Review

Epidemiological aspects of antibiotic resistance in respiratory pathogens

G. Mlynarczyk [a], A. Mlynarczyk [a],*, J. Jeljaszewicz [b],1

[a] *Department of Medical Microbiology, Medical University of Warsaw, 5 Chalubinskiego, 02-004 Warsaw, Poland*
[b] *National Institute of Hygiene, Warsaw, Poland*

Abstract

Respiratory infections are the most frequent reason for primary health care consultation. The main causes of respiratory tract infections in children are viruses and the most common types are upper respiratory tract infections: common cold, pharyngitis, otitis media and sinusitis. Pneumonia is much more serious. As well as viruses, bacteria are often involved in respiratory tract infections. Three bacterial species are most commonly isolated: *Streptococcus pneumoniae*, non-encapsulated *Haemophilus influenzae* and *Moraxella* (*Branhamella*) *catarrhalis*. The most common bacterial cause of pharyngitis is *Streptococcus pyogenes*. Bacteria isolated from community-acquired infection usually are sensitive to the majority of suitable drugs, but during the past two decades, significant antibiotic resistance has emerged. Resistance to penicillins has spread among *H. influenzae* and *S. pneumoniae*. The mechanism of penicillin resistance in *H. influenzae* is mainly by production of β-lactamases TEM-1 and ROB-1, whereas in *S. pneumoniae* resistance is an effect of the changes in penicillin binding proteins. Among respiratory pathogens, resistance to tetracyclines, macrolides, trimethoprim–sulphamethoxazole and fluoroquinolones has also appeared. Several mechanisms depending on changes in target, active efflux and modifying enzymes are involved. © 2001 Published by Elsevier Science B.V. and International Society of Chemotherapy.

Keywords: Streptococcus pneumoniae; *Streptococcus pyogenes*; *Moraxella catarrhalis*; *Haemophilus influenzae*; Respiratory pathogens; Drug resistance

1. Introduction

Respiratory infections are the most frequent reason for primary health care consultation. One fourth to one third of patients visiting general practitioners suffer from acute respiratory tract infection (ARTI). The most frequently recognised are pharyngitis, common cold, influenza, tonsillitis, nasopharyngitis, sinusitis and bronchitis. Paediatric respiratory tract infections are especially associated with significant morbidity and mortality. ARTI in children under 5 years of age are the most frequent cause of death from lung disease globally, causing more than 4 million deaths annually. Before bacterial but not viral infection, the carrier state in the upper respiratory tract is frequently established. The level of immune function is critical in many infections [1].

Potentially pathogenic bacteria are often present in nasopharyngeal samples taken from healthy children and the prevalence decreases with age. There is debate as to whether the use of nasopharyngeal samples to discriminate between bacterial and viral respiratory tract infection needs to be evaluated further in patients below 16 years [2].

The main causes of respiratory tract infections are viruses. Several viruses have been associated with the common cold syndrome. Rhinoviruses, adenoviruses, influenza and parainfluenza viruses, coronaviruses, respiratory syncytial viruses, enteroviruses and her-

* Corresponding author. Tel./fax: + 48-22-628-2739.
E-mail address: gmlynar@ib.amwaw.edu.pl (A. Mlynarczyk).
1 Professor Jeljaszewicz died in Warsaw on 7 May 2001.

pesviruses are more or less frequently recognised in upper and lower respiratory tract infections [3]. The frequency of a particular virus is different in different infections.

2. Upper respiratory infection

Among upper respiratory infections, the common cold, pharyngitis and otitis media are most frequently encountered in children [4].

Both viruses and bacteria cause acute pharyngitis. The group A β-haemolytic streptococcus causes about 15% of all pharyngitis and is the most important pathogen [5] often causing suppurative and non-suppurative sequelae [6]. Suppurative sequelae, peritonsillar abscess, otitis media, and acute sinusitis, are probably rare today in many countries because antibiotics are usually used at an early stage of disease. Of the non-suppurative sequelae, rheumatic fever is an autoimmunological reaction and glomerulonephritis is an immunological complex disease. Both are produced by specific types M protein of *Streptococcus pyogenes* [7].

