Title: Factors affecting antimicrobial resistance in *Streptococcus pneumoniae* following vaccination introduction

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Abstract:

Streptococcus pneumoniae is a major cause of pneumonia, meningitis and septicaemia worldwide. Pneumococcal antimicrobial resistance (AMR) has been highlighted by WHO as an important public health concern, with emerging serotypes showing resistance to multiple antibiotics. Indeed, although the introduction of pneumococcal conjugate vaccines (PCV) has been associated with an overall decline in pneumococcal AMR, there have been increases in prevalence of potentially disease-causing AMR serotypes not targeted by vaccination. Here we discuss a variety of evolutionary mechanisms at the host, pathogen and environmental levels that may contribute to changes in the prevalence of pneumococcal AMR in the post-vaccination era. The relative importance of these factors may vary by population, pneumococcal lineage, geography and time, leading to the complex relationship between vaccination, antibiotic use and AMR.

Pneumococcal Antimicrobial Resistance

With an estimated 3.7 million cases of severe pneumococcal disease in 2015 and 294000 deaths, *Streptococcus pneumoniae* remains a leading cause of death in children younger than 5 years, with substantial mortality and morbidity worldwide[1]. Reports of antimicrobial resistance (AMR) among clinical isolates of *Streptococcus pneumoniae* (pneumococcus) were first documented in the 1960s in the United States[2], with intermediate resistance to penicillin. By the 1970s and 1980s, penicillin-resistant pneumococci were reported globally[3]. *S. pneumoniae* colonises the human nasopharynx[4], is naturally competent and is therefore able to acquire genes conferring AMR from other pneumococci, and from other bacterial species occupying the same niche[5]. The first multidrug resistant (MDR) pneumococcal strain was identified in South Africa in 1977[6]. Subsequently, a number of MDR pneumococcal lineages with resistance to three or more antimicrobials have been shown to be circulating worldwide[3]. Antimicrobial-resistant pneumococcal infections have a substantial burden in terms of healthcare utilisation: in the US, for example, such infections account for ~20000 additional hospitalisations and $233 million in total cost[7]. Penicillin-resistant infections are also associated with worse patient outcomes by causing a significantly greater risk of in-hospital death due to pneumonia[8].

The introduction of **pneumococcal conjugate vaccines** (**PCV**, see Box 1, Glossary) into the routine immunisation schedule of more than 140 countries in the last two decades[i] has been associated with an overall decrease in infections caused by antimicrobial-resistant pneumococci[9,10]. This is primarily due to the reduction in incidence of serotypes targeted in the vaccine (vaccine types, VTs), which accounted for the majority of antimicrobial-resistant infections prior to vaccine introduction[10]. However, following vaccination, there have been

increases in frequencies of AMR among certain serotypes not targeted by vaccination (non-vaccine types, NVTs), in both carried and invasive pneumococcal isolates, which has countered the effects of vaccination[9,11–15]. Consequently, as shown by a recent global meta-analysis of AMR among paediatric pneumococcal invasive and carried isolates by Andrejiko *et al.*[9], the proportion of isolates overall which are non-susceptible to certain antibiotics (such as macrolides or tetracycline) remains unchanged following PCV implementation[9]. Furthermore, some PCV-introducing countries have seen an increased proportion of pneumococci showing resistance to one or more antibiotics. In Japan for example, the frequency of macrolide resistance genes among invasive pneumococci was observed to increase from 34% to 93% following PCV13 introduction[16], and an increase in the proportion of pneumococci showing resistance to macrolides and tetracycline has also been observed following PCV13 introduction in Argentina[14]. Within the meta-analysis by Andrejiko *et al.*[9], an increase in the estimated prevalence of non-susceptibility to macrolides and penicillin among invasive isolates was seen in Latin America & the Caribbean, from 1 year pre-vaccination to 3 years post-vaccination.

Pneumococcal population dynamics are highly complex and determining the relative importance of the factors responsible for these changes in AMR is far from straightforward. In this Opinion article, we discuss a variety of factors at the pathogen, host and environmental levels which play a role in AMR dynamics, in addition to stochasticity, secular trends, and artefacts such as "unmasking"[17] (Figure 1, Key Figure). We show that the relative importance of these factors may vary by population, pneumococcal lineage, geography and time, leading to the complex relationship between vaccination, antibiotic use and AMR.

