Correlation Between Erythromycin- Resistance Phenotypes of *Streptococcus Pneumoniae* and the Invitro Activity of Telithromycin and Azithromycin

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**ABSTRACT:**

Two principal mechanisms have so far been found to be responsible for acquired macrolide ,lincosamide and streptogramin B (MLSB) antibiotics resistance in *Streptococcus pneumoniae* : target site modification and active drug efflux ,the target site modification is due to methylase and prevents the binding of the antibiotic to its ribosomal target and can be expressed either in a constitutive (cMLSB phenotype) or inducible (iMLSB phenotype) manner. The macrolide efflux system, M phenotype, is mediated by a membrane protein responsible for the efflux resistance. Although the incidence of resistance to macrolides was low in the past, today the incidence reported by several countries shows a sensible increase. Thus it is necessary to search and test novel antimicrobial agents characterized by a spectrum of activity against the most common respiratory pathogens. This study compared the *invitro* activity (MIC and MBC) of telithromycin with activity of azithromycin against *Streptococcus pneumoniae* recently isolated from San Giovanni Battista Hospital (Turin, Italy). Erythromycin – resistance phenotypes were determined through a triple – disk test to correlate a potential different bacterial pattern to antimicrobial susceptibility. The incidence of erythromycin-resistance was 26.6%. In the group of Ery-R *Streptococcus pneumoniae* 58.33% strains belonged to cMLSB phenotype, 33.33% to M phenotype and 8.33% to iMLSB phenotype. Telithromycin presented MIC values lower than those detected with azithromycin against all isolated strains. Telithromycin appeared to be highly active against *Streptococcus pneumoniae*, in particular when resistance is mediated by the efflux system confirming its clinical efficacy among respiratory streptococcal infections.

**المستخلص:**

هناك النتائج أسستنا وجدنا أنهما، مستويات من المقاومة المكتسبة لمجموعة مضادات الماكروليدات الحيوية ومضادات الليموكوباسين والستريتوجراين ب الحيوية لبكتريا المكورات السريرية الرونية وها: تعديل موقع الهدف ودفق المضاد الفعال. ويرجع سبب تعديل موقع الهدف إلى إنزيم الميثايلاز الذي يمنع ارتباط المضاد الحيوي بهيدرو الريبوسمومي والذي يعبر عنه أما ب الجوهر (الأساسي) أو الثانوي (الفرعي). أما في نظام دفق المضاد أو الدعم المثليز الحيوية للماكروليدات فإن غشاء البروتين هو المسؤول عن مقاومة الدفق، وعلى الرغم من حدوث المقاومة للماكروليدات كانت قليلة في الماضي إلا أن حالات عديدة سجلت حالياً وزيادة ملاحظة لذلك في الوقت الاضطراري اختبار دراسة مضادات حيوية متعددة لعلاجها الواسعة ضد الممرضات البكتيرية التنفسية. هذه الدراسة تقارن بين الفعالية خارج الجسم الحي للتركيز الإثني المثليز والتركيز الإثني المثليز لنتائج المقاومة للماكروليدات لبكتريا سريرية الرونية واللازمات بويكاسين ضد المكورات السريرية الرونية التي عزلت من مستشفى سانت جويفانتي باتيسيا (تونس، إيطاليا). ان الامتصاص المظاهر المعاينة للمكورات السريرية الرونية حدد بطرقية اختياري انتخاب المقاومة للضارتين الثلاثة المتداخلة للمناخ المختلفة منحساسية البكتيريا للمضادات. ان نسبة المقاومة لللازمات بويكاسين كانت فيività بالانماط الثلاثة المحتملة لمضادات الإثني المثليز، ونسبة المقاومة لللازمات بويكاسين 33.33% بعد النوع الماكروليد و8.33% للنوع الثاني، ونسبة الفترات المثليز لللازمات بويكاسين نسبة ادنى من التي سجلت للازمات بويكاسين ضد كل العزلات البكتيرية. ان النتيجة في الدراسة كانت أكثر فعالية ضد بكتريا المكورات السريرية الرونية خاصة عندما تكون المقاومة عن طريق نظام الدفع مؤكدًا الفعالية السيربية ضد الأصباث التنفسية الناتجة عن المكورات السريرية الرونية.
INTRODUCTION:
Antimicrobial resistance has emerged as a major problem in *Streptococcus pneumoniae*. Increased resistance to macrolide in *Streptococcus pneumoniae* has been described worldwide. Mediterranean countries have the highest rates of Erythromycin-resistant pneumococci (1). Macrolide resistance in pneumococci is mainly mediated by two mechanisms: enzymatic target site modifications mediated by *erm* (B) methylase that confer the MLSB phenotype and active drug efflux pumps encoded by *mef* genes that confer the M phenotype (2). The target site modification is due to methylase, encoded by the *erm* genes, and prevents the binding of the antibiotic to its ribosomal target. It is well established that this resistance can be expressed either in a constitutive (cMLSBS, phenotype) or inducible (iMLSBS, phenotype) manner. The macrolide efflux system, M phenotype, is encoded by protein responsible for the efflux-mediated resistance (3,4). Although the incidence of resistance to macrolides was low in the past, today the incidence reported by several countries shows a sensible increase. Thus, it is necessary to search and test novel antimicrobial agents characterized by a spectrum activity against the most common respiratory pathogens. Ketolides are a new family of the MLSB class of antimicrobials, have shown to be more active in vitro than macrolides against various Gram-positive bacteria such as erythromycin-resistant *Streptococcus pneumoniae* strains. Telithromycin is the first ketolide developed for the clinical use. Telithromycin, a new antimicrobial agent, is a semi-synthetic derivative of erythromycin (5,6). This study compared the in vitro activity of telithromycin with the activity of azithromycin against *Streptococcus pneumoniae*. Erythromycin-resistance phenotypes were determined to correlate different bacterial patterns to antimicrobial susceptibility.

