

Streptococcus pneumoniae has always been an important pathogen for man and a big therapeutic success in the early years of antibiotherapy with penicillin.

For many years this species was perfectly susceptible and antibiotic susceptibility testing was not necessary. Then things changed dramatically about 25 years ago and resistance emerged in the South Hemisphere, spread to the world and now amounts to 50% of strains for penicillin as well as for other antibiotic families. This is a perfect example of emergence of new resistance.

Antibiotic testing of this species is now highly important and bioMérieux has developed a special VITEK2 card for this purpose with an appropriate selection of antibiotics.

Knowledge about the resistance of this species is important and we are pleased to offer you this new issue of our Identifying Resistance Newsletter to share this information.

Dr. Mark Mackowiak

VP for Research & Development,
bioMérieux sa

State-of-the-Art

■ Antibiotic and *S.pneumoniae*

The bioMérieux solution

■ Evaluation of the New VITEK® 2 System

Did you know?

- Web sites
- Identifying Resistance News
- NCCLS recommendations
- Antibiotics
- VITEK® 2 phenotypes

Practical advice

- why test *S.pneumoniae*?
- what are the key issues with *S.pneumoniae* infections?
- What is the reference method for testing *S.pneumoniae*?
- The epidemiology of *S.pneumoniae*

State-of-the-Art

Antibiotic and *S.pneumoniae*.....

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Beta-lactams

Although penicillin resistance was first described in the pneumococcus in 1967 (15) the emergence of unusual strains of pneumococci with very high level resistance to amoxicillin has recently been documented (7). These strains are unusual in that while the amoxicillin MIC's of

pneumococci are usually lower than those of penicillin, these strains are more resistant to amoxicillin than to penicillin. The strains were first isolated in France but have more recently also been found in the United States. Of recent concern also is the emergence of pneumococci with penicillin MIC's $\geq 8 \mu\text{g/ml}$. These strains remain rare,

Antibiotic and *S.pneumoniae*

Several antibiotics can inhibit *S.pneumoniae*.
And resistance exist for most of them.

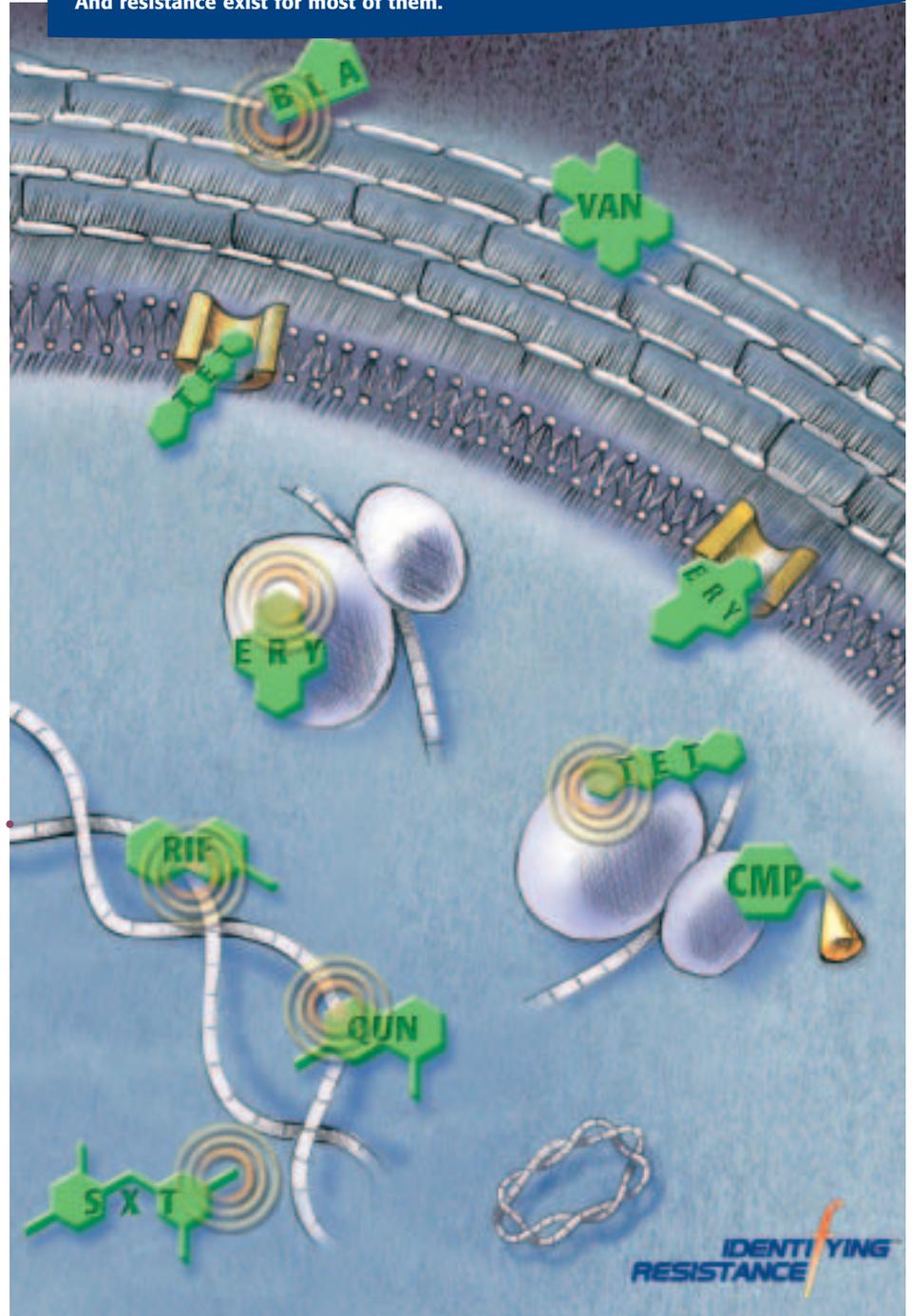


fig. 1

BLA b-lactams,
VAN vancomycin,
ERY erythromycin,
TET tetracyclin,
CMP chloramphenicol,
RIF rifampin,
SXT trimethoprim+sulfamethoxazole,
QUN quinolones

The three resistance mechanisms are:
target alteration,
efflux and antibiotic inactivation.

but there is evidence that they may be common in certain geographic areas of the United States and they have strong clonal origins. While changes in penicillin-binding proteins (PBP's) remain the basis of beta-lactam resistance in the pneumococcus by the transformation of pneumococcal *pbp* genes with DNA from viridans streptococci

to create mosaic genes (8), the discovery of the critical role played by *murM* in the biosynthesis of the branched chain precursors required to cross-link the cell wall of resistant pneumococci is providing new insight into non-PBP mechanisms that may be important in the development of beta-lactam resistance in this pathogen

(13). Within the setting of the specific PBP changes found in a highly penicillin-resistant Hungarian strain with an MIC of 16 µg/ml, it was found that *murM* played a critical role in the expression of that very high level of resistance (28). The role of *murM* is however complex, and an altered *murM* does not seem to play an essential role in the manifestation of all high-level penicillin resistant pneumococci (9).

Macrolides

The dominant mechanisms of macrolide resistance in the pneumococcus are the efflux mechanism (predominantly *mefA*) and the presence of the rRNA methylation gene, *ermB* which additionally confers resistance to lincosamides such as clindamycin and to streptograminB. Usually pneumococci have only one of these resistance genes but the concurrence of the genes was described in South Africa, in a global pneumococcal clone called the Taiwan19F – 14 clone (21). This clone now comprises a significant percentage of macrolide - resistant pneumococci in many other countries. Closely related versions of the *mef* and *erm* genes exist primarily in *Streptococcus pyogenes*. The *ermA* gene from *Streptococcus pyogenes* has been found in a pneumococcal strain from Greece (29) and together with the *ermB* gene in a pneumococcus from Spain (3). The *mefA* gene from the pneumococcus, formerly called *mefE*, is located in a transposable element called MEGA (macrolides efflux genetic assembly) (14), which lacks the genes for transposition in transposon1207.1 around the *mefA* gene from *Streptococcus pyogenes*, which has also been documented in *S. pneumoniae* (27). Of particular interest is the recent emergence of mutation based resistance to macrolides in the pneumococcus. The pneumococcus has four rRNA genes and there is a dose-response to increasing levels of resistance as mutations occur in the macrolide - binding areas of the RNA (30). Resistance to macrolides in pneumococci bearing these mutations have been found rarely, but are widely distributed, having been found in Europe, North America, South East Asia and Australia (11). It has also been

