Chapter

An Emerging Multidrug-Resistant Pathogen: *Streptococcus pneumoniae*

Khalid I. Alqumaizi and Razique Anwer

Abstract

Streptococcus pneumoniae (*S. pneumoniae*) has a multifaceted bond with its human host and causing several diseases in children and adults when host flexible immunity and bacterial acquisition factors allow them to invade essentially sterile spots, such as the middle ear spaces (causes otitis media), lungs (causes pneumonia), bloodstream (causes sepsis) and meninges (causes meningitis). In the early 1940s, management of pneumococcal infections used to be somewhat straightforward, and penicillin commonly was the antibiotic of choice. Soon after mainstreaming antibiotic usage, worldwide emergence of antibiotic resistance among *S. pneumoniae* isolates has changed this approach. Multiple factors, like prior antibiotic use, inappropriate usage of antibiotics especially in young age, and day care attendance are the most commonly identified risk features for the spread of penicillin resistance and other multiple-antibiotic resistance. Basic fundamental mechanisms of most pneumococcal resistances have been identified, several organizations like WHO, CDC, BSAC, EUCAST started campaigns for appropriate antibiotic use and also the introduction of pneumococcal conjugate vaccines have been recommended to limit the further emergence and spread of pneumococcal resistant.

Keywords: drug-resistant *S. pneumoniae*, World Health Organization, upper respiratory tract, β-lactam antibiotics, penicillin-binding proteins

1. Background

According to WHO, bacterial resistance to antibiotic drugs are now one of the most global events that threaten humanity; due to new resistant mechanisms acquired by bacteria that help them to evade both natural and chemical elimination systems that are, immune system and antibiotic drugs [1]. With the ability to acquire resistance, simple infections can create major clinical problems for different patients, leading to serious events that include death. Unfortunately, although warnings about the aimless use of antibiotic drugs have been made by medical experts since the 1940s, the expenditure of antibiotic drugs are still increasing [2]. This issue is not only related to certain countries like India and South Africa where antibiotics are available without prescriptions, but also worldwide [2]. This implies that restricted guidelines must be made by specialized health sectors in both hospitals and pharmacies. Not only that but also generating a public awareness forum

where people around the world are educated about the dangerousness of misusing antibiotic drugs. However, if increased consumption of antibiotics continues, doors for bacteria are going to be open, permitting them to enter an adaptive phase where mutations and among other things can take place; leading to deleterious consequences [3]. Indeed, the world today must reform the way antibiotics are being prescribed and utilized; not doing so, will impose a fast-rising threat which can be slowed down if certain behavior changes like a simple hand washing are applied [1]. Nevertheless, researchers in this field are facing a wide range of challenges which led to a major decrease in the discovery and development of new antibiotics; due to the widespread use of these drugs which have led to difficult new resistant bacteria families to appear [4]. This can be illustrated by looking back in time, for instance, approximately 47 new antibiotics were developed collectively in the period from 1983 to 2002, while from 2003 to 2012 almost seven new drugs only were developed [4]. This shows how close we are to reaching a post-antibiotic era where fear and trepidation from the simplest injuries and common infections are once again established. Therefore, the science community must come together and set up a focused system where only life-threatening resistant bacterium is targeted in order to safe major resources and develop better outcomes.

On one hand, we should also not forget to monitor and adjust the public behavior towards this topic, as it is the major fuel to this crisis. On the other hand, if this threat is left without a serious action, an estimation of nearly 10 million people will die every year in 2050 due to antimicrobial resistance, not to mention the huge cost burden with over 100 trillion USD [5]. In this chapter, we aim to establish a comprehensive understanding of defense mechanism of certain worrying and life-threatening bacteria (*Streptococcus* spp.) that have mastered new maneuvers to evade the immune system and antibiotic drugs; causing multiple of diseases that are hard to treat, and to investigate its impact on patients clinically. Not only that but also to scrutinize the general defects that allowed pathogens like bacteria to survive and conquer the human body, and to explore the drugs that used to fight such bacteria but eventually failed to do so. Under these circumstances, doors of opportunities are going to be open for researchers to grasp the most important knowledge that they need in order to innovate new ideas, to create new treatments and methods to minimize the risks of this crisis. For this reason, the scope of this chapter is going to be mainly focused on the problem of certain worrying bacteria that were categorized and prioritized by WHO.

2. Insights into antimicrobial emergence

It is well-known to scientists that bacteria are one of cleverest creatures that can not only generate new methods continuously to evade the immune system and antibiotic drugs, but also adapt to various situations to ensure its survival and growth. By knowing that, it is important to explore their mechanism in an attempt to have a better understanding of how they work and function. However, it would make sense to direct all efforts to certain worrying bacteria that are resistant by prioritizing it according to certain criteria. To do so, WHO has published a global priority list of resistant bacteria to antibiotic drugs in order to facilitate a path that will guide researchers all around the world where the urgency of finding new treatments is vital [6]. With the help of expert opinion and evidence-based data WHO-global priority pathogens list developed a multi-criteria decision analysis (MCDA) technique for prioritizing the research and development of new and effective antibiotic treatments. Following steps has been taken to set prioritization: (1) selection of antibiotic-resistant bacteria to be prioritized; (2) selection of criteria