Otitis media is, next to the common cold, the most commonly diagnosed and probably the most prevalent illness during the first 2 years of life. Although it has a mainly bacterial aetiology, some authors question the necessity of antimicrobial therapy. There is evidence that respiratory viruses have a crucial role in the aetiology and pathogenesis of this infection [8]. Among bacterial species most commonly isolated are *Streptococcus pneumoniae*, non-encapsulated *Haemophilus influenzae* and *Moraxella* (*Branhamella*) *catarrhalis*. Mixed infections are not infrequent.

A common disorder both in children and adults is sinusitis [9]. The agents of sinusitis vary according to the chronicity of infection, the age and underlying condition of the patient. The most common causes of sinusitis in children are *S. pneumoniae*, *H. influenzae* (unencapsulated) and *M. catarrhalis*. In chronic sinusitis anaerobes are often involved and infections are often mixed.

3. Lower respiratory tract infections

Infections of the lower respiratory tract, bronchitis and pneumonia, are often more serious than infections of the upper respiratory tract. Pneumonia is more common in children than in adults. The spectrum of organisms causing pneumonia is also different in children. Very specific agents can cause pneumonia in newborns and very young children such as *Chlamydia trachomatis* or Group B haemolytic streptococci. In older children the aetiological agents are the same as adults, but viral pneumonias are much more frequent [10]. In children younger than 3 years of age *H. influenzae* type b is much more common as a cause of pneumonia and meningitis.

The most frequent viral aetiological agents of community acquired pneumonia in children are RSV, parainfluenza and adenovirus. The most common bacteria are *S. pneumoniae* and *H. influenzae* [10]. Other bacteria such as *S. aureus*, *S. pyogenes*, Gram-negative bacteria, *M. pneumoniae* and *C. pneumoniae* are less frequent.

Different pathogens are associated with hospital acquired pneumonia or in children with cystic fibrosis. One of most common locations in the hospital for development of nosocomial infections is the Intensive Care Unit. Respiratory tract infections are the most common infections and are associated with ventilator therapy rather than the underlying medical condition. The most common isolates are Enterobacteriaceae and *S. aureus*. The predominant organisms in cystic fibrosis are *Pseudomonas* spp. and *S. aureus* [11].

As previously mentioned, the predominating agents in community acquired lower respiratory tract infections are *S. pneumoniae*, *H. influenzae* and *M. catarrhalis*. *S. pyogenes*' role in lower respiratory tract infection has declined in last years. During the past two decades, significant antibiotic resistance has emerged among all these bacteria [12].

4. S. pneumoniae

The most significant changes have been observed among strains of *S. pneumoniae*. In the 1940s, all *S. pneumoniae* were exquisitely susceptible to penicillin. Concentrations of penicillin less than 0.1 mg/l were bactericidal. In the 1960s, reports of strains of pneumococci with intermediate levels of penicillin resistance (MICs 0.1–0.6 mg/l) began to appear. In the 1970s strains possessing penicillin MIC values greater than 2 mg/l occurred. Unfortunately pneumococcal strains resistant to penicillin are usually also resistant to many other antibiotics like macrolides, tetracyclines, co-trimoxazole and even chloramphenicol [13,14]. High susceptibility of *S. pneumoniae* to quinolones is still seen, but increases in the prescription of these antibiotics increases resistance, up to 2% of strains in some areas [15].