Drivers of Pneumococcal AMR

A. Vaccination

There are several epidemiological observations which show a rise in the prevalence of resistant NVTs following vaccination in both carriage and disease isolates [11–14]. For example, increases in MDR NVT serotype 19A were widely documented following PCV7 introduction in many countries and more recently other AMR NVTs such as 8, 15A, 33F and 35B have been described[11–13]. A study by Lo *et al.*[12] among a global sample of invasive isolates found that serotype replacement post-PCV13 was largely due to expansion of NVTs within vaccine-type GPSCs (Global Pneumococcal Sequence Clusters), and such prevalent NVTs were associated with distinct lineages and AMR profiles in different countries.

However, the dynamics of NVTs following vaccination are non-linear and remain largely unpredictable[18]. It is noteworthy that the incidence of serotype 19A was increasing in various countries prior to PCV7 introduction[19] (e.g. South Korea[20], Spain[21], Belgium[21] and Israel[22]) and has increased in some countries without substantial PCV7 use (e.g. South Korea[20]). The global spread of particularly invasive/ transmissible pneumococcal serotypes is well documented and may account for the success of such pneumococci prior to vaccine introduction[19,21,23,24].

A number of mathematical models have attempted to predict the changes in pneumococcal AMR frequency and/or prevalence following vaccination (Table 1). Perhaps unsurprisingly, the predictions of these models differ widely, influenced by key assumptions of mechanisms

maintaining the co-existence of resistant and sensitive strains, as well as the specific impacts of vaccination including serotype coverage and effects on colonisation (Box 2). Some models predict an increase in resistant strains as the competitive balance between susceptible and non-susceptible strains, and between NVTs and VTs, is altered by vaccination[25–27] (Table 1). However, the intricacies of within-host dynamics, pneumococcal co-colonisation and mechanisms of immunity are not completely understood[28–30] (see Outstanding Questions Box). Indeed, a recent review by Atkins *et al*.[31] highlighted that mathematical models evaluating the effects of vaccination impact on pneumococcal AMR differ with regards to assumptions of co-colonisation and dual strain transmission – with the model results contingent on such assumptions. A recent study by Davies *et al*.[27] also highlighted that results differ substantially depending on the mechanism underlying the cost of resistance, the effects of vaccination on colonisation, as well as country-specific differences in pathogen transmission and disease burden (Box 2).

B. High carriage vs. low carriage settings

Increases in AMR post-vaccination have not been solely associated with increases in NVTs, particularly in countries with high colonisation rates. Studies of both carriage[32] and disease[33] isolates in Malawi, for example, have shown more limited indirect effects of PCV13 vaccination among unvaccinated groups, despite a high vaccine uptake and good adherence. High residual carriage of VTs and NVTs has been observed in Blantyre, Malawi, up to 7 years after PCV introduction, with a similar VT carriage prevalence half-life among both PCV-vaccinated and PCV-unvaccinated children[32]. No changes were observed in the frequency and serotype diversity of VTs in over-fives following PCV-13 in a carriage study from the Karonga district of Malawi[34] and there was no increase in serotype diversity in NVTs in any age group. Increases

in AMR among carried isolates in these cases can be partly attributed to a relative increase in non-susceptible VTs, rather than primarily increases in non-susceptible NVTs observed in other settings[11,13]. Such studies are largely based on carriage data in settings with high colonisation rates; it remains to be seen if the relative increase in AMR among carried VTs extends to a substantial increase in AMR among invasive isolates in these countries.

C. Antimicrobial use

Increased AMR levels among pneumococci have been associated with more intense use of antimicrobials[35,36]. Several mathematical models suggest that frequency of antimicrobial use and duration of exposure are important factors driving increases in AMR following vaccination[37–39]. These are in turn supported by epidemiological observations, for example, most of the genotypes within NVT 19A are resistant (at least moderately) to at least one antimicrobial. The clonal replacement of serotype 19A cc695 (intermediately penicillin resistant) by cc320 (highly penicillin resistant, also tetracycline and macrolide resistant) in the US[40] occurred during a period of intense macrolide use[41].