for prioritization; (3) data extraction and synthesis; (4) scoring of alternatives and weighting of criteria by experts; and (5) finalization of the ranking of pathogens. This list was created with the help of specialists all around the world and contains 12 most dangerous resistant bacteria families organized based on where exigency of new treatments is needed. The first three were sat as a **critical priority**, and those are *Acinetobacter baumannii*, CR, *Pseudomonas aeruginosa* CR and *Enterobacteriaceae* 3GCR [6]. The second six were sat as **high priority**, and those are *Enterococcus faecium* VR, *Staphylococcus aureus* MR&VR, *Helicobacter pylori* ClaR, *Campylobacter* FQR, *Salmonella* spp. FQR and *Neisseria gonorrhoeae* FQR [6]. The last three were sat as **medium priority**, and include *S. pneumoniae* PNS, *Haemophilus influenzae* AmpR and *Shigella* spp. FQR [6]. It is important to say that WHO has clearly pinpointed that *Mycobacterium tuberculosis* was excluded from the list because it has been reported worldwide as a priority and other initiatives are already devoted to finding new treatments for *Mycobacterium tuberculosis* [7]. In spite of that, those bacteria were selected based on 10 criteria, and those include "mortality, healthcare and community burden, a prevalence of resistance, a 10-year trend of resistance, transmissibility, preventability in the hospital and community settings, treatability and current pipeline" [8]. Each criterion was chosen by experts who have previous experience and knowledge, and evidence for those criteria was taken from various reliable sources; such as, systematic reviews of published literature and so much more. In the light of this, a one must bear in mind that sometimes establishing priorities have its drawbacks, which a large public forum like WHO should seek to abstain to avoid any wrongness that would rather cause a disaster; however, it is indeed an appreciated and understandable move as sometimes "the simplest messages are usually the most effective," and it will eventually help in addressing bacteria in a proper manner [9]. Nonetheless, the goal here is not only to create new treatments, but rather bring multiple sectors like governments, pharmaceutical companies, and experts together to ensure a successful procedure to face this great challenge both by raising awareness of communities and encouraging research [10, 11].

Furthermore, to face a great challenge like bacterial resistance a one must have a tremendous knowledge about their defense mechanism and the way they behave towards facing obstacles that are immune system and antibiotic drugs, and we attempt to review the accessible evidence and asses the relative importance of pathogens, and the status of drug-resistance *S. pneumoniae*, and their mechanisms and evolution of resistance to the various antibiotics.

3. Streptococcus pneumoniae

3.1 Classification, transmission, colonization and invasion

S. pneumoniae, is an important facultatively anaerobic Gram-positive coccal-shaped bacterium that occur in pairs or chains and surrounded by a polysaccharide capsule, belongs to Firmicutes phylum. Traditionally, classification based on their three distinctive patterns appear on blood agar, which are termed alpha (partial), beta (complete) and gamma (none) hemolysis. According to Rebecca Lancefield classification, the beta-hemolytic streptococci (BHS) species can be further classified by the cell wall carbohydrate [12]. Most of the BHS species are associated with human diseases, and are categorized under Lancefield Groups A, B, C and G. Group A and Group B are characterized by presence of antigen on particular species while Group C and G antigen occur on a small number of closely related species (collectively as termed "Group C/G") [13] (**Figure 1**). *S. pneumoniae* (also known as

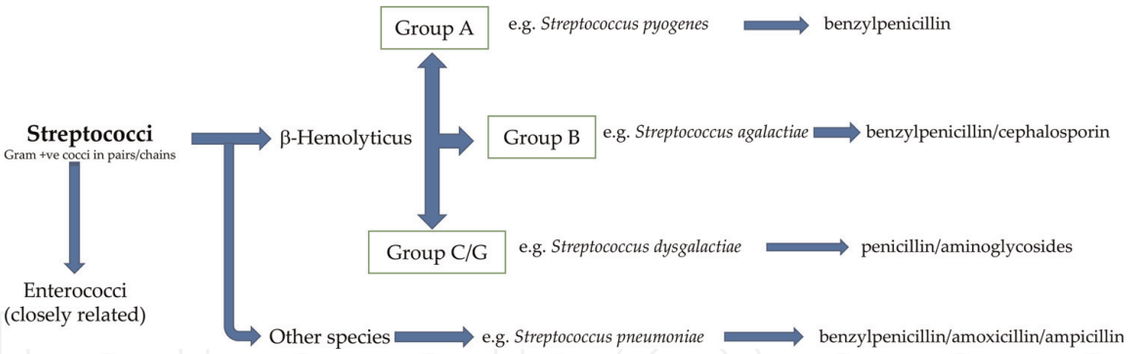

Figure 1.
Classification of Streptococcus spp. and drug of choice for causative agents.