MATERIALS AND METHODS:

Bacterial strains: Forty-five *Streptococcus pneumoniae* strains were collected from patients with respiratory infections in San Giovanni Battista hospital, (Turin, Italy) and between the period from January and March 2007. The isolated strains were tested for Gram stain morphology, colony morphology, hemolysis on sheep blood agar, optochin susceptibility, susceptibility in deoxycholate (bile), carbohydrate utilization, miniaturized manual systems such as the API 20 strept system (Biomerieux Italia, Rome, Italy) (7).

Determination of Erythromycin-resistant phenotype: Erythromycin resistance phenotype was determined by the triple-disk test described by Giovannetti *et al.* (8). Commercial disks (Oxoid, Basing stock, Hampshire, England) of erythromycin (15μg), clindamycin (2μg) and josamycin (30μg) were used. A disk of penicillin G (10 units, Oxoid) was added to confirm susceptibility of the isolated strains. The disks were placed 15-20 mm apart on Muller-Hinton agar supplemented with 5% sheep blood (Oxoid), which has been inoculated with a swab dipped into a bacterial suspension with a turbidity equivalent to that of a 0.5 MacFarland standard. After 18 h of incubation at 37°C in a 5% CO2 atmosphere, the absence of a significant zone of inhibition around the three disks was taken to indicate constitutive resistance, blunting of clindamycin and josamycin zone of inhibition proximal to the erythromycin disk was taken to indicate inducible resistance. The presence of the zone of inhibition around clindamycin and josamycin disks was taken to indicate the M phenotype.

Antimicrobial activity of telithromycin and azithromycin: Telithromycin (Aventis Pharma, Lainte, Italy) were dissolved in methanol (telithromycin) or 95% ethanol (azithromycin) at a concentration 128μg/ml and stored in a aliquots at -20°C until use. Determination of MIC was carried out using the microdilution broth method according to clinical and laboratory standard Institute (CLSI) with an inoculum of approximately 10°5 CFU/ml (9). Antimicrobial concentrations ranged from 0.003 to 64 μg/ml azithromycin and telithromycin. Results were observed after 18 h of incubation at 37°C in a 5% CO2 atmosphere. MBC was determined by plating 100μl from the wells.
showing no visible growth on agar plates and incubating for 18 h.

RESULTS:

**Erythromycin-resistance phenotypes:** on the basis of the erythromycin - clindamycin - josamycin triple - disk test, 33 out of 45 *Streptococcus pneumoniae* isolated strains were erythromycin-susceptible (73.33% Ery-S) and 12 (26.66%) were erythromycin-resistant (Ery-R). (figure 1).

**Antimicrobial activity of Telithromycin and Azithromycin:** MICs and MBCs of telithromycin and azithromycin were determined and compared. Homogeneous susceptibility patterns were observed among the Ery-S *Streptococcus pneumoniae* with low MIC values both for telithromycin and azithromycin. In fact, azithromycin MIC values ranged from 0.07 to 2 µg/ml and MICs of telithromycin ranged from 0.03 to 0.06 µg/ml for all the 33 Ery-S strains (table 1). The Ery-R *Streptococcus pneumoniae* showed azithromycin MIC values higher than Ery-S cocci, where they generally presented lower telithromycin MIC values. In particular, on the basis of the resistant phenotype patterns, the azithromycin MIC values ranged from 16-32 µg/ml for the 4 M phenotype strains and MICs ≥ 64 µg/ml to all the constitutive (7/7) and inducible (1/1) strains (table 1). Telithromycin presented a more heterogeneous susceptibility distribution in the three different Ery-R phenotypes: 42.8% (3/7) constitutive strains had MICs of telithromycin ranged from 16-32 µg/ml, where as MIC values were by lower in M phenotypes. In fact, in all M phenotype strains telithromycin MIC values ranged from 1-2 µg/ml and the only strain with inducible phenotype showed a MIC 0.12 µg/ml (table 1). Telithromycin and azithromycin MBC values were generally higher than the corresponding MICs, reflecting the same trend observed for MIC values (table 2). Among 12 resistant strains, 7/12 (58.33%) displayed the constitutive MLS phenotype. Figures (2, 3A), 4/12 (33.33%) had the M phenotype. Figures (2,3B) and 1/12 (8.33%) had inducible MLS phenotype (figure 2).