The changes in antibiotic use over time, however, make it difficult to interpret changes in AMR. Some countries have seen a decrease in antimicrobial prescriptions following vaccination – either as a result of public health campaigns or a decrease in infections caused by resistant pneumococci[42]. It follows that there are several examples of increasing AMR levels despite low antimicrobial use. For example, Norway has high rates of penicillin nonsusceptibility despite a low usage of antimicrobials historically[43], and increases in penicillin-resistant strains were noted in Iceland despite reductions in antibiotic consumption[44]. Increases in erythromycin MICs were

observed between 2001-2007 in Massachusetts despite a decline in antimicrobial prescriptions[45,46]. Furthermore, rates of antibiotic consumption were poorly correlated with rates of country-specific resistance in a modelling study of pneumococcal resistance to penicillins and macrolides across 20 European countries[47]. The global spread of particularly successful pneumococci, driven by other evolutionary processes, may account for these discrepancies[23,48]. If resistance doesn't impose a significant fitness cost, a decline in AMR might not necessarily be expected if antimicrobial use declines, but an increase in AMR pneumococci despite low antimicrobial use suggests other processes may also drive their success.

D. Pathogen Factors

There are particularly successful genotypes among NVTs that possess biological traits in addition to AMR which confer a competitive advantage that may have promoted their clonal expansion following vaccine introduction. These traits include: novel antigenic composition, metabolic genes conferring improved colonisation or increased transmissibility, and variation in other virulence factors such as pili and choline binding proteins[43,48].

Serotype 24F is an NVT associated with resistance to penicillin, erythromycin, clindamycin and tetracycline[49]. It has increased in prevalence following the introduction of PCV13 and is a predominant cause of in IPD in several countries[50,51]. Serotype 24F was at the upper end of the invasiveness spectrum in a meta-analysis by Balsells *et al.*[52], and has been associated with meningitis and bacteraemia in several studies[51,53]. Genomic characterisation of 24F isolates[54] has revealed it harbours many virulence genes which are conserved, including serine protease (*htrA*), hyaluronidase (*hysA*), streptococcal enolase (*eno*), choline binding proteins (*cbpD, cbpG,*

lytA, lytB, lytC, pce/cbpE, pspA, pspC/cbpA), fibronectin and laminin-binding proteins (*pavA, lmb*), in addition to genes involved in iron and manganese uptake (*piaA, piuA, psaA and cppA*). Such characteristics arguably have contributed to the success of this serotype in addition to AMR.

The poor control of serotype 3 further exemplifies this process, as although it is included in the PCV13 formulation, it has increased in prevalence despite routine vaccination, at least in part because current formulations do not provide effective antibody protection[55]. Azarian *et al.*[56] noted a recent expansion of the Clade II subgroup, which have a higher prevalence of AMR compared to other serotype 3 strains. Whole genome analysis of 616 serotype 3 isolates[48] concluded that the Clade II strains have a distinct antigenic profile, with 13 distinct antigenic markers (including NanA, StrH, PspC, and PspA), which may have facilitated immune escape from the host population, in addition to conferring other transmission advantages and virulence properties[48,56,57].

In addition to the polysaccharide capsule and protein antigens, some pneumococci also harbour pili on their outer surface, which bind to host cell components and facilitate colonisation and invasion[58]. Between 2000-2003, there was an increase in penicillin-nonsusceptible ST156 strains in Norway with the *rlrA* islet encoding type 1 pili (including the MDR Spain $9V^3$ clone[43]), on a background of low antimicrobial use. In animal models, 19F strains harbouring the *rlrA* islet outcompeted similar but non-piliated strains. Similarly, increases in piliated strains have been observed in the US following PCV7 vaccination. Before vaccination in 2000, PI-1 (encoding type 1 pili) was associated primarily with VTs. PI-1 decreased in prevalence with the declining VTs following vaccination, but re-emerged in 2004-2007 in association with NVTs, particularly

serotype 19A[59]. Similarly, there was a 40% increase in PI-2 (encoding type 2 pili) in serotype 19A following the introduction of PCV7 in Atlanta, Georgia[60].

Regions of the genome associated with AMR may also be under positive selection pressures for other evolutionary reasons. For example, in a cohort of 2518 IPD patients, Li *et al.*[61] showed that a mutation in the *pbp1b* gene, coding for a penicillin-binding protein, resulted in prolonged killing time and a 2.8 fold increased risk of meningitis. This specific mutation, which did not confer increased resistance, was rare among PCV13 serotypes and associated with NVTs and PPSV23 serotypes.

The global spread of the PMEN1 lineage provides an interesting example of how the acquisition of multiple genes may contribute to clonal success[23]. Wyres *et al.*[23] showed that, in addition to penicillin-resistance *pbp* genes, the PMEN1 clone donated genes associated with virulence and cell adherence to other highly prevalent pneumococci in 15 regions across the genome. These genes arguably may also have aided the global spread of PMEN1 as well as the recipients of these genes. Kadam *et al.* also showed that PMEN1 pneumococci possesses a unique gene regulatory system which confers high carriage rates *in vivo* through activation of the peptide *phrA*[62]. Activity of the *phrA* peptide system in response to galactose promotes the production of antibiotics, which would provide a competitive advantage to PMEN1 against other strains in the nasopharynx. It is also possible that the activation of *phrA* promotes nasopharyngeal colonisation by breaking down host mucins to release complex sugars[63].

E. Host Microbiome

The airway microbiome represents a rich network of bacterial social interactions among commensal and pathogenic organisms. There is widespread variability in the composition of the respiratory microbiome among individuals, which is increasingly recognised as a mediator of susceptibility to respiratory infection[64,65]. There are negative associations between pneumococcal carriage and certain bacterial species, including *Rothia, Gemella, Actinomyces, Dolosigranulum, Veillonella* and *Granulicatella*[64,66]. In contrast, other bacterial species seem to aid the growth of pneumococci[67] and enhance the effects of pneumococcal AMR. For example, *Moraxella* provides passive protection from beta-lactam antibiotic killing in polymicrobial biofilms through the production of beta lactamases[68]. The factors which determine the composition of the respiratory microbiome flora are highly complex and extend beyond vaccination and antibiotic use – including mechanism of birth, breastfeeding, early colonisation with particular pathogens, diet and host genetics[64].

Resident commensal species in the oral microbiome are thought to play an important role in the acquisition of AMR by pneumococci through horizontal genetic transfer (HGT). Early nucleotide sequencing studies from the 1990s provided the first evidence of interspecies HGT between *Streptococcus mitis* and the pneumococcus[69]. More recently, a high resolution analysis of HGT across multiple pneumococcal carriage serotypes has shown that pneumococcal serotypes that are commonly carried in the nasopharynx for long carriage durations[70,71], such as serotypes 6A, 13, 14, 16F, 19A, 19F, 23F, and 35B, were frequent recipients of *S. mitis pbp* fragments that confer reduced pneumococcal β-lactam susceptibility[5]. HGT requires co-carriage of donor and recipient lineages, and in the PCV era expanding NVT lineages are now more likely to encounter

commensal streptococci in carriage which may facilitate AMR HGT[11,34,72]. An example of an NVT lineage with evidence of AMR HGT in the post PCV era includes the beta-lactam resistant 35B (ST558) lineage that has expanded in the US causing IPD[73], and ST558 has acquired *S. mitis pbp* sequences that confer reduced pneumococcal β-lactam susceptibility[5]. Although the dynamics of interspecies HGT among streptococci are not well understood, it is likely that commensal streptococci are a source of AMR even for pneumococcal lineages that escape vaccine control.

F. Environmental Factors

Exposure to black carbon – a major component of air pollution – has been shown to induce significant changes in *S. pneumoniae* biofilm structure and function. Pneumococcal biofilms formed under exposure to black carbon are thicker with increased survival against penicillin[74].

Climate also has an effect on pneumococcal carriage and disease. Epidemics of pneumococcal meningitis, particularly serotype 1, have marked seasonality in West Africa and occur mainly during the hot, dry, dusty season[75]. It is thought that low humidity and dry Harmattan winds during these periods may lower mucosal defences[76]. Seasonal outbreaks of influenza in colder months in temperate countries have also been linked with an increased risk of IPD[77]. Lower absolute humidity in this analysis was also linked with an increased risk of IPD. Local increases in temperature have also been associated with increased rates of AMR in the US for *Escherichia coli, Staphylococcus aureus* and *Klebsiella pneumoniae* [78]. Although the study did not include *S. pneumoniae*, the mechanisms postulated - of increased rates of HGT and increased replication rates - are applicable to a range of bacterial pathogens.

G. Secular Trends, Stochastic Effects and Artefacts

In addition to the periodic epidemic nature of certain serotypes, pneumococci exhibit natural fluctuations in incidence over time[79,80], which should be borne in mind when interpreting trends in AMR. A number of VTs were increasing in Germany[81] (serotypes 1, 3 and 7F) as well as Belgium, Spain, England and Wales[21] (serotypes 1, 7F and 19A) several years prior to PCV7 introduction. A large scale study in Blantyre, Malawi, with data extending to 6 years prior to PCV13 introduction showed that a significant reduction in IPD preceded vaccine introduction[33]. Similarly, a 10% decrease in otitis media following vaccination in the US could be detected by pre-vaccine introduction trend analysis alone[82]. Therefore, a limitation of several studies investigating effects of PCV on IPD is the short period of time for which data is available prior to vaccine introduction, making it difficult to distinguish secular trends in IPD incidence from effects of vaccination[33]. Similarly, a process known as "unmasking" may have occurred following PCV introduction, through a reduction in VT prevalence thereby making it easier to detect resistant NVTs that were already present in the population. This will have the effect of overestimating serotype replacement in carriage[17]. Added to this, it is difficult to determine the extent to which natural fluctuations in prevalence are driven by stochastic processes. Stochastic dynamics in Pseudomonas *aeruginosa* for example, have been shown to play an important role in the emergence of AMR[83].