pneumococcus) is an opportunistic pathogen that colonizes the mucosal surfaces of the human upper respiratory tract (URT) and group in other species of streptococci. This microorganism survives and multiply in wet environments, colonize in respiratory tract, bloodstream, pleural fluid, peritoneum, surgical wounds and oropharynx secretions of infected individuals. It has also been shown to colonize the normally sterile site results in invasive infection. Despite the diversity of host sites of *S. pneumoniae* can survive for long periods on both dry and moist surfaces. Carriage of pneumococci in the nasopharynx is more common in young children. Carriage is generally asymptomatic; but it serves as the main source for invasive pneumococcal infections and also plays a role in transmission from person-to-person. Adherence is the main features that facilitate colonization in the host cells and tissues. Prerequisite factors (including influenza A virus and other bacteria) are required for *S. pneumoniae* to colonize and persist on the mucosal surface, after attaining of incubation duration sufficient transmission to occur. Nasal inflammatory response due to influenza A virus, that regulates the expression of pro-inflammatory chemokines, also upregulation of target epithelial receptors and damaged respiratory epithelium used for *S. pneumoniae* adherence and disintegrate the epithelium and that helps in providing nutrient. These combined effects of viral co-infection increase the susceptibility of the host to colonization of *S. pneumoniae* [14]. *S. pneumoniae* basically produced two enzymes, peptidoglycan-*N*-acetylglucosamine deacetylase and attenuator of drug resistance, that helps in the modification of their peptidoglycan and promote it resistant to the lytic effects of lysozyme, which are abundant on the mucosal surface of the upper respiratory tract [15]. Negatively charged capsular polysaccharides also aided *S. pneumoniae* access and attach to the surface of epithelial cells and avoiding entrapment in the nasal mucus [16]. *S. pneumoniae* also uses several surface components for binding, like virulence protein A & B, enolase, phosphorylcholine moieties on cell wall teichoic acid [17, 18]. The successful colonization of *S. pneumoniae* depends on their relationship with normal microbiota, which are very complex mechanism. Symbiotic relation with microbiota of nasopharynx is depends on competition or coordination in nature [19]. *S. pneumoniae* produces numbers of bacteriocins (pneumocins) and other related microbial peptides which helps in inhibit the growth of another microbiota [20].

3.2 Identified risk features of *S. pneumoniae*

Over the last 15 years, *S. pneumoniae*, designated as a "red-alert" human pathogen, primarily because of its exceptional ability to survive in the community environment and remarkable ability to upregulate or acquire resistance to antibiotics. *S. pneumoniae* imposes a huge disease burden as the leading cause of wide

range of infections, including community-acquired pneumonia, meningitis and sepsis in children and adults and causes otitis media in infants and young children. As all of these diseases are "dead ends" in the life cycle of the organism, the bacterial factors that cause invasive diseases must also be adaptive for colonization and/or transmission. *S. pneumoniae* is an opportunistic pathogen that colonizes the mucosal surfaces of the human upper respiratory tract. Up to 27–65% of children and <10% of adults are carriers of *S. pneumoniae* and carriage involves a commensal relationship between the bacterium and the host [21]. Dissemination of this microorganism through local spread, aspiration or seeding to the bloodstream results in many invasive diseases (**Figure 2**). Globally, pneumonia considered as leading cause of death in younger child whose age is <5 years, and it attributed 1.6 million deaths annually. According to the World Health Organization, pneumococcal disease continues to cause the most deaths among vaccine-preventable diseases [22]. Persons at higher risk for invasive pneumococcal disease include children below 2 years of age, adults above 65 years of age, those with underlying chronic conditions (cardiovascular or pulmonary diseases, etc.), and also who are immunocompromised like, congenital immunodeficiency, human immunodeficiency virus infection, leukemia, or systemic corticosteroid use, etc. [23].

3.3 Mechanisms of antimicrobial resistance

An organism is considered resistant when its growth *in vitro* is not inhibited by an antimicrobial agent. The causative agents for resistance differ greatly but often linked to empirical antimicrobial therapy, that's include inappropriate

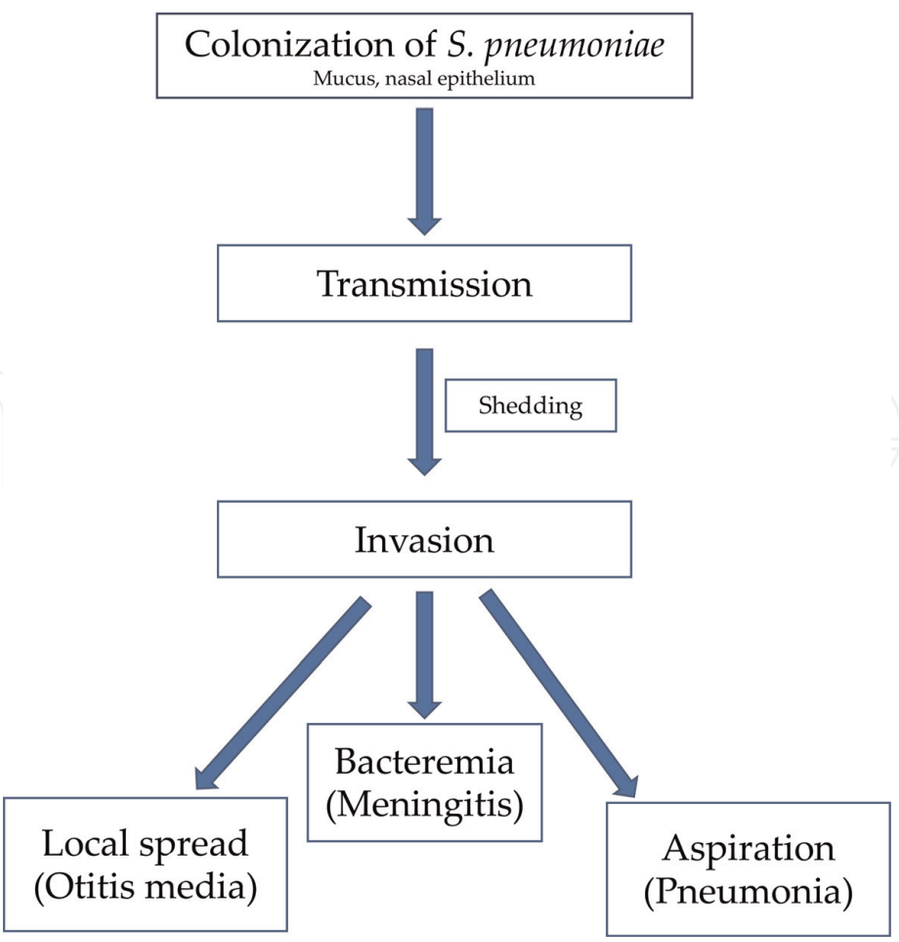

Figure 2.
Pathophysiology of Streptococcus pneumoniae.

