits of DNA gyrase and topoisomerase IV, with those in gyrA alone or gyrA/parC, conferring high-level resistance.[47,76] Resistance is also associated with horizontal transfer of the mutated genes.[85]

Acquisition of resistance to fluoroquinolone antibiotics also results from over-expression of genes encoding drug efflux pumps, specifically the PatAB ABC drug transporter,[86,87] as well as the Pmra transporter,[11] most likely achieved via horizontal gene transfer.

Non-Antibiotic Strategies to Overcome Antibiotic Resistance

Notwithstanding implementation of strategies targeted at overcoming risk factors for development of antibiotic resistance such as undiscerning use of antibiotics, smoking, immunosuppression and under-utilization of vaccines, pharmacological targeting of biofilm formation remains an attractive strategy to counter this ominous threat. In the case of the pneumococcus, encasement of this, as well as other types of respiratory pathogen, in biofilm promotes antibiotic resistance by several mechanisms. These include decreased bacterial metabolism and growth in the setting of exposure of pathogens to low concentrations of

antibiotics due to restricted permeation of these agents, a combination of circumstances which is highly conducive to development of resistance.[88] In addition, close proximity of antibiotic-susceptible organisms to resistant strains facilitates antibiotic resistance via horizontal gene transfer, while exposure of the pneumococcus to β-lactamase-producing organisms in polymicrobial biofilms may induce passive antibiotic resistance.[88]

As mentioned in detail in one of our earlier reviews on this topic,[88] pharmacological targeting of bacterial biofilm formation via development of inhibitors of quorum sensing mechanisms was then, and remains,[37,89,90] an attractive strategy to overcome biofilm formation. More recent strategies, include pharmacological targeting of two-component regulatory systems involved in initiation of biofilm formation.[91] To date, however, the only clinically available, biofilm-targeted strategy involves administration of nebulized, human recombinant DNAse1 to patients with cystic fibrosis infected with *P. aeruginosa*.[92] This enzyme, which targets both bacterial and human DNA, reduces sputum viscosity via dismantling of both biofilm and neutrophil extracellular traps (NETs).[92]

Impact of Antibiotic resistance in *S. pneumoniae*

A number of review articles published over several years has highlighted the emergence of antibiotic resistance among *S. pneumoniae* isolates worldwide, describing not only the epidemiology, mechanisms of resistance and risk factors, but also the clinical relevance and appropriate approach to antibiotic management.[93-98] Emerging resistance has been documented to all the major classes of antibiotics including β-lactams, macrolides and even fluoroquinolones. Data from the SENTRY Antimicrobial Surveillance Program, a continuously active global antibiotic resistance surveillance network, describes very succinctly the changes in antimicrobial resistance that have occurred among *S. pneumoniae* isolates between 1997 and 2016,[99-101] highlighting the impact of the introduction of PCV immunization of children on resistance evolution. Initially, between 1998 and 2001 among US isolates there was a decrease in susceptibility among pneumococcal isolates to

amoxicillin/clavulanate, penicillin and ceftriaxone (and other antibiotics), followed by improved susceptibility to beta-lactams during 2002 and 2003, attributed to introduction of PCV 7.[99] However, between 2004 to 2009 antimicrobial resistance among these beta-lactam antibiotics increased[99] and continued to increase further through to 2011.[100] The subsequent increase in antibiotic resistance that occurred a few years after introduction of PCV 7 was attributed to the emergence of serotype 19A, a serotype not covered by PCV 7, which expressed antimicrobial resistance.[102] However, in more recent years through to 2016, susceptibility of *S. pneumoniae* isolates from North America, Europe, the Asia Pacific region and Latin America has increased for many antibiotics and in all regions, attributable to the introduction of PCV 13 immunization in 2010.[101] However, there has been some debate as to whether antibiotic resistance is clinically relevant or whether there is a paradox between the reported *in vitro* sensitivity and clinical outcomes, with many studies failing to show a clear impact of antibiotic resistance on outcome, possibly as a consequence of methodological limitations.[93]

B-lactam resistance

A number of studies over several years has attested to the fact that the levels of penicillin and cephalosporin resistance in *S. pneumoniae* are such that they are unlikely to impact on beta-lactam resistance and on the outcome of patients with pneumococcal pneumonia.[103-110] On the other hand, a few studies have suggested that resistance to beta-lactam agents is indeed associated with worse outcomes in invasive pneumococcal pneumonia.[111-113] Turett and colleagues showed an independent association between pneumococci with an MIC to penicillin of $\geq$ 2µg/ml and mortality; however, 50% of the patients in that study were HIV-infected and the authors did not adjust for severity of illness.[111] Furthermore, only two patients in that study had actually received penicillin therapy (see discordant therapy below). Feikin and colleagues documented that when deaths in the first four days were excluded, mortality was significantly higher in isolates with a penicillin MIC $\geq$ 4 µg/ml (high level penicillin resistance) and cefotaxime MIC $\geq$ 2 µg/ml.[113] At least partly because of these

inconsistencies and the demonstration, using appropriate PK/PD principles, that adequate serum and tissue levels of parental β-lactams and oral amoxicillin could be achieved with appropriate dosing, the Clinical Laboratory Standards Institute (CLSI) increased the breakpoints for cefotaxime, ceftriaxone and amoxicillin for non-meningeal pneumococcal infections initially and subsequently for penicillin.[114,115] Yleyjeh and colleagues[116] evaluated 10 studies that examined the association between penicillin-non-susceptible pneumococci and outcome in pneumococcal pneumonia and found a significant difference in the mortality rate of 19.4% in the penicillin non-susceptible group and 15.7% in the in the penicillin-susceptible group. The authors indicated that despite these findings, they will not significantly affect our empiric treatment for CAP as current guidelines recommend using antibiotics effective against penicillin-resistant pneumococci.[114,116]

Other studies have suggested that patient-related factors such as older age and underlying comorbid illnesses,[113,117] severity of infection and do-not-resuscitate orders,[118] and clinical condition on presentation (shock and multilobar consolidation[119]) may be more important in predicting outcome than antimicrobial resistance.

Clearly antibiotic resistance can only be implicated as a cause of treatment failure if patients are treated with discordant therapy (therapy with an agent to which the pneumococcus is resistant). A prospective, international, observational study of 844 hospitalized patients with pneumococcal bacteremia, in which 15% of isolates had intermediate susceptibility to penicillin (MIC 0.12-1 µg/ml) and 9.6% were fully resistant (defined as an MIC $\geq$ 2µg/ml), documented that discordant therapy (defined as receipt for the first 2 days after the blood sample was obtained for culture of a single antibiotic that was inactive in vitro against the *S. pneumoniae* isolated) with the penicillins (penicillin, ampicillin, amoxicillin-clavulanate), cefotaxime and ceftriaxone was not associated with a higher mortality.[106] However, 11 patients were infected with what was considered to be cefuroxime-resistant pneumococci and eight of these cases, including all four cases that died, had been treated with cefuroxime at a dose of 750 mg 8 hourly. (p=0.0175). It has been indicated that clinical outcome is worse when *in vitro* testing suggests that the antimicrobial therapy would

be ineffective.[120] In this respect, an additional study suggested that intravenous cefuroxime given at a dose of 1500 mg every 8 hours would be effective therapy for bacteremic pneumococcal pneumonia with penicillin and cephalosporin-resistant isolates, at least for strains with a cefuroxime MIC of up to 4µg/ml, suggesting that the definition of cefuroxime resistance was of uncertain clinical relevance (1993 criteria; cefuroxime sodium MIC $\geq$ 2 µg/ml considered resistant).[121] A very recent study from Spain noted that despite an increasing prevalence of cefotaxime non-susceptible *S. pneumoniae* there was no evidence that patients hospitalized with bacteremic CAP and infected with non-susceptible strains had a worse outcome than patients infected with susceptible strains.[122]

Thus while bacteriological failures of less active penicillins (ticarcillin) and cephalosporins (cefazolin, cefuroxime and ceftazidime) have been documented there are also case reports of apparent failures of the more active cephalosporins.[123] One study documented the occurrence of pneumococcal meningitis in a child with sickle cell anaemia treated with vancomycin and cefotaxime.[124] However, low-dose cefotaxime was used and the patient was also immunocompromised. High-dose oral and intravenous amoxicillin, as well as high dose intravenous penicillin, ceftriaxone and cefotaxime, should achieve successful treatment of infections caused by pneumococcal isolates with penicillin MICs of $\leq$ 4µg/ml.[123] Another case of breakthrough bacteremia and meningitis was seen in a patient with pneumococcal pneumonia treated with cefotaxime; however, the antibiotic was changed to cefuroxime on the second day and immunocompromise was not excluded in the child.[125] Lastly, failure of treatment of cephalosporin therapy was noted in a child with pneumonia infected with a highly resistant pneumococcus.[126] However, initial treatment was with cefuroxime, followed by one dose of ceftriaxone followed by oral ceftibuten, which has poor activity against pneumococci and the patient developed a pleural effusion.

Macrolide resistance

The occurrence of macrolide resistance in *S. pneumoniae* isolates has been documented for many years and has recently been reviewed.[127] As opposed to β-lactam resistance, the

situation with macrolide resistance is much less clear. While there are studies demonstrating benefit of macrolides in the treatment of CAP, including macrolide-resistant *S. pneumoniae*, the discrepancy between clinical and bacteriological outcomes despite high MICs and expression of macrolide resistance genes (referred to as the in-vivo in-vitro paradox),[128,129] there are also numerous reports of macrolide failure in CAP, with both emergence of macrolide resistance ,as well as breakthrough bacteremia, in patients with pneumococcal pneumonia treated with macrolides.[130-139] In many of these studies, failure of macrolide therapy has occurred in the setting of both low-level (efflux mechanism) and high-level (ribosomal methylation mechanism) macrolide resistance. Cilloniz and colleagues recently documented that hospitalized patients with macrolide-resistant pneumococcal pneumonia were not more severely ill on hospital presentation nor had worse outcomes if treated with guideline-compliant antibiotic treatment regimens.[140] Nevertheless, because of increasing macrolide resistance and documentation of failure with both low- and high-level resistance, it has been recommended that macrolide monotherapy should not be used for CAP,[141] while others contend that these agents should still be considered for routine use in CAP, most commonly as part of combination therapy, together with beta-lactam antibiotics, and particularly in patients with severe CAP and sepsis, at least partly because of their non-antibiotic, pleiotropic effects.[142]

Fluoroquinolone resistance

There is also emerging evidence of fluoroquinolone resistance occurring in pneumococcal isolates.[143-145] Fluoroquinolones target mainly DNA gyrase or topoisomerase IV.[143] The main mechanism of fluoroquinolone resistance is the occurrence of mutations in the quinolone resistance-determining regions (QRDRs) of *par*C and *gyr*A, which encode topisomerase IV and DNA gyrase, respectively.[143,146] Fluoroquinolones, which possess dual activity against *S. pneumoniae,* are less likely to select for fluoroquinolone resistance than non-dual activity agents, since in the former, mutations in both DNA gyrase and topoisomerase IV are required for clinically relevant resistance.[143] In general, *par*C mutations confer resistance to

ciprofloxacin, but not to levofloxacin or moxifloxacin, while mutations in *gyr*A or both *par*C and *gyr*A confer resistance to the latter agents.[143] Low-level resistance occurs with one-step mutation in the target genes, whereas high-level resistance requires a second mutation, in the other target gene.[146] Isolates with a single *par*C mutation are usually reported as being susceptible to fluoroquinolones, because the MICS are at, or below, the CLSI breakpoints. Therefore, there is no test that accurately detects the presence of this resistance. Nevertheless, this one-step mutation increases the likelihood of the development of a second *gyr*A mutation, which is then associated with high-level resistance and therapeutic failure.[146] As such, failures of fluoroquinolone therapy have been regularly documented in patients with pneumococcal respiratory tract infections associated with fluoroquinolone resistance, with resistance being present either at the beginning of the infection, or emerging during treatment; risk factors for these infections have been determined in various studies and these infections have been noted to have a high mortality.[146-150]

Most of the reported treatment failures have occurred following administration of either ciprofloxacin or with levofloxacin at a dose of 500mg daily,[146,148] which is understandable given that the pharmacokinetic/pharmacodynamic parameters that predict the likely clinical response of an antibiotic.[143] Fluoroquinolones display concentration-dependent killing meaning that as the concentrations of these agents increase, so does their bactericidal activity.[143] The pharmacokinetic parameter that is commonly used as a correlate to bacteriological and clinical response of fluoroquinolones is the AUIC (area under the curve over the minimum inhibitory concentration), with a ratio of > 30 traditionally considered as being predictive of a good outcome in pneumococcal infections.[143] Ciprofloxacin does not achieve this breakpoint value and is, therefore, less likely to eradicate pneumococcal respiratory tract infections. Furthermore, studies by Schentag and colleagues have suggested that AUICs of over 125 should be targeted since values below 100 are associated with resistance development, regardless of whether the organism is Gram-positive or Gram–negative.[143,151] We have previously documented that higher doses of levofloxacin of 500mg

twice daily or 750mg daily, but not a lower dose of 500mg daily, are able to achieve these higher AUIC levels recommended (>125).[152]

In summary, a review of the current literature regarding the likely impact of antibiotic resistance in *S. pneumoniae* leads to the same conclusions that have been espoused in review articles published over the years.[123,153-157] In the case of the penicillins, aminopenicillins and cephalosporins, failures occur mainly with the use of agents that are poorly active against the pneumococcus, or with the use of doses of ostensibly efficacious antibiotics with PK/PD parameters that likely predict treatment failure, the latter being overcome with the use of more appropriate dosing. In the case of the macrolides, and despite the suggestion that there may be an *in vivo* / *in vitro* paradox, failures have occurred in patients infected with pneumococcal isolates with both low-level and high-level resistance, such that macrolide monotherapy is not recommended routinely in patients with CAP; however, the routine combination of a macrolide with standard beta-lactam therapy is recommended in sicker hospitalized and critically ill patients with CAP. In the case of the fluoroquinolones, while high-level resistance is likely to be associated with treatment failure in fluoroquinolone-resistant pneumococcal infections. However, an additional concern is being able to document those isolates harbouring a one-step mutation, and which are currently reported as susceptible on MIC testing, because they are more likely to develop a second mutation during fluoroquinolone therapy, thereby expressing high-level resistance, which may then be associated with treatment failure.

Impact of vaccination with PCV on pneumococcal antibiotic resistance

One development that has had a very positive impact on pneumococcal antibiotic resistance and which should ensure the ongoing efficacy of standard antibiotic therapy in patients with pneumococcal infections, has been the use of pneumococcal vaccines, particularly PCVs. Mechanisms by which vaccines may impact on antibiotic resistance include firstly, by eradicating the organisms, particularly the antibiotic-resistant serotypes, that are targeted by the vaccine and, secondly, by preventing infections, such as otitis media, for which

antibiotics would usually be prescribed.[158] A number of studies has shown a decrease in the rate of drug-resistant *S. pneumoniae* infections, in both younger children and older adults following introduction of PCVs.[159-162] In the case of PCV 7, invasive disease caused by penicillin non-susceptible strains decreased by 81% (95%CI 80-82%) in children under 2 years of age, and by 49% in adults ≥ 65 years.[159] Rates of resistance to many other antibiotics were also documented to decrease, as were the rates of multidrug- resistant strains.[159,160] There has been, however, an increase in resistant disease caused by non-vaccine serotypes, and in particular serotype 19A as mentioned above.[159] Following introduction of PCV 13, reductions in the rate of 19A infections, as well as infections with PCV 13 serotypes, decreased further in most age groups.[161,162] Clearly, ongoing surveillance of serotype frequency and antimicrobial resistance is required to assess the impact of broader us of PCV 13, as well as the use of any newer pneumococcal vaccines that may be introduced in the future.[161,162]

Outcome of pneumococcal pneumonia

Despite advances in medicine, particularly the availability of potent antimicrobial chemotherapeutic agents and even the establishment of intensive care unit (ICU) facilities, the mortality due to pneumococcal pneumonia remains high. A recent retrospective observational study in Barcelona, Spain, of hospitalized patients with pneumococcal pneumonia, conducted over a period of 20 years between 1997 and 2016, which was divided into four 5-year periods, noted that the 30-day mortality rate was 8% and did not change significantly between periods.[163] There was an increase in admissions to ICU and need for mechanical ventilation, and although the ICU mortality decreased between periods one and two, there was no significant difference with adjustments. Even in the propensity-adjusted multivariate analysis, 30-day mortality did not change. Another study of critically ill immunocompetent patients with pneumococcal pneumonia noted an in-hospital mortality of 18.9%.[164] Most studies such as these in pneumococcal CAP, as well as many others in all-

cause CAP, have arbitrarily examined short-term mortality in those patients, such as hospital mortality or 30-day mortality.[163-165]

It is only more recently that long-term mortality rates in patients with CAP have been investigated. These studies have revealed an unacceptably high long-term mortality, such that an episode of CAP is associated with a higher risk of long-term adverse events in comparison with age-matched subjects in the general population who have not suffered an episode of CAP.[165,166] Similar findings have been noted in patients with pneumococcal CAP. One earlier study of long-term survival in patients who had recovered from pneumococcal CAP noted that mortality was increased for up to 10 years and that the Pneumonia Severity Index (PSI) score on admission, and the presence of bacteremia, were risk factors associated with higher mortality.[167] Two more recent studies indicated similar findings.[168,169] The former study, in patients with invasive pneumococcal disease (IPD) and bacteremic and non-bacteremic pneumococcal pneumonia, documented that in patients with both non-pneumonia IPD and pneumococcal pneumonia who survived 30 days, approximately 40% died within the following 5 years.[168] The study documented that even non-invasive pneumococcal pneumonia (urine antigen test positive) had an impaired long-term outcome and that the increased long-term mortality was mainly associated with co-morbid disease. The latter study documented that adult patients who had survived an episode of invasive pneumococcal pneumonia died before their life expectancy, with only 9% of patients living longer than their life expectancy.[169]

While the causes of premature mortality in those who have recovered from a prior episode of IPD remain uncertain, the establishment of persistent, residual tissue reservoirs of pneumococcal antigens, particularly in the heart, lung and spleen, has been implicated in a number of studies.[170-179] Indeed, in the case of pneumococcal endocarditis, leakage of pro-inflammatory, pneumococcal antigens derived from dead and dying organisms has been reported to persist for up to seven years.[177] In this setting of persistent antigenemia, the interaction of pneumococcal capsular and cell wall components, as well as nucleic acids, with specific antibodies and Toll-like receptors expressed on cells of the innate immune

system, as well as structural cells, is likely to trigger or exacerbate chronic, mostly sub-clinical, systemic inflammation.[178,179] These events, in turn, predispose for development of a labile, pro-inflammatory/pro-coagulant phenotype, which may contribute to the pathogenesis of long-term cardiovascular events and other non-communicable diseases. [178,179]

Conclusions

The prevalence of pneumococcal infections appears to have declined significantly among adults in the US and is largely attributable to comprehensive childhood immunization with PCV 13 and its associated herd protection, as well as a decrease in cigarette smoking. However, in many other regions of the world pneumococcal infections are still highly prevalent, being associated with significant morbidity and mortality. While exposure to cigarette smoke is a well-recognized, major risk factor for pneumococcal infection and its associated antibiotic resistance, it is also concerning that emerging evidence is implicating vaping as a potential risk factor for pneumococcal infection. Although escalating antibiotic resistance has been a concern globally, clear evidence of a significant impact of current resistance rates on patient outcomes has not been forthcoming, largely because antibiotic guidelines for CAP take into account the possibility of antimicrobial resistance. While it is clear that the use of PCVs has resulted in a decrease in the prevalence of vaccine serotype-specific pneumococcal infections and their associated resistance, ongoing surveillance is essential to monitor for non-serotype disease and the possible emergence of antibiotic resistance in these serotypes.

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