The mechanism of pneumococcal resistance to penicillin G and other β-lactam antibiotics involves alteration in one or more of the penicillin binding proteins (PBPs) causing their lowered affinity to β-lactam antibiotics. In *S. pneumoniae* five high molecular weight PBPs are recognised: 1a, 1b, 2x, 2a and 2b. Presumably alteration of each of them could lead to increase in resistance to β-lactams [16]. Moreover, it has been shown that changes in the low molecular weight PBP3

have also been associated with resistance to β-lactams. The level of resistance is determined by how many and to what extent targets are modified [17]. Usually alterations in PBP2b are associated with an MIC > 0.1 mg/l and in many papers the *pbp2b* gene is nominated as being responsible for penicillin non-susceptibility [18]. However, several reports suggested that changes in other high-molecular weight PBPs could also increase penicillin MIC values in *S. pneumoniae*. The genes determining synthesis of the altered PBPs in pneumococci appeared by mutations and also were acquired from different species of streptococci, especially those of oral origen. The genes of resistant PBPs in pneumococci show specific mosaic structure, because they consist of segments of native pneumococcal DNA mixed with segments of foreign DNA [19].

In most cases, the extended spectrum cephalosporins and carbapenems possess greater activity than penicillins against strains with low level resistance although some pneumococci are more resistant to extended spectrum cephalosporins than to penicillin G. The resistance to penicillin G is primarily determined by changes in PBP2b, whereas elevated resistance to cephalosporins is determined rather by changes in PBP2x [20]. The acquisition of penicillin resistance by pneumococci is not accompanied by any reduction in pathogenicity.

The targets for quinolones are bacterial topoisomerases, enzymes that are essential to the cell for the regulation of DNA supercoiling levels and catenation and decatenation of the bacterial chromosome. The main targets are gyrase and topoisomerase IV. In pneumococci a majority of fluoroquinolones act preferentially through subunit A of topoisomerase IV. The resistance of *S. pneumoniae* to fluoroquinolones appears by mutation in genes determining topoisomerase IV (*parC*, *parE*) and in genes determining gyrase (*gyrA* and *gyrB*). Resistance to some fluoroquinolones can result from a single mutation in one or more of the genes encoding these enzymes [15]. A second mechanism of resistance to fluoroquinolones depending on multidrug efflux has been described in pneumococci [21].

Although, macrolide and lincosamide antibiotics still exhibit high activity against *S. pneumoniae*, in many countries the prevalence of the resistant isolates has rapidly increased. (e.g. 33, 24 and 31% in Spain, Italy and Belgium, respectively) [22]. At least five phenotypes of resistance to macrolides have been described in *S. pneumoniae*. Particular mechanisms depend on target modification or efflux [23]. The first, more common mechanism determines high level resistance and cross-resistance to macrolides, lincosamides and streptogramin B (MLS(B) phenotype) [24]. Phenotype MLS(B) is conferred by the presence of the *ermB* (*ermAM*) gene, encoding an enzyme, methylase of 23s rRNA. This enzyme changes 23s rRNA of the bacterial

ribosome by methylation of adenine (A2058) and makes it unsusceptible to all MLS(B) antibiotics [25]. More recently strains harbouring the *ermA* gene have been found with a similar phenotype to *ermB* [26]. In most cases production of methylase is inducible.

A second, well-known mechanism in *S. pneumoniae* depends on efflux of 14 and 15 membered macrolides. This confers the M phenotype which depends on low level resistance to 14 and 15 membered macrolides and susceptibility to 16 membered macrolides, lincosamides, streptogramins and ketolides. The efflux mechanism is mediated by the presence of gene *mefA* or *mefE* (both genes belong to the *mefA* group). Genes *mef* are believed to encode a hydrophobic membrane protein, which uses the energy of the proton motive force to pump macrolides outside the cell.

Recently four additional mechanisms (and phenotypes) connected with target modifications were described among clinical *S. pneumoniae* isolates. The first phenotype, MS (macrolide, streptogramin resistance) is an effect of a substitution of three aminoacids in a highly conserved region of ribosomal protein L4. A second related phenotype confers additional resistance to ketolides and is due to substitution of six aminoacids in the same protein. The phenotype ML (macrolide–lincosamide) is due to A2059-G change (numbering from *Escherichia coli*) in from two to four of four copies of 23S rRNA [26]. This phenotype is similar to laboratory-obtained mutants resistant to macrolides. Recently a new mechanism, depending on mutation in 23S rRNA was described [27]. The mutation in V domain of the four copies of 23 rRNA conferred resistance to 16 membered macrolides and streptogramins A and B. Strains remain susceptible to other macrolides, ketolides and lincosamides [27]. The most frequent is the first, MLSB phenotype and other are not found frequently.

Resistance to tetracyclines in *S. pneumoniae* is conferred by the presence of one of two determinants: *tetM* or *tetO* or both. These genes encode ribosomal protection proteins that have homology to elongation factors G and Tu. Both proteins have GTPase activity that appears to be important in the displacement of tetracycline from the ribosome. The *tetM* determinant is located on the conjugative transposons or plasmids; location of *tetO* is thought to be chromosomal, but is not definitely known [28]. The resistance to chloramphenicol is determined by synthesis of chloramphenicol acetylase, which inactivates the drug.

The resistance to both components of cotrimoxazole is conferred by modification of the target. Changed enzymes dihydropteroate synthetase (DHPS) in the case of sulphonamides and dihydrofolate reductase (DHR) in the case of trimethoprim are not susceptible to drug action, but are still active in metabolic pathways [29].

5. *S. pyogenes*

S. pyogenes is responsible for a variety of community acquired diseases and is probably one of the bacterial pathogens most often encountered in clinical practice. Penicillin is the drug of choice for treatment of infection with *S. pyogenes*, and macrolide antibiotics are recommended as an alternative treatment [6]. In spite of the extensive use of penicillin and other β-lactam antibiotics, *S. pyogenes* remains susceptible to these antibiotics. However, high rates of resistance to erythromycin have been reported in several countries. Moreover, resistance to other antibiotics like tetracyclines, chloramphenicol, sulphonamides and trimethoprim occur in some *S. pyogenes* strains. Resistance to macrolides as in *S. pneumoniae* may be conferred by several mechanisms. The most frequent depends on methylation of 23s rRNA leading to cross-resistance to macrolides, lincosamides and streptogramin B (MLSB). Two genes determining this resistance in *S. pyogenes* were recognised, e*rmTR* or e*rmAM* (*ermB*) [30]. The second mechanism known as phenotype M depends on macrolide efflux and is determined by the gene *mefA* [31].

Several strains are resistant to tetracyclines with identical mechanism as *in S. pneumoniae* determined by protein TetM, responsible for ribosome protection. Rare strains are resistant to chloramphenicol due to production of acetyltransferase (CAT). Resistance to sulphonamides and trimethoprim are determined by changes in the appropriate enzymes [6].

In the 1960s *H. influenzae* was uniformly susceptible to aminopenicillins. The first strains producing β-lactamases appeared in the 1970s, and the number of resistant strains is still growing. Some sources estimated the prevalence of *H. influenzae* strains producing β-lactamase to be as high as 30%. Resistance to tetracyclines, chloramphenicol, sulphonamides and trimethoprim and recently quinolones also occurs [13].

As in other bacteria, two mechanisms of tetracycline resistance have been described in *H. influenzae*. One depends on active efflux and is determined by the presence of one of two genes *tetB* or *tetK*, encoding hydrophobic membrane proteins which use the energy of the proton motive force to pump tetracyclines out of the cell. The second is connected with the presence of ribosome protecting protein TetM [32].

Resistance to chloramphenicol is determined by the synthesis of chloramphenicol acetyltransferase and resistances to sulphonamides and trimethoprim are the result of the presence of altered forms of appropriate enzymes.

Most strains of *H. influenzae* are still highly susceptible to fluoroquinolones, but recent reports show that resistant strains have already appeared and their frequency is growing proportionally to growth of fluoroquinolone usage. Resistance is the effect of mutations in genes determining bacterial topoisomerases. Several reports suggest that *H. influenzae* isolates require at least one amino acid substitution both in subunits of gyrase (GyrA) and topoisomerase IV (ParC) in order to attain significant levels of resistance to quinolones [33]. *H. influenzae* can produce β-lactamase type TEM-1 or ROB 1. Strains producing TEM β-lactamases are more frequent. Both types of β-lactamase are sensitive to inhibitors, like clavulanic acid. β-Lactamase production as well as resistance to other antibiotics in *H. influenzae* has been associated with the presence of large, chromosomally integrated, conjugative plasmids [34]. In the last few years the proportion of *H. influenzae* strains resistant to ampicillin but not producing β-lactamase has been growing. These BLNAR (β-lactamase negative, ampicillin resistant) strains possess changes in PBPs that are responsible for the lowered affinity to ampicillin [35]. The most frequent mutation is localised in the genes *ftsI* encoding PBP 3A and/or PBP 3B which are involved in septal peptidoglycan synthesis. The role of mutation in the gene *dacB* encoding PBP 4 has not been confirmed [36]. It is possible that other, yet unknown, mechanisms of ampicillin resistance exist among BLNAR *H. influenzae*.

In some countries, resistance to aminoglycosides in *H. influenzae* is as high as 16% and in most cases this is due to the production of APH(3′)-I [37]. Investigations of the efflux mechanisms of *H. influenzae* show that inactivation of genes homologous to *E. coli acrAB* confer hypersusceptibility to several antibiotics and dyes (e.g. erythromycin, rifampicin, novobiocin, ethidium bromide and crystal violet) [38].

6. *M. catarrhalis*

Before the 1970s, the majority of clinical isolates of *M. catarrhalis* were susceptible to penicillin G as well as aminopenicillins. Now over 90% of strains produce β-lactamases. The β-lactamases produced by *M. catarrhalis* are BRO-1 and BRO-2. The *bro* genes appeared to be located on the chromosome and encode for polypeptides that differ in only one aminoacid. Production of both types of enzyme is constitutive and they are expressed as lipoprotein associated with outer membrane. Lipoprotein precursors of β-lactamases are well known among Gram-positive bacteria, but are uncharacteristic in Gram-negative species [39]. Fortunately, this organism has remained almost uniformly susceptible to antibiotics other than penicillins, cephalosporins, macrolides, tetracyclines, β-lactamase/inhibitor combinations, fluoroquinolones and co-trimoxazole. Occasionally strains resistant to these agents appear. Most frequently resistance to tetracyclines is

determined by the TetB protein, causing an efflux of antibiotic [40].

7. Conclusions

Although the resistance of bacterial strains isolated in Poland from community acquired respiratory tract infections is not high, it is increasing. Although resistance is a much greater problem among hospital acquired infections, especially in the case of staphylococci and enterococci [41], resistance among community acquired microorganisms is also a problem especially in bacteria isolated from children. The risk is high because of high level of carriage of respiratory pathogens among children and easily spread in day nurseries, kindergartens and schools.

References

[1] Enarson DA, Chretien J. Epidemiology of respiratory infectious diseases. Curr Opin Pulm Med 1999;5:128–35.

[2] Gunnarsson RK, Holm SE, Soderstrom M. The prevalence of potential pathogenic bacteria in nasopharyngeal samples from healthy children and adults. Scand J Prim Health Care 1998;16:13–7.

[3] Cappelletty D. Microbiology of bacterial respiratory tract infections. Pediat Infect Dis J 1998;17:S55–61.

[4] Chonmaitree T, Heikkinen T. Role of viruses in middle-ear disease. Ann NY Acad Sci 1997;830:143–57.

[5] Markowitz M, Gerber MA, Kaplan EL. Treatment of streptococcal pharyngotonsillitis; reports of penicillins demise are premature. J Pediatr 1993;123:679–85.

[6] Gerber MA. Antibiotic resistance: relationship to persistence of group A streptococci in the upper respiratory tract. Pediatrics 1996;97:971–5.

[7] Brandt ER, Currie B, Mammo L, Pruksakorn S, Good MF. Can class I epitope of M protein be a diagnostic marker for rheumatic fever in populations endemic for group A streptococci? Lancet 1998;351:1860–1.

[8] Heikkinen T, Thint M, Chonmaitree T. Prevalence of various respiratory viruses in the middle ear during acute otitis media. N Engl J Med 1999;340:260–4.

[9] Jones NS. Current concepts in the management of paediatric rhinosinusitis. J Laryngol Otol 1999;113:1–9.

[10] SE-TU Study Group, Vuori E, Petola H, Kallio MJT, Leinonen K. Aetiology of pneumonia and other common childhood infections requiring hospitalization and parenteral antimicrobial therapy. Clin Inf Dis 1998;27:566–72.

[11] Tummler B, Kiewitz C. Cystic fibrosis: an inherited susceptibility to bacterial respiratory infections. Mol Med Today 1999;5:351–8.

[12] Doern GV. Trends in antimicrobial susceptibility of bacterial pathogens of the respiratory tract. Am J Med 1995;99:3S–5S.

[13] Powell M, McVey D, Kassim MH, et al. Antimicrobial susceptibility of *Streptococcus pneumoniae*, *Haemophilus influenzae* and *Moraxella* (*Branhamella*) *catarrhalis* isolated in the UK from sputum. J Antimicrob Chemother 1991;28:249–59.

[14] Jeljaszewicz J, Mlynarczyk G, Mlynarczyk A. Current threats of antibiotic resistance in bacteria. Blok Operacyjny 1998;3–4:49–55.

[15] Pan XS, Fischer LM. *Streptococcus pneumoniae* DNA gyrase and topoisomerase IV: overexpression, purification and differential inhibition by fluoroquinolones. Antimicrob Agents Chemother 1999;43:1129–36.

[16] Jeljaszewicz J, Mlynarczyk G, Mlynarczyk A. Present and future problems of resistance in gram positive cocci. Infection 1998;26:1–6.

[17] Mouz N, Gordon E, Di Guilmi AM, et al. Identification of a structural determinant for resistance to beta-lactam antibiotics in gram-positive bacteria. Proc Natl Acad Sci USA 1998;95:13 403–3 406.

[18] O'Neill AM, Gillespie SH, Whiting GC. Detection of penicillin susceptibility in *Streptococcus pneumoniae* by pbp2b PCR-restriction fragment length polymorphism analysis. J Clin Microbiol 1999;37:157–60.

[19] Hakenbeck R. Mosaic genes and their role in penicillin-resistant *Streptococcus pneumoniae*. Electrophoresis 1998;19:597–601.

[20] Zhao G, Yeh WK, Carnahan RH, et al. Biochemical characterisation of penicillin-resistant and sensitive penicillin binding protein 2x transpeptidase activities of *Streptococcus pneumoniae* and mechanistic implications in bacterial resistance to beta-lactam antibiotics. J Bacteriol 1997;179:4901–8.

[21] Gill MJ, Brenwald NP, Wise R. Identification of an efflux pump gene, *pmrA*, associated with fluoroquinolone resistance in *Streptococcus pneumoniae*. Antimicrob Agents Chemother 1999;43:187–9.

[22] Sahm DF, Jones ME, Hickley ML, Diakun DR, Mani SV, Thornsberry C. Resistance surveillance of *Streptococcus pneumoniae*, *Haemophilus influenzae* and *Moraxella catarrhalis* isolated in Asia and Europe 1997–1998. J Antimicrob Chemother 2000;45:457–66.

[23] Sutcliffe J, Tait-Kamradt A, Wondrack L. *Streptococcus pneumoniae* and *Streptococcus pyogenes* resistant to macrolides but sensitive to clindamycin: a common resistance pattern mediated by an efflux system. Antimicrob Agents Chemother 1996;40:1817–24.

[24] Mlynarczyk G. Molecular basis of bacterial resistance to antibiotics. Przegl Epidemiol 2000;54(Suppl. 1):18–25.

[25] Syrogiannopoulos GA, Grivea IN, Tait-Kamradt A, et al. Identification of *erm*(*A*) erythromycin resistance methylase gene in *Streptococcus pneumoniae* isolated in Greece. Antimicrob Agents Chemother 2001;45:342–4.

[26] Tait-Kamradt A, Davies T, Appelbaum PC, et al. Two new mechanisms of macrolide resistance in clinical strains of *Streptococcus pneumoniae* from Eastern Europe and North America. Antimicrob Agents Chemother 2000;44:3395–401.

[27] Depardieu F, Courvalin P. Mutation in 23S rRNA responsible for resistance to 16-membered macrolides and streptogramins in *Streptococcus pneumoniae*. Antimicrob Agents Chemother 2001;45:319–23.

[28] Widdowson CA, Klugman KP, Hanslo D. Identification of the tetracycline resistance gene, *tet*(*O*), in *Streptococcus pneumoniae*. Antimicrob Agents Chemother 1996;40:2891–3.

[29] Haasum Y, Strom K, Wehelie R, et al. Amino acid repetitions in the dihydropteroate synthase of *Streptococcus pneumoniae* lead to sulfonamide resistance with limited effects on substrate K(m). Antimicrob Agents Chemother 2001;45:805–9.

[30] Seppala H, Skurnik M, Soini H, Roberts MC, Huovinen P. A novel erythromycin resistance methylase gene (*ermTR*) in *Streptococcus pyogenes*. Antimicrob Agents Chemother 1998;42:257–62.

[31] Kataja J, Huovinen P, Skurnik M, Seppala H. Erythromycin resistance genes in group A streptococci in Finland. The finnish study group for antimicrobial resistance. Antimicrob Agents Chemother 1999;43:48–52.

[32] Roberts MC. Tetracycline resistance determinants: mechanisms of action, regulation of expression, genetic mobility and distribution. FEMS Microbiol Rev 1996;19:1–24.

[33] Georgiou M, Munoz R, Roman F, et al. Ciprofloxacin resistant *Haemophilus influenzae* strains possess mutations in analogous positions of GyrA and ParC. Antimicrob Agents Chemother 1996;40:1741–4.

[34] Livermore DM. Beta-lactamases in laboratory and clinical resistance. Clin Microbiol Rev 1995;8:557–84.

[35] Ubukata K, Shibasaki Y, Yamamoto K, et al. Association of amino acid substitutions in penicillin-binding protein 3 with beta-lactam resistance in beta-lactamase-negative ampicillin-resistant *Haemophilus influenzae*. Antimicrob Agents Chemother 2001;45:1693–9.

[36] Doern GV, Brueggemann AB, Pierce G, Holley HP Jr., Rauch A. Antibiotic resistance among clinical isolates of *Haemophilus influenzae* in the United States in 1994 and 1995 detection of beta-lactamase-positive strains resistant to amoxicillin-clavulanate; results of a national multicenter surveillance study. Antimicrob Agents Chemother 1997;41:292–7.

[37] Gomez-Lus R, Vargara Y. Aminoglycoside resistance in *Haemophilus influenzae*. J Chemother 1995;7:97–9.

[38] Sanchez L, Pan W, Vinas M, Nikaido H. The *arcAB* homolog of *Haemophilus influenzae* codes for a functional multidrug efflux pump. J Bacteriol 1997;179:6855–7.

[39] Bootsma HJ, Aerts PC, Posthuma G, et al. *Moraxella (Branhamella) catarrhalis* BRO beta-lactamase: a lipoprotein of Gram-positive origin? J Bacteriol 1999;181:5090–3.

[40] Roberts MC, Brown BA, Steingrube VA, Wallace RJ Jr. Genetic basis of tetracycline resistance in *Moraxella (Branhamella) catarrhalis*. Antimicrob Agents Chemother 1990;34:1816–8.

[41] Jeljaszewicz J, Mlynarczyk G, Mlynarczyk A. Antibiotic resistance in gram-positive cocci. Int J Antimicrob Agents 2000;16:473–8.