administration of subtherapeutic doses of antimicrobial agents, drug overuse or interrupted courses, and poor tissue-intake of the antimicrobial agent [24].

Antimicrobial resistance probably originated from horizontal resistance gene between bacterial species. These genes are acquired rapidly by the mechanism of plasmid promoted-conjugation, transformation or virus-induced transduction process, that all process contribute to the development of antimicrobial resistance. Due to these mechanisms some of the genes are inherited, some change to random DNA mutations in bacteria, and others are imported from related or distant bacteria [25] (**Figure 3**). Repeatedly use of antibiotic has been shown to be the strongest risk factor for the carriage and spread of resistant pneumococci, at both the individual and the community levels [26]. Evidence showed that antimicrobial resistance developed in *S. pneumoniae* may indication of transmission of the organism among patients and may be predictive of an impending outbreak of *S. pneumoniae* infections.

S. pneumoniae modify its genome through the uptake and incorporation of exogenous DNA from other pneumococci or closely related oral streptococci has facilitated the spread of antibiotic resistance and evasion of vaccine-induced immunity. Identification of resistant pneumococci based upon genetic features, culture-based phenotypic susceptibility methods are the gold-standard approach in clinical laboratories. Interpretations to evaluate antibiotic resistance in *S. pneumoniae* have been established by several organizations, such as WHO, CDC, Clinical and Laboratory Standards Institute (CLSI), the (BSAC), British Society for Antimicrobial Chemotherapy (BSAC), and the European Committee on Antimicrobial Susceptibility Testing (EUCAST). Culture of clinical specimens and subsequent antibiotic susceptibility testing to suggest treatment options are helps in recognition of antibiotic resistance in *S. pneumoniae*. The microbial identification and diagnosis of the infecting microorganisms are prerequisites for efficient treatment and hospital/community infection control and helps in control the spreading antibiotic resistance strains. These procedures are time consuming, laborious, and require well-trained technicians for correct interpretation of results. However, effective, immunological microbial identification methods have been developed for only a small number of bacterial species [27]. Molecular-based methods such as ribosomal RNA sequencing and MALDI-TOF are available and considered as powerful tool to

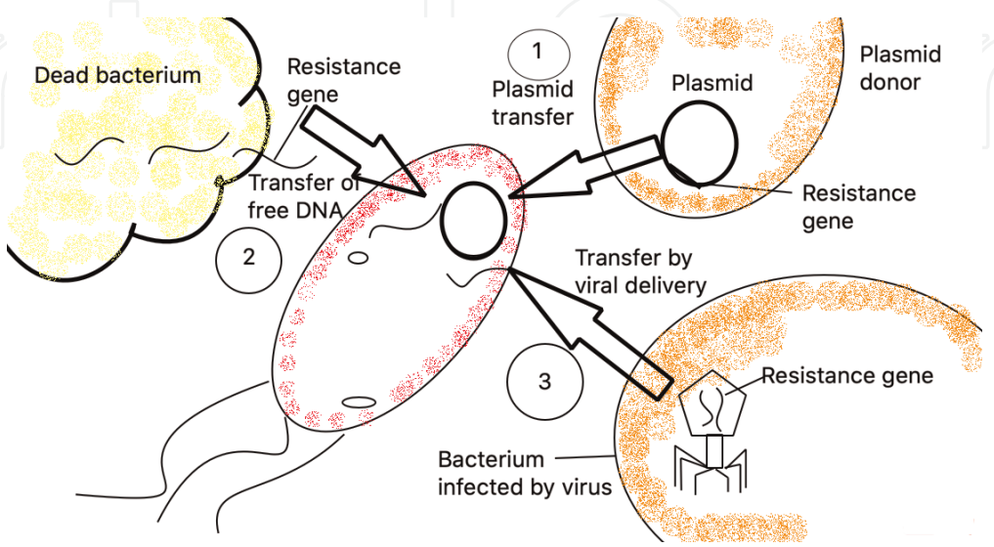

Figure 3.
Bacterial acquiring resistance genes. Three major methods for resistant gene acquisition: (1) donor cells transfer plasmid containing one or more genes into another bacteria, (2) bacteria integrate gene through transformation process and, (3) a virus acquires a resistance gene from a bacterium and injects it into a different bacterial cell.

improve detection from clinical specimens [28, 29]. Biomolecular factors for antibiotics resistance have also led to the development of a variety of molecular assays to detect the presence of resistance genes in pneumococcal isolates (PCR) and also directly from clinical specimens (MALDI-TOF) [30, 31]. According to Metcalf et al., they developed a promising whole-genome sequencing (WGS) based "typing pipeline" for rapid automated predictions of pneumococcal serotypes, MICs, genotypes, and additional features [32]. Enhanced bioinformatic tools such as ARG-ANNOT (antibiotic resistance gene annotation) for querying WGS data greatly expand the depth of laboratory-based strain surveillance efforts and provides a periodically updated database for known accessory resistance genes to screen bacterial whole-genome sequence data [33].

Antibiotics have been a basis of pneumococcal disease treatment and either by decreasing or eradicating the bacterial load from host body [34]. As production of penicillin started in the mid-1940s, after that treatment of pneumococcal infections has relied heavily upon penicillin and other β-lactam antibiotics, which showed most effective antibiotics against this bacterium. In 1912, a first antimicrobial-resistant pneumococcal infections were documented when optochin resistance in experimental mice was described [35]. Five years later acquired optochin resistance was seen in humans [36]. In 1967, the first clinical isolate in a pediatric patient in Australia reported with reduced penicillin susceptibility [37]. During the period of 1970–1980, pneumococci resistant to penicillin, erythromycin, and trimethoprim-sulfamethoxazole (TMP-SMX) spread rapidly globally, including many developed nations [38]. Tetracycline, chloramphenicol and fluoroquinolone resistances were also documented at relatively low levels compared to those for the above-mentioned antibiotics [39]. More than 40% of isolates are penicillin resistant in several countries that lack significant conjugate vaccine coverage [40, 41]. Only few studies have been conducted on the acquisition of multidrug resistance however, these studies have found that extremes of age (i.e., <5 years and more than 65 years of age), previous use of β-lactam antibiotics by patients with noninvasive disease, antibiotic use in the last month by patients with nasopharyngeal colonization, population density, geographic location, and pneumococcal seven-valent conjugate vaccine (PCV7) serotype are all independent risk factors [42].

Typical therapy for the treatment of pneumococci disease (including invasive) are β-lactam antibiotics (benzylpenicillin, amoxicillin or ampicillin). Soon after mainstreaming antibiotic usage, multi-resistant pneumococcal clones emerged and disseminated worldwide. Penicillin resistant *S. pneumoniae* strains emerged globally, including macrolide and tetracycline, that elucidates the potential of this microorganism to respond selectively in environmental changes. Regulated mechanisms of innate resistance or acquisition of foreign determinants that have also brought *S. pneumoniae* as one of the organisms threatening the current antibiotic era. Nearly, 90 serotypes of *S. pneumoniae* have been identified like 6B, 9V, 14, 19F, or 23F were high level resistant to β-lactam, were first reported in children via nosocomial transmission [43]. In European Union countries, multidrug resistance was observed among isolates of serotypes 19A, 14, 1, 19F, and 23F [44]. In the United States, serotypes of 15A, 15B, 15C, 6C, 23A and 35B showed less multidrug resistance if the person had conjugate vaccine, taken 14 years ago. Multi-resistant serotype 19A isolates still showed the highest MICs for β-lactams, macrolides, lincosamides, tetracycline, and co-trimoxazole [45].

Another cause of β-lactam resistance is due to phenotypic expression of penicillin resistance alterations that results in modification of penicillin-binding proteins (PBPs), consequently reducing peptidoglycan synthesis. This loose affinity causes cell lysis and bacterial cell death [46]. As peptidoglycan serves important roles in maintenance of cell integrity, cell expansion, cell division, cellular diffusion and

surface anchoring. Gram positive bacterium pneumococcal peptidoglycan is composed of alternating glucosamine and *N*-acetylmuramic acid residues, directly cross-linked by transpeptidases between two *N*-acetylmuramic acid residues via short pentapeptides (L-Ala-γ-D-Glu-L-Lys-D-Ala-D-Ala) between the L-Lys and the last D-Ala of an adjacent loop. Structural similarity of the β-lactam binds to the D-Ala-D-Ala terminus of the peptidoglycan stem peptide, that causes β-lactams irreversibly bind transpeptidases at their active site. Binding of β-lactams to the transpeptidase active site of these penicillin-binding proteins (PBPs) thus blocks cross-linking of muropeptide chains to prevent cell wall synthesis [47]. Pneumococcal strain reveals reflective changes in corresponding key PBP genes, and a very wide range of "resistant" PBP gene alleles [48]. It has never been observed within pneumococcal strains how β-lactamases, introduced either mobile genetic elements or expressed from the core genome. Structural alterations that causes prevention of binding to analogs (β-lactams) of their normal substrates is expressed from the core genome serve their essential biosynthesis for resistant PBP [49].

Six PBPs genes have been described in *S. pneumoniae*. Three PBP alterations (PBP1a, 2x, and 2b) strongly associated with β-lactam resistance. All three of these PBPs share a penicillin-sensitive N-terminal transpeptidase domain that contains three conserved motifs: SerXXLys, containing the active-site serine that is bound (acylated) by PBPs; SerXAsn; and LysSer(or Thr)Gly [50]. In contrast to PBP2b and PBP2x, PBP2a has been associated with decreased susceptibility and higher MICs which causes β-lactam resistance [51]. PBP gene substitutions that appear to affect the polarity, charge distribution, and flexibility of the region neighboring the active site to decrease PBP-binding affinities for penicillin and/or other β-lactam classes in non-susceptible pneumococci [52].

As discussed earlier, PBP genes (*PBP1a*, *PBP2b*, and *PBP2x*) have been clearly demonstrated to be required for high-level β-lactam resistance in clinical isolates. In some instances, low-level resistance is also dependent upon proteins that are not directly targeted by β-lactams. Sometime due to different PBP allele combinations shows different β-lactam resistance phenotypes, and this complication leads to PBP genes from certain strains were not transform wild-type strains to the same high level of resistance [53]. One study showed that strains exhibiting identical PBP transpeptidase domain sequences exhibited penicillin MICs ranging from 0.25 to 2.0 μg/ml [54]. Another cause for resistivity is due to unaltered *murM* genes. *murM* gene inactivation, effects in the lack of branching activity, subsequently the synthesis of peptidoglycan consisting of only linear muropeptides. The finding suggested that MurM aminoacyl ligase appears to be required for penicillin resistance, that appeared a direct role of aminoacyl ligase branching activity in penicillin resistance [55]. One study also showed another type of resistant mechanism, peptidoglycan *O*-acetyltransferase encoded by the *adr* gene, attenuates PBP variant causes penicillin resistance [56]. Though, recent studies showed that most penicillin-resistant pneumococci are effectively treated by high doses of parenteral β-lactams.

4. Conclusion

With the advent of more advanced laboratory techniques, including whole-genome sequencing, and continued, high-quality surveillance of antimicrobial resistance, we can continue to further expand our understanding of this area. Special program and campaigns run by various organization like, WHO, CDC, BSAC, EUCAST should continue to be in all countries to decrease not only the burden of disease but also antimicrobial-resistant pneumococci. Also more focus

on pneumococcal conjugate-vaccines because the new conjugate vaccines target these resistant serotypes, the implementation of use of these vaccines is expected to have an important role in limiting the spread of antibiotics-resistant *S. pneumoniae* strains.

Conflict of interest

The author declares that there is no conflict of interest.

Author details

Khalid I. Alqumaizi[1] and Razique Anwer[2*]

1 Department of Family Medicine, College of Medicine, Imam Mohammad Ibn Saud Islamic University (IMSIU), Riyadh, Saudi Arabia

2 Department of Pathology, College of Medicine, Imam Mohammad Ibn Saud Islamic University (IMSIU), Riyadh, Saudi Arabia

*Address all correspondence to: razainuddin@imamu.edu.sa

References

[1] WHO. Antimicrobial Resistance: Global Report on Surveillance 2014. Geneva: World Health Organization; 2014. Available from: http://apps.who.int/iris/bitstream/10665/112642/1/9789241564748_eng.pdf?ua=1

[2] Honigsbaum M. Superbugs and us. Lancet. 2018;**391**(10119):420. DOI: 10.1016/S0140-6736(18)30110-7

[3] Giedraitienė A, Vitkauskienė A, Naginienė R, Pavilonis A. Antibiotic resistance mechanisms of clinically important bacteria. Medicina. 2011; **47**(3):137-146

[4] Li B, Webster TJ. Bacteria antibiotic resistance: New challenges and opportunities for implant-associated orthopaedic infections. Journal of Orthopaedic Research. 2018;**36**(1): 22-32. DOI: 10.1002/jor.23656

[5] O'Neill J. Antimicrobial Resistance: Tackling a Crisis for the Health and Wealth of Nations. Review on Antimicrobial Resistance. 2014. Available from: https://amr-review.org/sites/default/files/AMR%20Review%20Paper%20-%20Tackling%20a%20crisis%20for%20the%20health%20and%20wealth%20of%20nations_1.pdf

[6] Tacconelli E, Carrara E, Savoldi A, Harbarth S, Mendelson M, Monnet DL, et al. Discovery, research, and development of new antibiotics: The WHO priority list of antibiotic-resistant bacteria and tuberculosis. The Lancet Infectious Diseases. 2018;**18**(3): 318-327

[7] WHO. Publications on TB Drug Resistance. Available from: http://www.who.int/tb/publications/drug-resistance/en/ [Accessed: 18 December 2017]

[8] Luepke KH, Suda KJ, Boucher H, et al. Past, present, and future of antibacterial economics: Increasing bacterial resistance, limited antibiotic pipeline, and societal implications. Pharmacotherapy: The Journal of Human Pharmacology and Drug Therapy. 2017;**37**:71-84

[9] The Pew Charitable Trusts. Antibiotics Currently in Clinical Development. 2017. Available from: http://www.pewtrusts.org/~/media/assets/2017/05/antibiotics-currently-in-clinical-development-03-2017.Pdf?la=en [Accessed: 2 June 2017]

[10] The Wellcome Trust. What We Do. Available from: https://wellcome.ac.uk/what-we-do [Accessed: 17 May 2017]

[11] BARDA. Biomedical Advanced Research and Development Authority. Available from: https://www.phe.gov/about/BARDA/Pages/default.aspx [Accessed: 17 May 2017]

[12] Lancefield RC. A serological differentiation of human and other groups of hemolytic streptococci. Journal of Experimental Medicine. 1933; **57**:571-595

[13] Facklam R. What happened to the streptococci: Over-view of taxonomic and nomenclature changes. Clinical Microbiology Reviews. 2002;**15**:613-630

[14] McCullers JA, Rehg JE. Lethal synergism between influenza virus and *Streptococcus pneumoniae*: Characterization of a mouse model and the role of platelet-activating factor receptor. The Journal of Infectious Diseases. 2002;**186**:341-350

[15] Davis K, Akinbi H, Standish A, Weiser J. Resistance to mucosal lysozyme compensates for the fitness deficit of peptidoglycan modifications by *Streptococcus pneumoniae*. PLoS Pathogens. 2008;**4**:e1000241