shown that mutations in the L4 and L22 proteins can confer clinically relevant macrolide resistance in the pneumococcus (23, 31) and combinations of mutation in these genes and rRNA can confer resistance to the streptogramins as well as to the ketolides (26).

Oxazolidinones

The first reports of linezolid - resistant pneumococci have been made (12) and the molecular basis of the resistance is being sought.

Tetracycline

Most tetracycline resistance in the pneumococcus is conferred by the presence of the *tetM* gene and although the *tetO* gene has been described in two discrete geographic locations (South Africa and USA), these strains remain rare (19, 34).

Rifampin

The molecular basis of resistance to the rifamycins is mutation in the beta subunit of RNA polymerase *rpoB*. A number of mutations in the gene associated with resistance have been described (10, 25).

Trimethoprim-Sulfamethoxazole

The molecular basis of resistance to these agents are base mutations, insertions and deletions in the genes encoding

the enzyme substrates of the drugs (1, 18).

Chloramphenicol

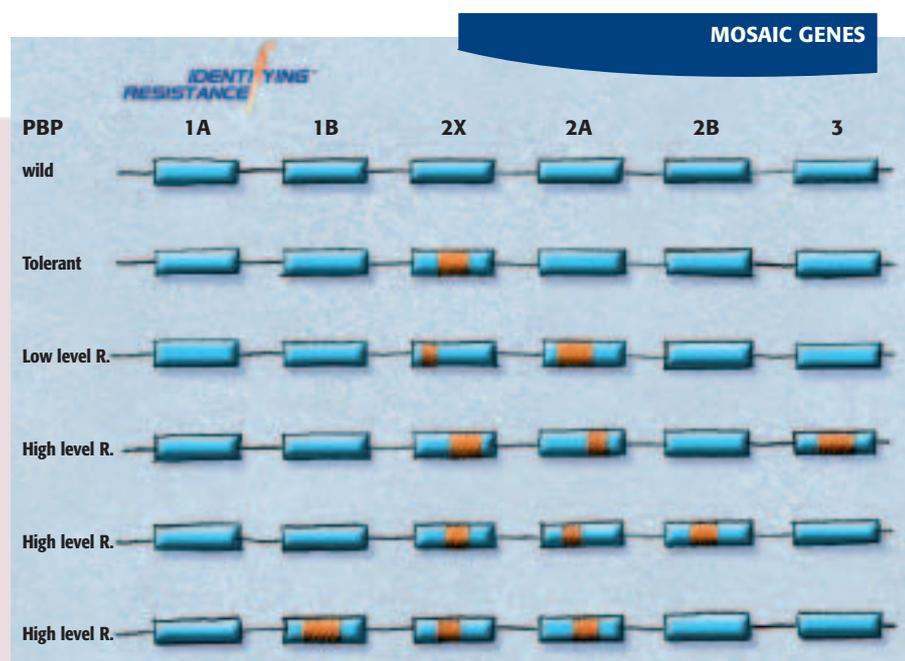
Resistance to chloramphenicol in the pneumococcus is mediated by the acquisition of a gene encoding chloramphenicol acetyl transferase. An interesting aspect of this mechanism is that the gene is located on a plasmid, probably derived from the staphylococcus, which has entered the pneumococcus by linearization and inclusion on a transposable element (33).

Vancomycin

Although no vancomycin-resistant pneumococcus has been described, there are reports of a tolerance phenotype, the clinical role of which remains to be fully established (22, 24).

Fluoroquinolones

The emerging resistance to fluoroquinolones is mediated mostly by changes in the QRDR of the topoisomerase enzymes. There is also efflux mediated resistance that is thought only to be clinically relevant mostly at low levels of resistance. Although there is evidence for clonal spread of fluoroquinolone resistance in Hong Kong (16), most of the emerging resistance in North America has been sporadic mutation in strains isolated from patients with a previous history



of fluoroquinolone exposure (4). There is however potential for the rapid emergence of fluoroquinolone resistance, as this phenotype has now been documented in a number of well-recognized international global clones in Europe (20) and in the US (5, 17). A recent report of interest is the demonstration that a proportion of fluoroquinolone-resistant pneumococci appear to have acquired the resistance genotype by horizontal transfer from viridans streptococci (2).

Optochin

Although not used clinically since the beginning of the last century, optochin is widely used in the laboratory identification of pneumococci. The molecular basis of resistance to the agent, related to the quinone class of antimalarials is a single base mutation in the *atpC* gene (6). It is of concern that the important phenotype for pneumococcal identification, of optochin – sensitivity, can be lost by a single base mutation.

Summary

The multiply-resistant pneumococcus continues to evolve. Knowledge of the molecular basis of antibiotic resistance can give insights into the likely evolution of resistance in the organism. The introduction of conjugate pneumococcal vaccine has been shown in the U.S. to reduce the prevalence of antibiotic-resistant pneumococci (32) and this vaccine may slow the emergence of newly acquired resistance determinants in strains belonging to conjugate vaccine serotypes. Surveillance of the emergence of resistance in non – vaccine serotypes is therefore of utmost importance.

fig. 2

***S.pneumoniae* has six different PBPs named: PBP 1A, PBP1B, PBP2A, PBP2B, PBP2X to build its cell wall. They are targets of b-lactam antibiotics. Their sequences can partially change following genetic exchanges with other bacterial species, giving these "mosaic genes". Red sections are imported sequences, and combinations of the exchanged sections give various levels of resistance.**

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Evaluation of the New VITEK® 2 System for Determination of the Susceptibility of Clinical Isolates of *Streptococcus pneumoniae*

Monica Chavez, Jose Luis Garcia Lopez, Julian Coronilla, Anastasio Valverde, M. Carmen Serrano, Rosa Claro, Estrella Martin Mazuello (Sevilla, Spain) *Chemotherapy* 2002; 48:26-30

214 clinical isolates

The VITEK 2 allows rapid determination of the antimicrobial susceptibility of S.pneumoniae and demonstrates a good degree of agreement with the Sensititre method of the antimicrobials tested.

Evaluation of the VITEK 2 System for Susceptibility testing of *Streptococcus pneumoniae* isolates

W.H.F. Goessens, N. Lemmens-den Toom, J. Hageman, P.W.M. Hermans, M. Sluijter, R. de Groot, H.A. Verbrugh (Rotterdam, the Netherlands) *Eur J Clin Microbiol Infect Dis* (2000) 19:618-622

In conclusion, VITEK 2 shows good agreement with the reference method, as demonstrated by the low number of major errors, but it has a tendency to overestimate MICs, resulting in minor errors

200 isolates.

50 penicillin-susceptible and 150 intermediate or penicillin-resistant isolates.

Rapid Automated Antimicrobial Susceptibility Testing of *Streptococcus pneumoniae* by Use of the bioMérieux VITEK 2

James Jorgensen, Arthur L. Barry, M.M. Traczewski, Daniel F. Sahn, M. Leticia McElmeel, and Sharon A. Crawford (San Antonio, Wilsoville, St Louis, USA) *J. Clin. Microbiol.* Aug 2000, p. 2814-2818

The VITEK 2 provided rapid, reliable susceptibility category determinations with both the challenge and clinical isolates examined in this study.

54 challenge strains and 407 and 423 clinical isolates.

WEB SITES

<http://www.cdc.gov>

<http://www.cdc.gov/ncidod>

- **Streptococcus pneumoniae Disease Technical Information and Additional Information**
- **Drug-resistant Streptococcus pneumoniae Disease**

<http://www.cdc.gov/ncidod/dbmd/abcs>

- **Active Bacterial Core Surveillance (ABCs) Report**

Identifying Resistance Symposium in Korea (No.3)



On November 18th, 2004 was held the third **Identifying Resistance** Symposium in Seoul.

The guest speaker was Prof. Roland Leclercq from the Academic Hospital of Caen, France.

He presented the current knowledge on resistance to macrolides. Dr J.P. Marcel focused on Identifying Resistance.

Prof Wee-Yeal Lee described glycopeptide resistance to enterococci, Prof Mi-Na Kim, Linezolid resistance in enterococci and Staphylococci in Korea.

Prof D-H Shin updated knowledge on Antimicrobial resistance in biofilm. Audience was around 80 people.

These symposiums are the opportunity to gather top opinion leaders on antibiotic resistance in Korea, especially from Seoul National Hospital and Yonsei University. Guest speakers mark the event as they are top specialists from Europe. In previous years guest speakers were Prof. P. Courvalin then Prof. C. Poyart.



Laboratory PathCare in CapeTown (South Africa)

This lab has been the first VITEK2 user in Africa.

They are using it for routine testing, and are happy with it, especially with detection of mechanisms of resistance.

nccls recommendations

ref: M100-S14 (M7) January 2004

Table 2G lists MIC Interpretive Standards for *S.pneumoniae*

Relevant antibiotics are assigned to four classes (A, B, C, oral)
Class A only contains three molecules

1. penicillin G

S results can be extended to 19 other molecules (comment 4)
I/R results should not lead to test a series of six drugs (comment 1)

2. erythromycin

S or R results can be extended to three closely related antibiotics (comment 11)

3. trimethoprim-sulfamethoxazole

for CSF isolates,

reporting should be restricted to five antibiotics: penicillin, cefotaxime, ceftriaxone, meropenem and vancomycin. (comment 1)

specific low breakpoints (S<0.5, I=1, R>2) should be used for cefotaxime and ceftriaxone. (comment 7)
and cefepime should not be reported in the USA for such isolates (no FDA-approved indication) (comment 6)

Antibiotics

several families of antibiotics can be used against *S.pneumoniae* infections.

b-lactams	Penicillin G, Ampicillin, Imipenem, Ertapenem, Cefotaxime, Ceftriaxone, Cefepime, Cefpirome, Cefuroxime-axetil
aminoglycosides	Streptomycin, Gentamicin, Kanamycin
chloramphenicol	Chloramphenicol
tetracyclines	Tetracycline
macrolides	Erythromycin, Lincomycin, Telithromycin Pristinamycin
oxazolidinones	Linezolid
vancomycin	Teicoplanin, Vancomycin
trimethoprim-sulfamethoxazole	Trimeth. + Sulfamethoxazole
fluoroquinolones	Sparfloxacin, Trovafloxacin, Levofloxacin, Gatifloxacin, Moxifloxacin, Fosfomicin
rifampin	Rifampin

VITEK® 2 Phenotypes V4.0

Phenotypes identified with the four main families

b-lactams	alteration of PBP High Level resistance alteration of PBP Low Level resistance
macrolides	resistant (MLSb) resistant (efflux) resistant to streptogramins
quinolones	quinolone resistance
glycopeptides	(no resistance so far)

for more information : identifying.resistance@eu.biomerieux.com

why test *S.pneumoniae*?

Resistance (vs penicillin) reaches 50% in some countries the spread is continuing.

what are the key issues with *S.pneumoniae* infections?

Severe infections such as meningitis.
Specific breakpoints have been established to easily identify intermediate and resistant strains in these csf infections.

What is the reference method for testing *S.pneumoniae*?

Broth MicroDilution (BMD) and not agar dilution. (nccls)

The epidemiology of *S.pneumoniae*

For several decades *S.pneumoniae* was totally susceptible to antibiotics. Resistance started in 1977 and spread widely in the 90's to reach very high level in some countries.

This spread is mainly clonal.

Accurate testing enables to measure the spread of resistance.

The fight against this pathogen also includes vaccines.

IDENTIFYING™
RESISTANCE

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