susceptibility testing, a fear of infection with resistant organisms leads to the use of antibiotics which are described in the literature as having a very broad spectrum while being very easy to administer, i.e. quinolones orally and ceftriaxone intramuscularly once daily.

The ceftriaxone resistance was due to an extended-spectrum beta-lactamase (ESBL), which could be shown to be plasmid mediated, since resistance could be transferred during in vitro transconjugation experiments. Therefore, there is a concern that this resistance could spread easily to other commensal and pathogenic bacteria in Cambodia. The possibility that this observed high resistance rate in *E. coli* represented clonal dispersion, due to cross-contamination of the doctors during the training course, could be excluded by DNA fingerprinting by means of arbitrarily primed PCR [1], by which only two of the six isolates appeared to have identical fingerprints.

These findings are rather disturbing, although they are probably not representative of the entire population, since probably only the doctors and the wealthy can afford self-treatment and treatment with the newer and more expensive antibiotics. These data illustrate that resistance can be very high even in developing countries and suggest that laboratory facilities may form an essential part of medical progress, especially in developing countries and suggest that laboratory facilities may form an essential part of medical progress, especially for diagnostic and therapeutic strategies. In a recent study, ESBLs were detected in 26% of Enterobacteriaceae isolated in a hospital in Thailand [2]. These data and our limited experience illustrate that studies on susceptibility of bacteria in developing countries should be performed urgently and the results disseminated widely in order to enhance appropriate use of antibiotics. Possibly, the treatment advocated by the WHO, using cheap antibiotics, may be insufficient in certain situations.

*Geert Claeys,* *Mario Vaneechoutte,*
*Gerda Verschraegen*
Laboratory for Bacteriology and Virology, University Hospital, Gent, Belgium

Accepted 16 July 1997

**References**


---

### Phenotypes of resistance to macrolide and lincosamide antibiotics in *Staphylococcus* species

*Clin Microbiol Infect* 1997; 3: 702–705

Macrolide, lincosamide and streptogramin (MLS) antibiotics are chemically distinct, but are alike in their mode of action, which is inhibition of protein synthesis by binding to the 23S component of the 50S ribosomal subunit. Three mechanisms of MLS resistance are most frequently encountered: (1) enzymatic target modification by an N⁶-dimethylase [1–3]; (2) enzymatic inactivation by (a) 3-lin 4-cty O-nucleotidyltransferase (linA), (b) streptogramin A acetyltransferase (saa), and (c) streptogramin B hydrolyase (sbh) [4–6]; and (3) active efflux resulting in hampered erythromycin permeability (erpA) or macrolide streptogramin permeability (msrA) [7–9]. In staphylococci, N⁶-dimethylase activity can either be constitutively expressed, or be induced by the presence of a macrolide. Although in the former situation (constitutive expression) resistance to all macrolide, lincosamide and streptogramin B antibiotics is readily detected by agar diffusion, isolates resistant to 14- and 15-membered macrolides may still appear susceptible to clindamycin in the latter (inducible expression) [10]. The National Committee for Clinical Laboratory Standards (NCCLS) guidelines for performing susceptibility testing by disk diffusion do not specifically address this issue [11], and have been shown to be unreliable in detecting inducible N⁶-dimethylase activity [12]. To overcome this problem, a modification with the addition of lincomycin has recently been suggested [13].

During a 3-month period our laboratory augmented the standard susceptibility testing procedure (NCCLS; erythromycin and clindamycin disks not apposed) by including a lincomycin disk on a separate agar plate (Mueller–Hinton 2 cation-adjusted agar, bioMérieux, Crapone, France). Erythromycin (15 μg; Becton Dickinson, Meylan Cedex, France) and clindamycin (2 μg; Becton Dickinson, Meylan Cedex, France) zone diameters were interpreted according to the NCCLS guidelines [11]. Interpretations of lincomycin (15 μg; bioMérieux, Marcy l’Etoile, France) zone diameters were those provided by the manufacturer (susceptible, ≥21 mm; intermediate, 18–20 mm; resistant, ≤17 mm).

Staphylococci were defined as catalase-positive, Gram-positive cocci in clusters. *Staphylococcus aureus* and coagulase-negative staphylococci (CNS) were differentiated on the basis of DNase activity and free coagulase production. Doubtful results were confirmed by *Staphylococcus aureus* Accuprobe (Gen-Probe, San Diego, CA). CNS were not further identified to the species level.
Figure 1 Modified disk diffusion method in which the erythromycin disk (E; 15 μg) was placed in between the clindamycin (CC; 2 μg) and lincomycin (L; 15 μg) disks respectively: (a) Erythromycin N^6-dimethylase, constitutive expression (erm). (b) Erythromycin N^6-dimethylase, inducible expression (erm). (c) 3-lin 4-cli O-nucleotidytransferase (linA). (d) Erythromycin permeability (erpA) or macrolide streptogramin permeability (mutA). (e) Streptogramin A acetyltransferase (saa) or streptogramin B hydrolase (dbh).
In total, 444 consecutive patient isolates (324 S. aureus, and 120 CNS) that were considered clinically significant were investigated. The augmented screening procedure detected 59 (13.3%) isolates revealing reduced susceptibility to any of the three antibiotics comprising 18 (5.5%) S. aureus and 41 (34.2%) CNS, with the following susceptibility patterns: erythromycin, lincomycin and clindamycin resistant, n=27; erythromycin monoresistant, n=24; lincomycin monoresistant, n=2; intermediate to lincomycin, n=1; lincomycin resistant and intermediate to clindamycin, n=5. Thus, three isolates (S. aureus, n=1; CNS, n=2) showing reduced susceptibility to lincomycin were only detected by the augmented screening.