Concluding Remarks

Fortunately, the decline in prevalence of infections caused by antimicrobial resistant pneumococci overall following the introduction of routine infant vaccination currently outweighs the increases

in antimicrobial resistant NVTs in many countries[84]. Nonetheless, AMR is emerging and the possibility of large-scale increased AMR remains a global concern[ii]. A holistic approach to the interpretation of post-vaccine AMR amongst pneumococci and other mucosal microbes is required, as bacterial dynamics are determined by a complex combination of genetics, host factors, co-infections and environmental influences in addition to vaccination and antimicrobials use. Even if these additional factors have a small magnitude of effect, they are cumulative. There are several ongoing studies which will provide more information on the plethora of mechanisms which shape the post-vaccination population structure of *S. pneumoniae*[85]. Studies of inter-species interactions in the microbiome in different populations, including the nature of competitive interactions and acquisition of AMR genes, are key to understanding AMR trends.

Box 1: Pneumococcal conjugate vaccines, serotype replacement and AMR

A series of pneumococcal conjugate vaccines (PCV) have been introduced to combat the capsular types, or serotypes, responsible for the highest disease burden and AMR, each targeting 7, 10 or 13 serotypes. Out of >90 serotypes[86], only ~6-11 accounted for >70% of cases of invasive pneumococcal disease (IPD) in children in Europe and North America before the first PCV vaccination, PCV7, was introduced[24]. Similarly, most clinical isolates with high-level penicillin resistance belonged to only 5-10 serotypes[87]. Although a decline in IPD has been observed in many countries following introduction of PCVs, pneumococcal carriage rates remain largely unchanged, in part due to high residual carriage of vaccine serotypes (particularly in high burden settings)[32] and in part due to an increase in prevalence in serotypes which have not been targeted by vaccination (non-vaccine types: NVTs)[88]. This process, known as serotype replacement, has occurred in invasive disease in addition to carriage, thus eroding the effects of vaccination[72].

Box 2: Conceptual frameworks exploring changes in AMR post-vaccination

A number of theoretical frameworks have been used to investigate changes in pneumococcal AMR prevalence and/or frequency following vaccination (Table 1) with a range of mechanisms explored. Different key mechanisms are responsible for the changes in AMR observed in different models, including: the duration of antibiotic exposure[37]; rates of antimicrobial use[37,39,42,89]; ecological processes including within-host competition and rates of co-colonisation by sensitive and resistant strains[25–27]; serotype coverage in PCV vaccination[37,42]; variability in antimicrobial consumption among sub-populations and rates of contact between them[27]; and diversifying selection on pneumococcal subtypes[27]. Each model differs with regards to the mechanisms maintaining the co-existence of resistant and sensitive strains prior to vaccination, including assumptions of co-colonisation and fitness costs of resistant strains, as well as mechanisms through which vaccination in implemented in the population. Changes to these assumptions have led to pivotal differences in results yielded[31]. For example, simulations by Davies *et al.*[27] predict that, where sensitive strains have a within-host growth advantage in the absence of antimicrobials, vaccines which block the acquisition of VTs in vaccinated hosts lead to decreased frequencies of AMR. However, they also show that frequencies of AMR are decreased following vaccination by vaccines which operate through shortening the duration of carriage in the host. This is supported by Lehtinen *et al.*[70] who show that the fitness advantage of resistant strains may be maintained by a longer duration of carriage. Frequencies of AMR are also decreased in simulations by Davies *et al.* following acquisition-blocking vaccination where resistant strains are assumed to have a transmission cost. Davies *et al.* also explored the effects of variability between countries in parameters other than antibiotic use and found that predictions of overall

vaccine impact were similar. They also explored the effects of vaccination in high transmission settings, such as in Sub-Saharan Africa[32], for which they found that a higher vaccine efficacy is needed to achieve a reduction in AMR pneumococcal carriage. These differences in model predictions highlight the importance of the mechanism of vaccination on colonisation, as well as the cost of resistance and country-specific differences, on pneumococcal dynamics and AMR frequencies.

Table 1: Conceptual frameworks exploring changes in AMR frequencies following pneumococcal vaccination

Reference	***Maintenance of AMR frequency pre-vaccination***	***Mechanism of vaccination***	***Impact of vaccination on AMR frequency***
Davies *et al.*[27]	Four mechanisms are included: <u>Treatment diversity model and pathogen diversity model:</u> In the treatment diversity model, antimicrobial treatment rates differ among assortatively-mixing subpopulations (geographic regions, socioeconomic status, host age or risk classes). Subpopulations with higher rates of antimicrobial consumption have higher frequencies of AMR and vice versa. In the pathogen diversity model, diversifying selection maintains subtypes with different durations of carriage. Such subtypes have differing frequencies of AMR, with greater selection for AMR in strains with longer duration of carriage. <u>Treatment competition model and growth competition model:</u> AMR frequency is maintained by frequency-dependent selection; individuals can be colonised with	Two types of vaccine are included: - An acquisition-blocking vaccine (prevents pneumococcal acquisition by a certain value). - A clearance-accelerating vaccine (shortens duration of carriage by a certain value). Both vaccines decrease carriage frequency.	<u>Acquisition-blocking vaccine:</u> Treatment competition model: Reduced co-colonisation following vaccination results in decreased resistance frequencies, as within-host competition favours resistant strains. Growth competition model: Decreased co-colonisation overall favours the promotion of resistant strains, as within-host competition favours susceptible strains. Treatment diversity and pathogen diversity models: Vaccination results in decreased resistance frequencies.

	both sensitive and resistant strains and the rate of colonisation compared to co-colonisation is key to determining co-existence. In the treatment competition model, resistant strains have a transmission cost. In the growth competition model, sensitive strains are able to outcompete resistant strains in the absence of antimicrobials, due to a within-host growth advantage.		<u>Clearance-accelerating vaccine:</u> Such vaccines have the overall effect of inhibiting resistance, as they result in shorter carriage duration.
De Celles *et al*.[42]	Two models are included: one in which transmission/invasiveness differences are introduced between VTs and NVTs, and another in which differences are introduced between susceptible and non-susceptible strains. Penicillin-resistant pneumococci have a cost of resistance, with lower transmissibility and lower invasiveness. This model simulated pneumococcal meningitis only.	Acquisition-blocking vaccines with a range of serotype coverages are simulated.	The scenarios with reductions in antibiotic-use, low VT coverage of vaccination and high AMR frequency, led to a higher meningitis incidence with penicillin-susceptible strains.
Mitchell *et al.*[26]	Variable co-infection of 2 (out of 3) susceptible and non-susceptible strains is permitted (including a resistant NVT, a resistant VT and a susceptible NVT). The growth advantage for resistant strains varies between 1.0 and 1.05%.	90% of the entrants into the model are vaccinated, with a reduction in transmissibility of VTs to vaccinated hosts by 50%.	Vaccination opens up niche spaces for both resistant and susceptible NVTs, and increases in dual carriage allow for greater spread of the resistant NVT strain within the population.
Obolski *et al*.[25]	Resistant strains have a longer duration of carriage, weighed against the cost of resistance through lower infectivity. Co-infection of susceptible and non-susceptible strains is inhibited by a factor ψ owing to ecological competition.	Vaccine serotype “a” is completely removed from the population (the frequency of such strains becomes zero at the point of vaccination introduction).	Vaccination results in rapid increase in frequency of pre-existing resistant NVTs due to the removal of competition from VTs.

Temime *et al.*[37]	Over a threshold of antimicrobial usage, resistant strains have a growth/transmission advantage relative to susceptible strains.	A fraction of children <2 years are vaccinated, with vaccine protection lasting for an average time before they move to unvaccinated compartments as adults. Two types of vaccines are simulated: A 7-valent vaccine targeting PCV7 serotypes, with resistance frequencies reflecting those of France at the time. An "optimised" 11-valent vaccine, targeting all resistant strains to penicillin. Vaccinated hosts were susceptible only to carriage with NVTs for an average time, and co-colonisation is not permitted.	The 7-valent vaccine has a marginal effect on the frequency of AMR. The 11-valent vaccine resulted in lower AMR frequencies initially, however over time the effects of the vaccine were eroded by the emergence and transmission of resistant NVTs. Antimicrobial use favours the growth of resistant NVTs (longer exposure leads to a greater proportion of resistant strains).
Temime *et al.* [89]	Over a threshold of antimicrobial usage, resistant strains have a growth/transmission advantage relative to susceptible strains. This model simulated pneumococcal meningitis only.	A fraction of children <2 years are vaccinated with a heptavalent vaccine, with vaccine protection lasting for an average time before they move to unvaccinated compartments as adults. Vaccinated hosts are susceptible only to carriage with NVTs for an average time, and co-colonisation is not permitted.	The effect of vaccination depends on antibiotic exposure: in settings with low use of antibiotics, PCV vaccination prevents penicillin resistant pneumococcal meningitis cases, whereas in settings with greater exposure to antibiotics, vaccination does not lead to a substantial decrease in such cases.
Van Effelterre *et al.*[39]	There is a serotype-specific "fitness cost" of sensitive and resistant serotypes, which may affect transmission. All resistant strains	Vaccinated hosts have a lower serotype-specific risk of colonization and/or of IPD if colonized by	Without vaccination, an increase in the prevalence of IPD caused by resistant 19A strains

	(regardless of serotype) are slightly less able to cause IPD compared to a susceptible strain of the same serotype.	VTs, compared to non-vaccinated hosts. PCV7 effectiveness against non-PCV7-serotype IPD was assumed to be 0%.	was significant but only slightly lower than if vaccination was introduced. In the absence of antimicrobials, the increase in the prevalence of resistant 19A strains was significantly lower, suggesting that antimicrobial use is a more important contributing factor than vaccination.

Figure 1, Key Figure: Factors contributing to pneumococcal AMR

There are several factors and evolutionary processes at the level of the environment, host and pathogen itself which may contribute to the ongoing changes in antimicrobial resistance among pneumococci.

Glossary

Non-vaccine type: a serotype which is not targeted in a multivalent vaccine targeting several serotypes

Pneumococcal conjugate vaccine (PCV): These vaccines include specific pneumococcal polysaccharides conjugated to a protein carrier (such as the cross-reacting material of diphtheria toxin) in order to boost T cell immunity. There are currently PCVs targeting 7, 10 and 13 serotypes available.

Serotype: The type of polysaccharide present in the capsule surrounding each pneumococcus determines its capsular type or serotype. Individual serotypes prompt unique immune responses

with varying levels of cross-immunity across certain serotypes. There are >90 serotypes documented.

Serotype replacement: the process by which NVTs increase in frequency in the population following strain-targeted PCV vaccination, thereby "replacing" the VTs.

Resources

[i]https://view-hub.org/sites/default/files/2022-04/VIEW-hub_Report_March2022_0.pdf

[ii]https://apps.who.int/iris/handle/10665/112642

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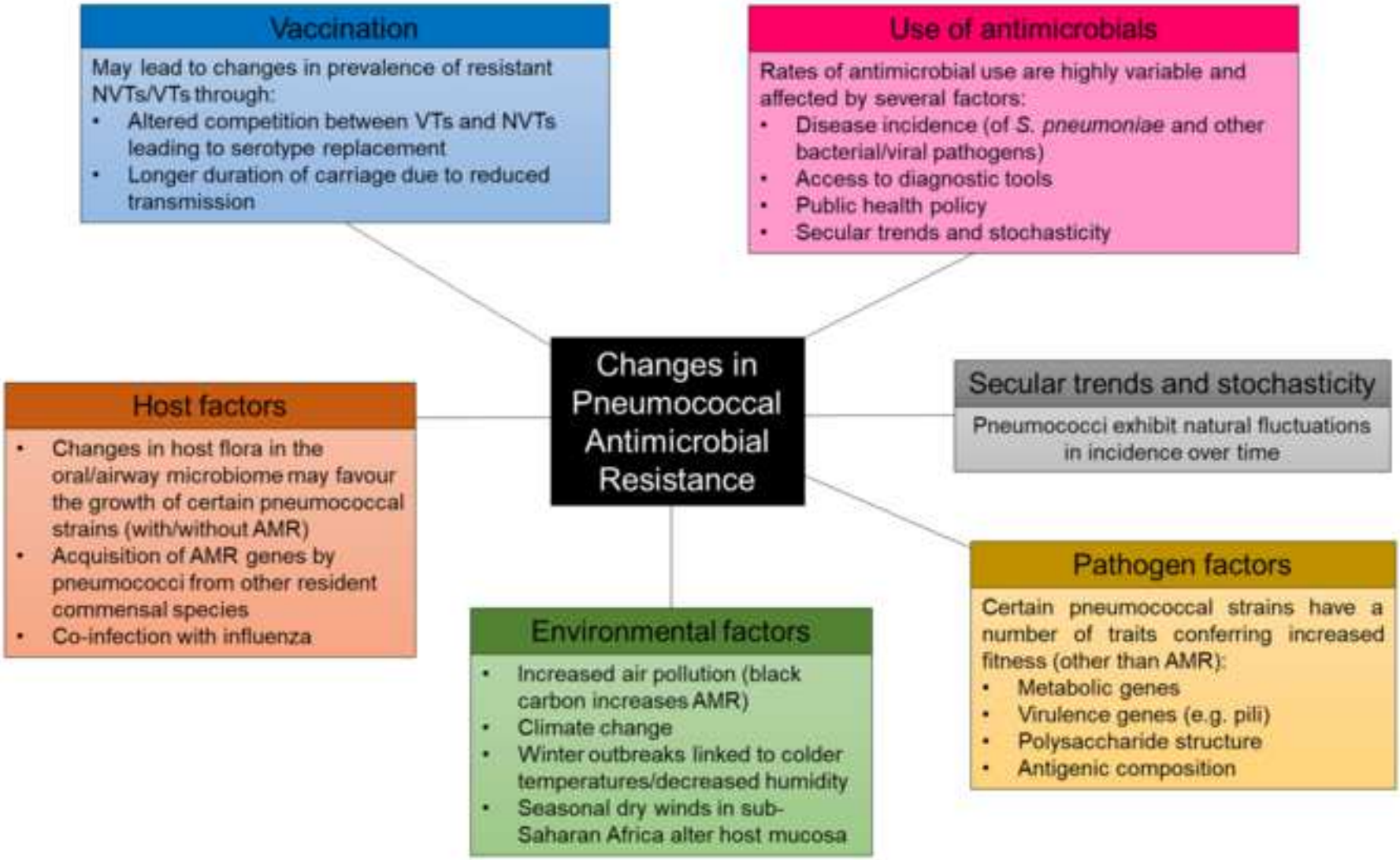
Figure



Highlights

- Recent studies investigating the long-term impacts of pneumococcal conjugate vaccination have found that the proportion of pneumococci showing resistance to first-line antimicrobials has decreased following vaccination. However, increased rates of resistance to particular antimicrobials such as macrolides have been observed in several countries, particularly among serotypes not targeted by vaccination.
- Pneumococcal conjugate vaccines targeting 7, 10 and 13 serotypes have been introduced in many countries over the last two decades. Newer vaccines with greater valency targeting 15 and 20 serotypes are expected to be licensed in 2022 and 2023 respectively. However, these vaccines do not provide protection against several lineages associated with AMR.
- Recent insights into the upper respiratory tract microbiome, including acquisition of genes conferring AMR from commensals, have revealed the importance of inter-species dynamics in pneumococcal AMR in the host.
- Mathematical models simulating the effects of pneumococcal vaccination on AMR have highlighted the importance of mechanisms of vaccination, inter-strain competition and rates of co-colonisation, exposure to antibiotics, as well as the cost of resistance and country-specific differences on changes in AMR frequencies.

Outstanding Questions

- What are the precise ecological processes which underpin interstrain pneumococcal competition? What is the relative importance of immunological competition (including duration and strength of serotype-specific immunity vs. cross-immunity) and ecological competition (for metabolic/host resources)? To what extent does interstrain competition operate through decreased acquisition of competitor strains relative to increased clearance?

- How important are biological factors other than antimicrobial use and vaccination in promoting the spread of resistant NVTs? Is there a favourable genetic basis to clonal success in resistant strains other than AMR alone? How do these strains compete with susceptible strains in the host?

- What are the dynamics of the interactions between *S. pneumoniae* and other species in the same upper respiratory tract niche? How do commensals and other pathogens in the upper respiratory tract render the host either more or less susceptible to pneumococcal carriage and disease? Do such microbial interactions affect some pneumococcal strains more than others?

- Commensals are donors, recipients, and reservoirs of ARGs (antibiotic resistance genes): after every course of antimicrobials taken, the whole microbiome experiences selection for AMR, with ARGs potentially transferring from commensals to pathogens by horizontal gene transfer. How important is this bystander effect?

- To what extent is the perceived increase in pneumococcal AMR a surveillance artefact?

How important is “unmasking” - through which a reduction in VT prevalence following vaccination makes it easier to detect low frequency NVTs that were already present in the population before vaccination?