[16] Nelson AL et al. Capsule enhances pneumococcal colonization by limiting

mucus-mediated clearance. Infection and Immunity. 2007;**75**:83-90

[17] Jensch I et al. PavB is a surface-exposed adhesin of *Streptococcus pneumoniae* contributing to nasopharyngeal colonization and airways infections. Molecular Microbiology. 2010;**77**:22-43

[18] Cundell DR, Gerard NP, Gerard C, Idanpaan-Heikkila I, Tuomanen EI. *Streptococcus pneumoniae* anchor to activated human cells by the receptor for platelet-activating factor. Nature. 1995;**377**:435-438

[19] Shak JR, Vidal JE, Klugman KP. Influence of bacterial interactions on pneumococcal colonization of the nasopharynx. Trends in Microbiology. 2013;**21**:129-135

[20] Bogaardt C, van Tonder AJ, Brueggemann AB. Genomic analyses of pneumococci reveal a wide diversity of bacteriocins—Including pneumocyclicin, a novel circular bacteriocin. BMC Genomics. 2015; **16**:554

[21] Bogaert D, De Groot R, Hermans PW. *Streptococcus pneumoniae* colonisation: The key to pneumococcal disease. The Lancet Infectious Diseases. 2004;**4**:144-154

[22] WHO Global Immunization Data 2014. Geneva, Switzerland: World Health Organization; Available from: http://www.who.int/immunization/monitoring_surveillance/global_immunization_data.pdf?ua1

[23] Robinson KA, Baughman W, Rothrock G, Barrett NL, Pass M, Lexau C, et al. Active bacterial core surveillance/emerging infections program network. Epidemiology of invasive *Streptococcus pneumoniae* infections in the United States, 1995-1998: Opportunities for prevention in the conjugate vaccine era. JAMA. 2001;**285**:1729-1735. DOI: 10.1001/jama.285.13.1729

[24] Livermore DM. Bacterial resistance: Origins, epidemiology, and impact. Clinical Infectious Diseases. 2003;**36** (Suppl 1):S11-S23

[25] Mah MW, Memish ZA, Cunningham G, Bannatyne RM. Outbreak of *Acinetobacter baumannii* in an intensive care unit associated with tracheostomy. American Journal of Infection Control. 2001;**29**(5):284-288

[26] Dowell SF, Schwartz B. Resistant pneumococci: Protecting patients through judicious use of antibiotics. American Family Physician. 1997;**55**: 1647-1648

[27] Gray LD, Fedorko DP. Laboratory diagnosis of bacterial meningitis. Clinical Microbiology Reviews. 1992;**5**: 130-145

[28] Avni T, Mansur N, Leibovici L, Paul M. PCR using blood for diagnosis of invasive pneumococcal disease: Systematic review and meta-analysis. Journal of Clinical Microbiology. 2010; **48**:489-496

[29] Cherkaoui A, Hibbs J, Emonet S, Tangomo M, Girard M, Francois P, et al. Comparison of two matrix-assisted laser desorption ionization-time of flight mass spectrometry methods with conventional phenotypic identification for routine identification of bacteria to the species level. Journal of Clinical Microbiology. 2010;**48**(4):1169-1175. DOI: 10.1128/JCM.01881-09

[30] Fukushima KY, Yanagihara K, Hirakata Y, Sugahara K, Morinaga Y, Kohno S, et al. Rapid identification of penicillin and macrolide resistance genes and simultaneous quantification of *Streptococcus pneumoniae* in purulent sputum samples by use of a novel real-time multiplex PCR assay. Journal of

Clinical Microbiology. 2008;**46**: 2384-2388. DOI: 10.1128/JCM.00051-08

[31] Seng P, Drancourt M, Gouriet F, La Scola B, Fournier PE, Rolain JM, et al. Ongoing revolution in bacteriology: Routine identification of bacteria by matrix-assisted laser desorption ionization time-of-flight mass spectrometry. Clinical Infectious Diseases. 2009;**49**(4):543-551. DOI: 10.1086/600885

[32] Metcalf BJ, Gertz RE, Gladstone RA, Walker H, Sherwood LK, Jackson D, et al. Strain features and distributions in pneumococci from children with invasive disease before and after 13-valent conjugate vaccine implementation in the USA. Clinical Microbiology and Infection. 2016;**22**:60.e9-60.e29. DOI: 10.1016/j.cmi.2015.08.027

[33] Gupta SK, Padmanabhan BR, Diene SM, Lopez-Rojas R, Kempf M, et al. ARG-ANNOT, a new bioinformatic tool to discover antibiotic resistance genes in bacterial genomes. Antimicrobial Agents and Chemotherapy. 2014;**58**:212-220. DOI: 10.1128/AAC.01310-13

[34] Jacobs MR, Koornhof HJ, Robins-Browne RM, Stevenson CM, Ver-Maak ZA, Freiman I. Emergence of multiply resistant pneumococci. The New England Journal of Medicine. 1978;**299**: 735-740. DOI: 10.1056/NEJM197810052991402

[35] Moore HF, Chesney AM. A study of ethylhydrocuprein (optochin) in the treatment of acute lobar pneumonia. Archives of Internal Medicine. 1917;**19**:611

[36] Ross RW. Acquired tolerance of pneumococcus to M. & B. 693. Lancet. 1939;**1**:1207-1208

[37] Hansman D, Bullen MM. A resistant penumococcus. Lancet. 1967;**2**:264-265

[38] Klugman KP, Koornhof HJ, Kuhnle V. Clinical and nasopharyngeal isolates of unusual multiply resistant pneumococci. American Journal of Diseases of Children. 1986;**140**: 1186-1190

[39] Jones RN, Sader HS, Mendes RE, Flamm RK. Update on antimicrobial susceptibility trends among *Streptococcus pneumoniae* in the United States: Report of ceftaroline activity from the SENTRY antimicrobial surveillance program (1998-2011). Diagnostic Microbiology and Infectious Disease. 2013;**75**:107-109. DOI: 10.1016/j.diagmicrobio.2012.08.024

[40] Doern GV, Pfaller MA, Kugler K, Freeman J, Jones RN. Prevalence of antimicrobial resistance among respiratory tract isolates of *Streptococcus pneumoniae* in North America: 1997 results from the SENTRY antimicrobial surveillance program. Clinical Infectious Diseases. 1998;**27**:764-770. DOI: 10.1086/514953

[41] Song JH, Lee NY, Ichiyama S, Yoshida R, Hirakata Y, Fu W. Spread of drug-resistant *Streptococcus pneumoniae* in Asian countries: Asian network for surveillance of resistant pathogens (ANSORP) study. Clinical Infectious Diseases. 1999;**28**:1206-1211. DOI: 10.1086/514783

[42] Brandileone MC, Casagrande ST, Guerra ML, Zanella RC, Andrade AL, Di Fabio J. Increase in numbers of β-lactam-resistant invasive *Streptococcus pneumoniae* in Brazil and the impact of conjugate vaccine coverage. Journal of Medical Microbiology. 2006;**55**:567-574. DOI: 10.1099/jmm.0.46387-0

[43] Jacobs MR. Clinical significance of antimicrobial resistance in *Streptococcus pneumoniae*. South African Medical Journal. 2007;**97**:1133-1140

[44] European Centre for Disease Prevention and Control. Annual

epidemiological report 2012: Reporting on 2010 surveillance data and 2011 epidemic intelligence data. Solna, Sweden: European Centre for Disease Prevention and Control; 2013

[45] Centers for Disease Control and Prevention. Antibiotic Resistance Threats in the United States, 2013. Atlanta, GA: Centers for Disease Control and Prevention; 2014

[46] Percheson PB, Bryan LE. Penicillin-binding components of penicillin-susceptible and -resistant strains of *Streptococcus pneumoniae*. Antimicrobial Agents and Chemotherapy. 1980;**18**: 390-396. DOI: 10.1128/AAC.18.3.390

[47] Tipper DJ, Strominger J. Mechanism of action of penicillins: A proposal based on their structural similarity to acyl-D-alanyl-D-alanine. Proceedings of the National Academy of Sciences of the United States of America. 1965;**54**: 1133-1141. DOI: 10.1073/pnas.54.4.1133

[48] Coffey TJ, Dowson CG, Daniels M, Zhou J, Martin C, Spratt BG, et al. Horizontal gene transfer of multiple penicillin-binding protein genes, and capsular biosynthetic genes in natural populations of *Streptococcus pneumoniae*. Molecular Microbiology. 1991;**5**: 2255-2260

[49] Kim L, McGee L, Tomczyk S, Beall B. Biological and epidemiological features of antibiotic-resistant *Streptococcus pneumoniae* in pre- and post-conjugate vaccine eras: A United States perspective. Clinical Microbiology Reviews. 2016;**29**:525-552

[50] Contreras-Martel C, Dahout-Gonzalez C, Martins ADS, Kotnik M, Dessen A. PBP active site flexibility as the key mechanism for β-lactam resistance in pneumococci. Journal of Molecular Biology. 2009;**387**:899-909

[51] Cornick JE, Bentley SD. Streptococcus pneumoniae: the evolution of antimicrobial resistance to beta-lactams, fluoroquinolones and macrolides. Microbes and Infection. 2012;**14**(7):573-583

[52] Gordon E, Mouz N, Duée E, Dideberg O. The crystal structure of the penicillin-binding protein 2x from *Streptococcus pneumoniae* and its acyl-enzyme form: Implication in drug resistance. Journal of Molecular Biology. 2000;**299**:477-485

[53] Hakenbeck R. β-Lactam resistance in *Streptococcus pneumoniae*: Penicillin-binding proteins and non-penicillin-binding proteins. Molecular Microbiology. 1999;**33**:673-678

[54] Chesnel L, Carapito R, Croizé J, Dideberg O, Vernet T, Zapun A. Identical penicillin-binding domains in penicillin-binding proteins of *Streptococcus pneumoniae* clinical isolates with different levels of β-lactam resistance. Antimicrobial Agents and Chemotherapy. 2005;**49**:2895-2902

[55] Filipe SR, Tomasz A. Inhibition of the expression of penicillin resistance in *Streptococcus pneumoniae* by inactivation of cell wall muropeptide branching genes. Proceedings of the National Academy of Sciences of the United States of America. 2000;**97**:4891-4896

[56] Crisóstomo MI, Vollmer W, Kharat AS, Inhülsen S, Gehre F, Buck-Enmaier S, et al. Attenuation of penicillin resistance in a peptidoglycan O-acetyl transferase mutant of *Streptococcus pneumoniae*. Molecular Microbiology. 2006;**61**:1497-1509