**DISCUSSIONS&CONCLUSIONS:** - The burgeoning problem of resistance to antibiotics in *Streptococcus pneumoniae* has attracted the attention of researchers all over the world. Two principal mechanisms of macrolide resistance have been described, target modification is mediated by rRNA erythromycin resistance methylase and coded by the erm (erm B or erm TR) gene (2). Resistance can be expressed either constitutively (cMLS b phenotype) or inducibly (iMLS B phenotype). The M phenotype involves an active efflux pump, which removes both 14-membered and 15-membered macrolides from the bacterial cell (10). By using the triple - disk test we showed that 58.33% of Ery-r strains belonged to cMLS phenotype, 33.33% were resistant to macrolides by the activation of an efflux pump (M phenotype) and 8.33% belonged to iMLS phenotype. Telithromycin, the first member of ketolides, has a good spectrum of activity against respiratory pathogens as well as a high bactericidal activity (11). In this study, the in vitro activity of telithromycin against clinical isolates of *Streptococcus pneumoniae* was compared to that of azithromycin, the telithromycin presented a good antibacterial activity against *Streptococcus pneumoniae* strains tested. The MICs for constitutive strains had MICs of telithromycin ranged from 8-16 µg/ml, and in all M phenotype strains telithromycin MICs values ranged from 1-2 µg/ml and the only strain with inducible phenotype showed a MIC 0.12 µg/ml compared with AL-Tiemei study who found that the MICs for constitutive strains were >16 µg/ml, and the MICs for M phenotype strains were 0.5-4 µg/ml (2). While Kaida reported that for 55 isolates of Ery-resistance *Streptococcus pneumoniae* MICs ≥ 1 carrying the M phenotype (11). The MICs for *Streptococcus pneumoniae* constitutive and M phenotype strains were ranged from 0.25-64 µg/ml and for inducible strains were 0.008-2 µg/ml in Morosini study (5). The present study showed that MICs values for Azithromycin ranged from 16-32 µg/ml for the 4 M phenotype strains and MICs ≥ 64 µg/ml to all the constitutive and inducible strains in Hoffman study the MICs values for M phenotype were...
study the MICs values for M phenotype were 32 µg / ml while for constitutive and inducible strains the MICs ≥ 64 µg / ml (12). Also in our study, for all 33 Ery-susceptible strains the azithromycin MICs values ranged from 0.07 – 2 µg / ml and MICs of telithromycin ranged from ≤ 0.003 – 0.06 µg / ml, while the telithromycin MICs values ranged from 0.008 – 0.064 µg / ml in Bingen study (13). Streptococcus pneumoniae is the most common cause of community-acquired pneumonia, macrolide antibiotics remain a viable first choice for empirical treatment of community-acquired pneumonia in outpatients. Our study shows that telithromycin appeared to be highly active against all Ery-r strains of Streptococcus pneumoniae, in particular when resistance is mediated by the efflux system, indicating its clinical efficacy in the treatment of respiratory Streptococcal infections. Moreover, the different pattern shown by Ery-r phenotypes to antibiotics indicates that the triple-disk test is a simple and reliable alternative method, suggesting the need for laboratories to introduce it into laboratory routine.
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Table 1: MICs of erythromycin-susceptible and erythromycin-resistant isolates to TEL, chloramphenicol, and azithromycin.

Table 2: MICs of erythromycin-susceptible and erythromycin-resistant isolates to TEL, chloramphenicol, and azithromycin.

**Definitions:**
- TEL = chloramphenicol
- AMX = azithromycin
- MZM = erythromycin
- ERY = erythromycin-resistant
- ERY-FX = erythromycin-susceptible
- MDR = multidrug-resistant
- CR = completely resistant
- CNS = constitutive resistance
- IRR = inducible resistance
Figure (1) Percentage of erythromycin -susceptible and erythromycin - resistant clinical isolates of *Streptococcus pneumonia*.

Figure (2) Distribution (%) of erythromycin -resistance phenotypes in isolates of erythromycin -resistant *Streptococcus pneumonia*. 
Figure 3: *S. pneumoniae* ery-R phenotypes obtained by the triple disk test. In each plate, the erythromycin disk (E; 15 μg) is at the center, with the clindamycin disk (DA; 2 μg) on the right and josamycin (JO; 30 μg) on the left. Penicillin (P; 10 Units) is on the bottom of the plate.

A: eMLS-constitutive resistance; B: M-M resistance; C: iMLS-inducible resistance.
REFERENCES: