Molecular mechanisms and epidemiology of resistance in Streptococcus pneumoniae in the Middle East region

Grace El Moujaber,† Marwan Osman,† Rayane Rafei,† Fouad Dabboussi and Monzer Hamze*

Abstract

Purpose. Streptococcus pneumoniae is a commensal bacterium that normally colonizes the human nasopharyngeal cavity. Once disseminated, it can cause several diseases, ranging from non-invasive infections such as acute otitis media and sinusitis through to invasive infections with higher mortality, including meningitis and septicaemia. Since the identification of the first S. pneumoniae strain with decreased susceptibility to penicillin in the 1960s, antibiotic resistance among S. pneumoniae has increased disturbingly and the mechanisms of resistance have begun to unfold.

Methodology. This work briefly reviewed the available data on the molecular mechanisms underlying antimicrobial resistance and its epidemiology among pneumococcal strains in Middle Eastern countries.

Key findings. Both intrinsic and acquired mechanisms (mutations, acquisition of novel mobile genetic elements and sometimes gene duplication and overexpression) affect susceptibility to a large variety of antibiotics. In Middle Eastern countries, including Lebanon, Iran, Saudi Arabia and Turkey, surveillance showed a disturbing increase in the strength and prevalence of resistance to antibiotics over the years, especially in the last decade. However, no surveillance reports were found in other Middle Eastern countries, such as Syria and Iraq.

Conclusion. In order to better survey, control and prevent the emergence of multidrug- and extremely drug-resistant S. pneumoniae strains, antimicrobial stewardship, national surveillance and public awareness programmes should be developed urgently in Middle Eastern countries.

INTRODUCTION

Streptococcus pneumoniae remains a significant public health problem [1]. In 2000, up to 14.5 million severe episodes of pneumococcal disease were reported, with around 826 000 deaths in children younger than 5 years old [2]. Pneumococcus exclusively inhabits human beings and it is a commensal nasopharyngeal and oropharyngeal bacterium [3, 4]. It is transmitted by droplets and aerosols either from infected patients with pneumococcal disease or from healthy carriers of the bacteria [1, 5].

Conventional identification uses optochin susceptibility, bile solubility and latex agglutination tests [6]. The lytA gene is considered to be a specific molecular target for pneumococcus identification, where its restriction by BsaI provides in addition specific signatures for reliable diagnosis of S. pneumoniae infections, especially those caused by non-typeable strains [7]. Moreover, real-time PCR targeting lytA was recommended as a rapid and reliable non-culture assay to complement the gold standard culture technique for S. pneumoniae detection [8]. Nevertheless, a new study showed that misidentification of S. pneumoniae can occur [9].

Many predisposing factors can increase the probability of pneumococcal colonization: young age (especially <5 years old), old age (>65 years old) and immunocompromised populations [2, 10]. Pneumococcal colonization is also affected by socio-economic factors, crowding, alcoholism, smoking, anaesthesia, viral infections affecting the respiratory tract, chronic diseases such as chronic obstructive pulmonary disease, corticosteroid therapy and lack of pneumococcal vaccination [10, 11]. Even though pneumococcal colonization is usually asymptomatic, it can progress and disseminate into usually sterile body sites, causing immune system dysregulation. This can cause
different pneumococcal diseases, ranging from less serious infections such as sinusitis, conjunctivitis and acute otitis media through to more serious and mortal infections, such as invasive pneumococcal disease (IPD) and community-acquired pneumonia [10].

MECHANISMS OF RESISTANCE IN STREPTOCOCCUS PNEUMONIAE

The first antibiotic-resistant S. pneumoniae strain that drew attention was isolated in the late 1960s in Australia [12]. Soon after, resistance rates to different antimicrobial agents among S. pneumoniae strains increased disturbingly and they have become a worldwide problem [13]. The carriage prevalence of resistant strains has also extended and this has amplified the issue [14, 15]. Furthermore, S. pneumoniae has intrinsic resistance to a large group of antibiotics, including polypeptides, aminoglycosides and first-generation quinolones [16]. The molecular mechanisms underlying antimicrobial resistance in S. pneumoniae are reviewed here and are summarized in Table 1.

Beta-lactam resistance

Beta-lactams are antibiotics that are responsible for the inhibition of cell-wall synthesis through binding to specific enzymes called penicillin-binding proteins (PBPs) [17]. There are six PBPs in S. pneumoniae, of which only three are associated with resistance: PBP1a, PBP2x and PBP2b (Table 2).

Altered PBP genes acquired from related species (such as Streptococcus mitis and Streptococcus oralis) through recombinational events are the main beta-lactam resistance mechanism in S. pneumoniae [18]. A low level of penicillin resistance can be based mainly on alterations in PBP2x and -2b, which produce penicillin-nonsusceptible S. pneumoniae (PNSP). However, a high level of resistance is achieved through a combination of three altered PBPs: PBP1a, -2b and/or -2x.

Recently, PBP2x seems to have been the primary target for piperacillin and not PBP2b [19]. Another recent genome-wide association study identified a set of 301 single nucleotide polymorphisms, from which 73 caused amino acid alterations in cell-wall synthesis genes [20].

Additionally, MurM and MurN proteins, encoded by a murMN operon and responsible for an abnormality in cell-wall synthesis through replacing linearized muropeptides with atypical branched ones, have also been associated with penicillin resistance [21, 22]. Although MurM alone is not enough to cause penicillin resistance, it is important in the acquisition of the highest level of resistance to both penicillin and cephalosporin [23].

Aminoglycoside resistance

Once inside the bacteria, aminoglycoside inhibits protein synthesis through binding to the 30S ribosomal subunit [24]. To complete its antimicrobial activity, aminoglycoside demands an active aerobic metabolism to penetrate inside the cell. Since S. pneumoniae is a facultative anaerobic bacterium, the electron transport chain is incomplete, conferring a natural low-level resistance to this antimicrobial agent [24, 25].

Nevertheless, high-level resistance to aminoglycoside can also occur through the acquisition of the mobile genetic elements Tn1545 or Tn6003, which were identified in the chromosome of a pneumococcal strain in 1986 [26] and 2007 [27], respectively. Tn1545 was shown to carry resistance determinants for three different antimicrobial families: 3’-aminoglycoside phosphotransferase type III (aphA-3’), which was reported for the first time in 1984 [28], erythromycin ribosome methylase B (ermB) and the tetracycline resistance protein (tetM), which confer a high level of resistance to aminoglycosides, macrolides and tetracycline, respectively [26]. However, Tn6003 is a novel genetic element formed by the insertion of the macrolide-aminoglycoside-streptothricin element within Tn6002 [27, 29].

Resistance to macrolide and related antibiotics

Ribosomal modification was the first resistance mechanism described in S. pneumoniae caused by the erm gene carried by Tn1545 [26] alongside another Tn917 [30]. ermB encodes for a methylase responsible for the dimethylation of a specific adenine in 23S rRNA, A2058 in domain V, leading to a decreased affinity to macrolides [31]. In 2001, ermA was described in a macrolide-resistant strain isolated in Greece [32]. Both ermA and ermB confer resistance to the macrolide-lincosamide-streptogramin B families (MLSb) [30, 32]. This form of co-resistance can be expressed in two different ways, either constitutive (cMLSb) or inducible (iMLSb). These resistance phenotypes do not depend on a resistance-determinant gene type but on the regulatory region [33].