Thirty-eight of the 59 resistant isolates (erythromycin, lincomycin, and clindamycin resistant, n=19; erythromycin monoresistant, n=14; lincomycin monoresistant, n=1; intermediate to lincomycin, n=1; lincomycin resistant and intermediate to clindamycin, n=3) were available for retesting both by the NCCLS standard procedure and by a modified disk diffusion method in which the erythromycin disk was placed in between the clindamycin and lincomycin disks (distance 15 mm). From susceptibility patterns (Figure 1) the isolates were classified according to the putative resistance mechanisms [12]: (a) constitutive N6-dimethylase, n=18 (S. aureus, n=3; CNS, n=15); (b) inducible N6-dimethylase, n=14 (S. aureus, n=3; CNS, n=11); (c) linA, n=4 (S. aureus, n=3; CNS, n=1); (d) msrA or erpA, n=1 (CNS); (e) ssa or sbh, n=1 (CNS). Differentiating ssa from sbh and erpA from msrA was not possible, since streptogramins were not tested [12].

Comparison of the modified to the NCCLS standard procedure revealed unequivocal results regarding the 18 isolates harboring constitutively expressed N6-dimethylase and one isolate showing msrA or erpA activity. The modified procedure identified inducible N6-dimethylase activity in 14 additional isolates, irrespective of the lincosamide tested (Figure 1b); linA and ssa or sbh activity, respectively, in one isolate each, however, were only detected by lincomycin (Figure 1c,e). By the NCCLS standard procedure (erythromycin and clindamycin disks not apposed), 13 of 14 isolates with inducible N6-dimethylase activity appeared to be clindamycin-susceptible (Figure 2a), but apposing clindamycin next to erythromycin disks resulted in the detection of lincosamide resistance conferred by this mechanism in all 14 isolates (Figure 2b). Although there is still controversy over the clinical significance of clindamycin resistance conferred by inducible N6-dimethylase activity, relapse in patients with staphylococcal infections who were treated with clindamycin has been reported [14].

While it reliably detected inducible N6-dimethylase activity when apposed next to the erythromycin disk, clindamycin alone detected only three of four isolates with resistance conferred by linA (Figure 1c). The proper detection of this resistance mechanism, which greatly impairs the activities of both lincomycin and clindamycin in vitro [13], is a prerequisite for assessing its clinical significance, and, since clindamycin
is still an important drug for the treatment of staphylococcal infections, the detection of any mechanism conferring resistance to this drug may be important for predicting the clinical outcome.

Our findings confirm that the reliable detection of MLS resistance conferred by inducible N'-dimethylase activity in staphylococci by disk diffusion requires the apposition of the lincosamide next to the erythromycin disk; the substitution of lincomycin for clindamycin further increases the sensitivity of the resistance screening.

Guido Stirnimann, Sara Droz, Lukas Matter, Thomas Badner
Institute for Medical Microbiology, Bern, Switzerland

Revised version accepted 5 July 1997

References


Nasopharyngeal carriage of Streptococcus pneumoniae in children's day-care centers: 10-month follow-up study in Nice, France

Clin Microbiol Infect 1997; 3: 705–708

Streptococcus pneumoniae (SP) is one of the most frequently encountered bacterial species in respiratory tract infections, occasionally leading to potentially life-threatening complications (meningitis, septicemia). Since 1987, a steady increase in resistance has been observed in France, with rates reaching 32% in 1994 [1]. Every child carries SP at some time during the year and this organism is responsible for the majority of bacterial infections between 3 months and 3 years of age [2]. However, epidemic processes within day-care centers do not appear to have been investigated to date. The object of this study was to assess the prevalence and incidence of nasopharyngeal carriage of penicillin-resistant pneumococci (PRP), and identify risk factors associated with carriage in a cohort of children attending day-care centers in south-eastern France.

A prospective longitudinal study was conducted between September 1994 and June 1995 in three day-care centers in the town of Nice, France. Two of these were hospital day-care centers catering for children of hospital staff (DCCs A and P), while the third was a private center (DCC S). A nasopharyngeal sample was collected monthly, starting at inclusion and for as long as the child attended the center. Care-givers and parents were interviewed each time to investigate recent upper or lower respiratory tract infections and/or antimicrobial treatment. Samples were collected with a flexible Vygon 522.06 tube fitted onto a 1-mL syringe and introduced into one of the child’s nostrils. SP strains were tested for susceptibility to oxacillin by the Kirby–Bauer method. Susceptibility to penicillin was