In the late 1990s, a different pattern was detected: resistance to 14- and 15-membered macrolides but susceptibility to clindamycin and streptogramin B. This phenotype, called the M phenotype, was detected in both S. pneumoniae and Streptococcus pyogenes and did not seem to be caused by target modification, since erm genes were not detected [34]. Soon after, mefE was identified (with almost 90 % similarity to the mefA found in S. pyogenes) as a necessary element for the efflux system involved in erythromycin resistance [35]. mefE was originally found to be associated with the msr gene encoding an ATP-dependent efflux pump, and located on a macroline efflux genetic assembly (mega) [36]. In 2004, mefE was detected, along with the tetM gene, on a transposon related to the Tn916-family, Tn2009, in two pneumococcal strains [37]. Another transposon, Tn2010, was also shown to carry mefE, but together with ermB, making it a dual-macrolide resistance gene carrier transposon [38]. mefA was also characterized in S. pneumoniae and was found to be carried by Tn1207.1, a portion of the original transposon described in S. pyogenes Tn1207.3 [39]. In 2005, a new mef subclass, mefI, was identified in S. pneumoniae [40]. Moreover, mefI was found to be located on the right-hand side of a newly characterized non-mobile composite structure, the 5216fQ complex, adjacent to
<table>
<thead>
<tr>
<th>Antibiotic resistance</th>
<th>Mechanism of resistance</th>
<th>Associated enzyme or gene</th>
<th>Phenotype</th>
<th>Origin</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aminoglycosides</td>
<td>Low cell-wall permeability</td>
<td>–</td>
<td>Low level of aminoglycoside resistance</td>
<td>Intrinsic</td>
<td>[25]</td>
</tr>
<tr>
<td>Aminoglycoside modification</td>
<td>(APH)(3')5'-III</td>
<td>High level of resistance to kanamycin and closely related aminoglycosides</td>
<td>Tn1545 and Tn6003</td>
<td>[26, 28, 76]</td>
<td></td>
</tr>
<tr>
<td>Beta-lactams and cephalosporins</td>
<td>Mosaic genes</td>
<td>PBP2x, PBP2b and PBP1a</td>
<td>Resistance to cefotaxime, piperacillin or contributors to high level of resistance to penicillin</td>
<td>Transformation from related species</td>
<td>[122]</td>
</tr>
<tr>
<td></td>
<td>Point mutations</td>
<td>murM</td>
<td>Contributor to beta-lactam and cephalosporin resistance</td>
<td>Sporadic</td>
<td>[23, 125]</td>
</tr>
<tr>
<td></td>
<td>Point mutations</td>
<td>mraA</td>
<td>Resistance to piperacillin</td>
<td>Sporadic</td>
<td>[126]</td>
</tr>
<tr>
<td>Macrolides</td>
<td>Ribosomal modification</td>
<td>ermB</td>
<td>High level of resistance to macrolides, lincosamides and streptogramins (MLS{B} phenotype)</td>
<td>Tn1545, Tn917, Tn6003, Tn6002, Tn2017 and Tn2010</td>
<td>[26, 29, 30, 76]</td>
</tr>
<tr>
<td></td>
<td>Efflux system</td>
<td>mefE</td>
<td>Low level of resistance to macrolides (resistance to 14- and 15-membered macrolides) (M phenotype)</td>
<td>mega, Tn2009 and Tn2010</td>
<td>[36–38]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>mefA</td>
<td>Low level of resistance to 14- and 15-membered macrolides and lincosamide (M-L phenotype)</td>
<td>Tn5253</td>
<td>[42]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>23S rRNA</td>
<td>Resistance to 14-, 15- and 16-membered macrolides and streptogramins B (M16-S{B} phenotype)</td>
<td>Tn207.1</td>
<td>[39]</td>
</tr>
<tr>
<td></td>
<td>Amino acid substitution or insertion</td>
<td>Ribosomal proteins L4 and L22</td>
<td>Resistance to macrolides and streptogramin B (M-S{B} phenotype)</td>
<td>Sporadic</td>
<td>[44]</td>
</tr>
<tr>
<td>Fluoroquinolones</td>
<td>Target alteration</td>
<td>parC</td>
<td>Low level of resistance to fluoroquinolones</td>
<td>Sporadic</td>
<td>[46]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>parC and gyrA</td>
<td>High level of resistance to fluoroquinolones</td>
<td>Sporadic</td>
<td>[45]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>parF or gyrB</td>
<td>Contributors to fluoroquinolones resistance</td>
<td>[49–51]</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Efflux mechanism</td>
<td>pmrA</td>
<td>Resistance to norfloxacin, ciprofloxacin, acriflavine and ethidium bromide</td>
<td>Overexpression</td>
<td>[54]</td>
</tr>
<tr>
<td></td>
<td>ABC efflux mechanism</td>
<td>patA/patB</td>
<td>High level of resistance to fluoroquinolones</td>
<td>Overexpression</td>
<td>[56]</td>
</tr>
<tr>
<td>Glycopeptides</td>
<td>Point mutations</td>
<td>meS</td>
<td>Vancomycin tolerance</td>
<td>Sporadic</td>
<td>[65]</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>Chloramphenicol modification</td>
<td>cat</td>
<td>High level of resistance to chloramphenicol</td>
<td>Tn5253</td>
<td>[42, 87]</td>
</tr>
<tr>
<td>Rifampicin</td>
<td>Point mutations</td>
<td>rpoB</td>
<td>Resistance to rifampicin</td>
<td>Sporadic</td>
<td>[82, 83]</td>
</tr>
<tr>
<td>Cotrimoxazole</td>
<td>Point mutations</td>
<td>DFR or DHPS</td>
<td>High level of resistance to cotrimoxazole</td>
<td>Sporadic</td>
<td>[69, 70]</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>Ribosomal protection</td>
<td>tetM</td>
<td>High level of resistance to tetracycline</td>
<td>Tn1545, Tn2009, Tn3872, Tn2010, Tn2017 and Tn6003</td>
<td>[76]</td>
</tr>
<tr>
<td></td>
<td>Mutations</td>
<td>npsF and patA</td>
<td>Resistance to tetracycline</td>
<td>Sporadic</td>
<td>[81]</td>
</tr>
<tr>
<td></td>
<td>Overexpression of thiamine biosynthesis pathway</td>
<td>spr0634 and spr0638</td>
<td>Resistance to tetracycline</td>
<td>Overexpression</td>
<td></td>
</tr>
</tbody>
</table>
the msr and catQ genes [41]. Recently, it was proved that this IQ element is actually inserted within a larger genetic element, conjugative transposon Tn5253, an integrative conjugative element [42].

Mutations in ribosomal sites engaged in macrolide activity can participate in the emergence of other macrolide resistance phenotypes, such as M16S$_B$ caused by a mutation, A2062C, conferring resistance to 16-membered macrolide and streptogramins [43]. The ML phenotype can be caused by another mutation, A2059G, conferring resistance to 14-, 15-, 16-membered macrolides and lincosamides [44]. Likewise, mutations occurring in ribosomal protein L4 or L22 confer macrolide resistance [44].

Fluoroquinolone (FQ) resistance

Over the years, a large number of mutations in the quinolone-resistance-determining regions (QRDRs) of gyrA, gyrB, parC and parE have been identified as the main cause behind the development of quinolone resistance in S. pneumoniae. Not all amino acid substitutions cause the same level of resistance; mutations in parC alone, such as Ser79-Phe, Ser79Tyr, Ser79Ala, Asp83Gly and Asp83Tyr, confer a low level of resistance to FQ. When combined with mutations in gyrA such as Glu85Lys, Ser84Tyr and Ser84Phe, some mutations confer high-level resistance [45, 46]. In addition, it was confirmed that parC is the primary target for FQ, and mutations in this subunit were shown to be the crucial first step for the acquisition of a high level of resistance [45, 47]. gyrA or parC mutations can also predispose to further substitutions associated with an increase in resistance level [48]. Amino acid substitutions in gyrB (Glu474Lys) [49] and parE (Asp435Asn, Ile-460-Val) [50, 51] were also identified as contributors to resistance, but seemed to have a minimal effect [52].

Although target alterations were thought to be the only mechanism causing FQ resistance, a pneumococcal multidrug resistance gene, pmrA, coding for an efflux pump, was identified and showed more than 20% similarity to the Bacillus subtilis multidrug resistance (bmr) and Staphylococcus aureus norfloxacin efflux pump (norA) genes [53, 54]. More recently, the overexpression of patA and patB genes was associated with FQ resistance in clinical isolates of S. pneumoniae and their inactivation restored susceptibility to FQ [55]. The patA (spr1887) and patB (spr1885) genes belong to the same operon and work together as heterodimers to make a functional ABC transporter [56]. Even though upregulation of patAB has been observed in many laboratory mutants and clinical isolates, the regulatory mechanisms controlling expression of these genes are still unknown and may be due to mutations in rho-independent terminator or patAB gene duplication [57, 58].

Although interspecies gene transfer from commensal streptococci was confirmed in vitro, it was less common in vivo [59–61].

Sulfonamide resistance

Cotrimoxazole-resistant S. pneumoniae were isolated in 1972 [66]. Several studies demonstrated that a single amino acid substitution, Ile100Leu, in dihydrofolate reductase (DHFR) was responsible for a major increase in trimethoprim and cotrimoxazole resistance [67, 68]. Additional mutations were also identified within DHFR and although their roles were unclear, they seem to enhance and increase the resistance level [68]. Recently, an unusual new resistance genotype was detected in more than 30% of cotrimoxazole-resistant strains, characterized by an Asp92Ala substitution alongside the wild-type substitution in position 100 in DHFR [69]. Furthermore, different mutations were also identified in dihydropteroate synthase (DHPS) [70].

Tetracycline resistance

Tetracycline resistance is usually associated with the acquisition of tet genes such as tetA, tetB, tetC and tet31, coding for efflux proteins, and tetT, tetW, tetM and tetO, encoding for proteins involved in ribosomal protection [71].

In early analysis, the tet gene found in S. pneumoniae strains was shown to be part of a foreign DNA insertion that included another resistance gene coding for chloramphenicol resistance, cat, and transferred by a plasmid-free mechanism [72]. This genetic element was first named omega cat-tet or Ω (cat-tet) [73], and was then renamed Tn5253 [74]. Tn5253 is composed of two distinct conjugative transposons, each displaying its own conjugal proprieties: (1) Tn5251 (related to the Tn916

<table>
<thead>
<tr>
<th>Mutations</th>
<th>Resistance</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>pep2x</td>
<td>Thr550Ala</td>
<td>Cefotaxime</td>
</tr>
<tr>
<td></td>
<td>T338A</td>
<td>Penicillin</td>
</tr>
<tr>
<td>pep2b</td>
<td>Thr464Ala</td>
<td>Piperacillin</td>
</tr>
<tr>
<td></td>
<td>T451A</td>
<td>Penicillin</td>
</tr>
<tr>
<td>pep1a</td>
<td>Mosaic gene</td>
<td>Penicillin and cephalosporin</td>
</tr>
<tr>
<td>cpoA</td>
<td>Gly12Val</td>
<td>Piperacillin</td>
</tr>
<tr>
<td>ciaH</td>
<td>Thr230Pro</td>
<td>Cefotaxime</td>
</tr>
<tr>
<td></td>
<td>Ala203Val</td>
<td></td>
</tr>
<tr>
<td>murM</td>
<td>Mosaic gene</td>
<td>Penicillin and cephalosporin</td>
</tr>
</tbody>
</table>

Table 2. Mutations associated with betalactam resistance

Glycopeptide tolerance

A combination of vancomycin and gentamicin can be used as an effective alternative to treat penicillin- and cephalosporin-resistant S. pneumoniae strains by increasing the intracellular concentration of gentamicin [62]. Although vancomycin resistance has not yet been detected among S. pneumoniae, the emergence of vancomycin-tolerant strains was reported in association with the loss of function mutation of vncS, a histidine kinase gene [63, 64]. This mutation was thought to be the main mechanism behind vancomycin tolerance leading to autolytic activity suppression. A study conducted in the late 1990s reported that the vncS mutant not only exhibited vancomycin tolerance, but also was capable of developing tolerance to many other antibiotics, including quinolones and beta-lactams [65].

The msr and catQ genes [41]. Recently, it was proved that this IQ element is actually inserted within a larger genetic element, conjugative transposon Tn5253, an integrative conjugative element [42].
family), the 18 kb tet-carrier transposon inserted within (2) Tn5252, the cat-carrier that comprises the rest of Tn5253 [74]. Moreover, Tn5251 has shown the potential for independent transfer to a variety of bacterial species after excision from Tn5253 and a capacity to combine with other resistance-determinant carriers [75]. Other transposons were also identified as carriers of the tetM gene, including Tn1545 [26], Tn2009 [37], Tn3872, Tn2010 and Tn2017 [76].

However, numerous studies reported tetracycline susceptibility among tetM-harbouring strains [77]. In 2012, framing errors or frameshift mutations in seven tetracycline-susceptible S. pneumoniae strains, caused by either deletion or insertion, were identified as the reasons behind a low transcription level of tetM and therefore the susceptible profile [78]. Resistant strains presented truncated tetM promoter that resulted in an increase in the gene transcription, thus causing a higher level of resistance.

Although tetM is the dominant tetracycline resistance gene found in S. pneumoniae, tetO was first reported in South Africa in 1996 [79] and then in Washington State, USA in 1998 [80]. While the former suggested a clonal spread, the latter suggested a non-clonal dissemination, since tetO was reported in five different unrelated serotypes. In contrast to the tetM gene, tetO does not seem to be carried by a mobile genetic element and so cannot be transferred horizontally [80].

In addition, recent research proved that another tetracycline resistance mechanism is present in S. pneumoniae, which is not associated with the acquisition of tet genes [81]. Since both tetM and tetO were not detected, genomic analysis of tetracycline-resistant strains indicated the presence of mutations within the rpsJ gene, coding for the ribosomal protein S10 located near the tetracycline site of action, along with overexpression of the ABC transporter due to mutations in the patA gene. Both mutations were confirmed to be contributors to tetracycline resistance. Moreover, transcriptomic analysis also linked the overexpression of genes involved in the thiamine synthesis pathway to tetracycline resistance, including the spr0634 and spr0638 genes, coding for the thiaminase and phosphomethyl pyrimidine kinase, respectively.

Rifampicin resistance

Rifampicin-resistant S. pneumoniae strains have been isolated in South Africa and analysis demonstrated that these strains contained mutations in cluster I of rpoB (Asp415Glu and His425Asn) that were equivalent to mutations previously identified in Escherichia coli, alongside mutations in cluster III [82]. Mutations in clusters N and II were also detected in rifampicin-resistant strains disseminated in HIV patients and children with conjunctivitis from four different Spanish hospitals [83]. In addition, the detection of a high level of variation within rpoB suggested a horizontal transfer origin, with the viridans group as potential donors [83].

Chloramphenicol resistance

Although chloramphenicol resistance mechanisms have been described, the acquisition of chloramphenicol acetyltransferase (Cat) encoded by the cat gene remains the major mechanism among bacteria, including S. pneumoniae. Cat causes enzymatic modification, which is associated with chloramphenicol acetylation and the generation of inoperative O-acetoxy chloramphenicol products, further hampering its successful binding to ribosomes [84, 85]. In S. pneumoniae, the cat gene is carried by Tn5253, a composite transposon identified in 1991 [74]. In 2000, a linearized version of pC194, a cat carrier plasmid originally described in Staphylococcus aureus, was found integrated into the S. pneumoniae chromosome [86]. Recent nucleotide analysis of Tn5253 demonstrated the presence of Δcat, a newly described genetic element containing a copy of the linearized plasmid [87]. A second cat gene, catQ, was reported for the first time in pneumococcal strains [41] and was later found to also be located on Tn5253. However, the strain harbouring Tn5253 with a catQ gene lacked the presence of pC194, which is usually used as a cargo gene for Tn5253 [42]. catQ is an A-type cat gene with more than 90% DNA similarity to the first catQ originally described in Clostridium perfringens [88].

Epidemiology of resistance in the Middle East region

Pneumococcal infections are usually treated with antibiotics. Consumption of antibiotics has increased significantly – by more than 30% over a period of 10 years [89]. This high consumption is primarily due to overprescribing or sales without a medical prescription, especially in urban areas where antibiotics are more readily available [89]. In Middle Eastern countries, antibiotic misuse and overuse has also been documented. Perioperative and postoperative antibiotics, over-the-counter antibiotic sales and even the use of antibiotics in the food industry has led to the rapid emergence and dissemination of antibiotic-resistant strains in these areas [90].

In recent years, various epidemiological studies aiming to establish the prevalence of resistant S. pneumoniae strains in several Middle Eastern countries have been conducted and they have served as surveillance of antibiotic resistance evolution in this region. This part of the paper reviews the epidemiology of pneumococcal resistance in Middle Eastern countries (Table 3).

We systematically searched the PubMed/Medline and Web of Science databases for investigations that reported on the epidemiology of S. pneumoniae with regard to Middle Eastern countries. However, to the best of our knowledge, there has been no surveillance of antibiotic resistance among S. pneumoniae from the last 15 years in Syria, Qatar and Iraq.

In Lebanon, a national prospective study reported that 30% of clinical strains were isolated from children aged less than 5 years old. The resistance rates to penicillin, erythromycin...
<table>
<thead>
<tr>
<th>Years of survey</th>
<th>Targeted population (age)</th>
<th>Number of isolates</th>
<th>Setting</th>
<th>Resistance (%) to PEN*</th>
<th>ERY*</th>
<th>CMN*</th>
<th>FQs*</th>
<th>SXT*</th>
<th>VAN*</th>
<th>TET*</th>
</tr>
</thead>
<tbody>
<tr>
<td>2005-2011</td>
<td>Clinical invasive isolates (all age groups)</td>
<td>257</td>
<td>Nationwide studies</td>
<td>17.4</td>
<td>29.3</td>
<td>0.5</td>
<td>66.1</td>
<td>0</td>
<td>[91]</td>
<td></td>
</tr>
<tr>
<td>2008-2010</td>
<td>Clinical invasive isolates (all age groups)</td>
<td>65</td>
<td>Local study (Alexandria)</td>
<td>70</td>
<td>25</td>
<td>4</td>
<td>55</td>
<td>0</td>
<td>[92]</td>
<td></td>
</tr>
<tr>
<td>2011</td>
<td>Clinical invasive isolates (children aged &lt;5 years)</td>
<td>203</td>
<td>Local study (Kuwait City)</td>
<td>61.3</td>
<td>38.7</td>
<td>20.6</td>
<td>48.5</td>
<td>0</td>
<td>31</td>
<td>[93]</td>
</tr>
<tr>
<td>2013-2014</td>
<td>Healthy children (2-60 months)</td>
<td>175</td>
<td>Local studies (Istanbul)</td>
<td>39.9</td>
<td>19</td>
<td>9</td>
<td>0</td>
<td>100</td>
<td>0</td>
<td>[94]</td>
</tr>
<tr>
<td>2016</td>
<td>Healthy children (from 2 to 6 years)</td>
<td>66</td>
<td>Local study (Kashan)</td>
<td>66.7</td>
<td>42.6</td>
<td>0</td>
<td>6.2</td>
<td>2.1</td>
<td>26.8</td>
<td>[95]</td>
</tr>
<tr>
<td>2017</td>
<td>Healthy children (from 7 to 19 years)</td>
<td>291</td>
<td>Local study (Kashan)</td>
<td>32</td>
<td>39</td>
<td>15.4</td>
<td>0</td>
<td>36.1</td>
<td>0</td>
<td>[96]</td>
</tr>
<tr>
<td>2018</td>
<td>Healthy children (from 2 to 6 years)</td>
<td>195</td>
<td>Local study (West Bank)</td>
<td>42</td>
<td>9.5</td>
<td>77.5</td>
<td>0</td>
<td>30.9</td>
<td>0</td>
<td>[97]</td>
</tr>
<tr>
<td>2019</td>
<td>Healthy children (from 2 to 6 years)</td>
<td>195</td>
<td>Local study (West Bank)</td>
<td>42</td>
<td>19.5</td>
<td>62.6</td>
<td>0</td>
<td>33.3</td>
<td>0</td>
<td>[98]</td>
</tr>
<tr>
<td>2020</td>
<td>Healthy children (from 2 to 6 years)</td>
<td>195</td>
<td>Local study (West Bank)</td>
<td>42</td>
<td>9.5</td>
<td>77.5</td>
<td>0</td>
<td>30.9</td>
<td>0</td>
<td>[99]</td>
</tr>
<tr>
<td>2021</td>
<td>Healthy children (from 2 to 6 years)</td>
<td>195</td>
<td>Local study (West Bank)</td>
<td>42</td>
<td>19.5</td>
<td>62.6</td>
<td>0</td>
<td>33.3</td>
<td>0</td>
<td>[100]</td>
</tr>
<tr>
<td>2022</td>
<td>Healthy children (from 2 to 6 years)</td>
<td>195</td>
<td>Local study (West Bank)</td>
<td>42</td>
<td>9.5</td>
<td>77.5</td>
<td>0</td>
<td>30.9</td>
<td>0</td>
<td>[101]</td>
</tr>
</tbody>
</table>

*Penicillin with MIC >0.06 μg L⁻¹ (PEN), erythromycin (ERY), clindamycin (CMN), fluoroquinolones (FQs), trimethoprim-sulfamethoxazole (SXT), vancomycin (VAN), tetracycline (TET). Community-acquired respiratory tract infections (CARTI), not determined (ND), UAE, United Arab Emirates

Table 3. Epidemiology of pneumococcal resistance in Middle Eastern countries

El Moujaber et al., Journal of Medical Microbiology 2017;66:847–858
and cotrimoxazole were 17.4, 29.3 and 54 % respectively; resistance to levofloxacin did not surpass 1 % and no resistance to vancomycin was documented [91]. However, a higher level of resistance to macrolides, penicillin, tetracycline and cotrimoxazole was reported in another survey [92]. Among the macrolide-resistant strains, \textit{ermB} was the most detected macrolide resistance determinant (36 %), followed by dual carriers of \textit{erm} and \textit{mef} genes (32 %), and then by \textit{mef} carriers (18 %) [92]. The remaining strains (14 %) did not carry any resistance determinant, suggesting the presence of a mutation in either L4 protein or 23S rRNA [92]. Moreover, a retrospective study reported an increase in erythromycin and clindamycin resistance between 2011 and 2013. In addition, a slight increase in penicillin resistance was found. Cotrimoxazole and FQ resistance were shown to be stable in this period [93]. The latest report showed moderate prevalence of resistance to penicillin and erythromycin, but no strain was resistant to FQ [94]. Although the prevalence of FQ-resistant \textit{S. pneumoniae} strains in Lebanon was not high [91, 93], first-step mutations were detected in QDRs of \textit{parC} (two isolates) and \textit{parE} (one isolate) among 45 levofloxacin-susceptible clinical strains isolated from north Lebanon [95]. These mutations are usually followed by other mutations that can eventually lead to a high level of FQ resistance.

In Egypt, a recent study targeted a different population formed of 600 healthy subjects [96]. A prevalence of 29 % pneumococcal carriage with high rates of antibiotic resistance was reported; 70, 40, 25, 49, 55 and 0 % of strains were resistant to penicillin, erythromycin, clindamycin, tetracycline, cotrimoxazole and vancomycin, respectively [96]. When compared to the rates from two previous studies conducted in the mid-2000s, these rates show substantial increases in antibiotic resistance, especially with regard to penicillin and erythromycin [97, 98].

In Saudi Arabia, between 2005 and 2009, 66 and 62 % of clinical isolates from sterile sites in children were resistant to penicillin and erythromycin, respectively [99]. In another study conducted between 2007 and 2009, 47 cases of \textit{S. pneumoniae} infection were identified as having a high level of resistance to penicillin and cotrimoxazole and a moderate resistance rate to chloramphenicol. A high multidrug resistance rate was also documented, while vancomycin tolerance was not observed [100]. It is important to note that cotrimoxazole resistance in Saudi Arabia is the highest within the region, reaching up to 100 % in patients of all ages with invasive and non-invasive diseases [101, 102]. This represents a disturbing increase from the resistance rates observed in the early 2000s from paediatric patients [103]. However, from 2009 and 2012, in a group of children with suspected pneumonia and meningitis, a high level of penicillin and erythromycin resistance was reported, with no FQ or vancomycin resistance documented [101]. In a recent retrospective study [104], \textit{S. pneumoniae} was the fourth most common keratitis-causing bacteria. In contrast to previous studies, where pneumococcal strains were isolated from sterile body sites such as cerebrospinal fluid, blood and synovial fluid [99–102], this study only included corneal isolates, and of these the pneumococcal strains expressed lower resistance rates to penicillin and erythromycin, although no resistance to FQ or vancomycin was observed.

In Turkey, surveillance reports from the mid-2000s showed low resistance rates to both penicillin and erythromycin [97, 98]. A following study indicated a decrease in penicillin and erythromycin susceptibility in clinical isolates between 2003 and 2006 [105]. The cMLS\textsubscript{B} phenotype was predominant (82 %), followed by the M phenotype (18 %), and resistance to cotrimoxazole, tetracycline and chloramphenicol were also detected. Furthermore, from 2008 to 2010, an additional decrease in antimicrobial susceptibility among children with respiratory diseases was remarked and no resistance to FQ or vancomycin was detected [106]. In the most recent survey of isolates recovered from five Turkish medical centres between 2011 and 2013, 61.9 % were resistant to penicillin, 48.9 % to erythromycin and 35.1 % to clindamycin, while 1.8 % were resistant to levofloxacin [107].

In Palestine, the first national study conducted in 2013 in the West Bank showed that 55.7 % of healthy children aged less than 2 years old attending day care centres were positive carriers of \textit{S. pneumoniae}, from which 66.8, 30.3 and 45.9 % of isolates were resistant to penicillin, erythromycin and cotrimoxazole, respectively [108].

In Iran, several recent studies targeted various populations, including healthy populations and patients of different ages and showed lower antimicrobial resistance rates in comparison with other Middle East countries. Since 2007, among healthy Iranian children, penicillin, erythromycin, cotrimoxazole and tetracycline resistance fluctuated between different surveys [109–111]. However, Iran was the only Middle Eastern country that reported tolerance or resistance to vancomycin among healthy and sick populations [110, 112]. Within clinical isolates from sterile body sites, a variable percentage of resistance to penicillin, erythromycin, FQ and cotrimoxazole was also reported [112–115].

In the United Arab Emirates (UAE), a study that was conducted between 2004 and 2006 reported antibiotic resistance to penicillin (43 %), erythromycin (31 %), clindamycin (23 %), tetracycline (18.6 %), cotrimoxazole (97 %) and chloramphenicol (3 %) among \textit{S. pneumoniae} strains isolated from patients. Further, 20 % of strains showed triple resistance to tetracycline, cotrimoxazole and erythromycin. In 2016, surveillance reports showed an increase in antibiotic resistance rates, especially to penicillin, erythromycin and FQ [94].

In Oman, the most recent surveillance report indicated a high level of penicillin and erythromycin resistance, with slight FQ resistance [94]. Before this study, the last report on antibiotic pneumococcal resistance was prepared in 2007. This study included 15 positive pneumococcal isolates, of which 50 % were resistant to penicillin; however, no
resistance to vancomycin, linezolid, FQ or tigecycline was detected [116].

In Jordan, according to studies on S. pneumoniae strains isolated from blood and cerebrospinal fluid in the mid-2000s, resistance to penicillin and erythromycin was low [97, 98]. In another study conducted between 2005 and 2006 among healthy children, 19.5% were S. pneumoniae carriers, from which the majority expressed resistance to penicillin and tetracycline. High resistance to clindamycin and erythromycin and slight resistance to chloramphenicol and ciprofloxacin were also documented. Of macrolide-resistant strains, the majority (58%) had the M phenotype and the rest had the MLSB one; 54% were mefA carriers, 21% were ermB carriers, 17% were dual carriers and the rest (8%) did not carry any macrolide resistance determinant [117]. Recently, a high level of resistance to penicillin, erythromycin, cotrimoxazole and tetracycline was reported among healthy Jordanian children. Resistance to clindamycin was also reported, but at a lower rate, while no resistance to levofloxacin or vancomycin was detected [118].

In Bahrein, the sole surveillance study performed [94] on a collection of 100 clinical isolates showed that 40% were resistance to penicillin, while only 16% were resistance to erythromycin, and no resistance to either levofloxacin or vancomycin was detected [118].

In Kuwait, a rise in antibiotic resistance was documented in a study conducted over three distinct periods of time (1997–2001, 2002–2005 and 2006–2007), and this included resistance to penicillin, cotrimoxazole, tetracycline and clindamycin. No resistance to rifampicin or vancomycin was documented [119]. In another study carried out between 2001 and 2004, similar resistance rates were documented. In both studies, no resistance to vancomycin was reported [120].

CONCLUSION
Increasing rates of antibiotic resistance are a worldwide concern. According to the World Health Organization, in 2015 none of the eastern Mediterranean region countries had national plans or progress reports for the last 5 years concerning antimicrobial resistance. Furthermore, only 38% (8 of 21%) of these countries performed surveillance of bacterial resistance. Thus, additional surveillance reports and studies among bacteria, including S. pneumoniae, are needed, especially in view of the current public health problems strongly linked to the conflicts happening in the region, the high spread of counterfeit medicines and the misuse and overuse of antibiotics [121].

In the case of S. pneumoniae, antimicrobial resistance reports should be coupled with the number of pneumococcal cases and serotyping surveillance in each country. Insights into the current pneumococcal distribution can contribute to future development of strong prevention programmes on a global, regional and national level.

In addition, antimicrobial stewardship, national surveillance and public awareness programmes in combination can be of major importance in preventing or limiting the spread of multidrug-resistant strains.

Funding information
The authors received no specific grant from any funding agency.

Acknowledgements
We would like to thank Hussam Khaled for critical reading of the manuscript.

Conflicts of interest
The authors declare that there are no conflicts of interest.

References:


38. del Grosso M, Northwood JG, Farrell DJ, Pantosti A. The macrolide resistance genes erm(B) and mef(E) are carried by Tn2010 in dual–gene Streptococcus pneumoniae isolates belonging to clonal complex CC271. Antimicrob Agents Chemother 2007;51:4184–4186.


48. Gillespie SH, Voelker LL, Ambler JE, Traini C, Dickens A. Fluoroquinolone resistance in Streptococcus pneumoniae: evidence that gyrA mutations arise at a lower rate and that mutation in


84. Dang-van A, Tiraby G, Acrar JF, Shaw WV, Bouanchaud DH. Chloramphenicol resistance in Streptococcus pneumoniae:


