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Correlation Between Erythromycin- Resistance Phenotypes of *Streptococcus Pneumoniae* and the *Invitro* Activity of Telithromycine and Azithromycine

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ABSTRACT:-

Tow 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 ,prevents the binding of the antibiotic to its ribosomal target and can be expressed either in a constitutive (cMLS phenotype) or inducible (iMLS 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 26.66% .In the group of Ery-R *Streptococcus pneumoniae* 58.33% strains belonged to cMLS phenotype, 33.33% to M phenotype and 8.33% to iMLS 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.

العلاقة بين الانماط المظهرية المقاومة للارثرومايسين لبكتريا المكورات السبحية الرئوية والفعالية خارج خلوية للتليثرومايسين والازثرومايسين

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المستخلص:-

هناك اليتان اساسيتان وجدتا انهما مسؤولتان عن المقاومة المكتسبة لمجموعة مضادات الماكروليدات الحيوية ومضادات اللنكومايسين والستربتوجرامين ب الحيوية لبكتريا المكورات السبحية الرئوية وهما: تعديل موقع الهدف ودفق المضاد الفعال . ويرجع سبب تعديل موقع الهدف الى انزيم الميثايليز الذي يمنع ارتباط المضاد الحيوي بهدفه الرايبوسومي والذي يعبر عنه اما ب الجوهري (الاساسي) او الثانوي (الفرعي) .اما في نظام دفق المضاد او النمط المظهري للماكروليد فان غشاء البروتين هوالمسؤول عن مقاومة الدفق .وعلى الرغم من حدوث المقاومة للماكروليدات كانت قليلة في الماضي الا ان حالات عديدة سجلت حاليا وبزيادة ملحوظة .لذلك وجد من الضروري اختبار ودراسة مضادات حيوية تمتاز بفعاليتها الواسعة ضد الممرضات البكتيرية التنفسية. هذه الدراسة تقارن بين الفعالية خارج الجسم الحي للتركيز الادنى المثبط والتركيز الادنى القاتل للمضادات التليثرومايسين والازثرومايسين ضد المكورات السبحية الرئوية التي عزلت من مستشفى سان جوفاني باتيسيا (تورين،ايطاليا) .ان الانماط المظهرية المقاومة للارثرومايسين حددت بطريقة اختبار الاقراص الثلاثة للمقارنة بين النماذج المختلفة من حساسية البكتريا للمضادات . ان نسبة المقاومة للارثرومايسين كانت 26.66% ، ووجد ان 58.33% يعود الى النوع الجوهري او الاساسي بينما 33.33% يعود للنوع الماكروليدي و8.33% للنوع الثانوي او الفرعي .مثلت قيم التركيز الادنى المثبط للتلثرومايسين نسب ادنى من التي سجلت للازثرومايسين ضد كل العزلات البكتيرية . ان التيليثرومايسين كان اكثر فعالية ضد بكتريا المكورات السبحية الرئوية خاصة عندما تكون المقاومة عن طريق نظام الدفق مؤكدا الفعالية السريرية ضد الاصابات التنفسية الناتجة عن المكورات السبحية الرئوية .

INTRODUCTION:-

Antimicrobial resistance has emerged as a major problem in *Streptococcus pneumoniae* ,increased resistance to macrolide in *Streptococcus pneumoniae* has been described world wide ,Mediterranean countries have the highest rates of Erythromycin –resistant pneumococci [1]. Macrolide resistance in pneumococci is mainly mediated by tow 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 (cMLSB phenotype) or inducible (iMLSB 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 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 invitro 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) ,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 – resistance phenotype: Erythromycin – resistance phenotype was determined by the triple –disk test described by Giovanetti *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 suspention with a turbidity equivalent to that of a 0.5 Mcfarland standard .After 18 h of inoculation 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 as 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≥ 64µg / 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 for 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 MIC, 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%) has 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- memberd and 15-memberd macrolides from the bacterial cell(10). By using the triple –disk test we showed that 58.33% of Ery-r strains belonged to cMLS phenptype, 33.33% were resistant to macrolides by the activation of an efflux pump (M phenotype) and 8.33% belonged an i MLS phenotype. Telithromycin ,the first member of ketolides ,has a good spectrum of activity against respiratory pathogens as well as a high bactericidal activity [6] .In this study ,the *invitro* 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 >16µg / ml ,and the MICs for M phenotype strains were 0.5-4 µg / ml [2] .,while Kaieda 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 0.5->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 out patients .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.

Table (1) MICs of erythromycin-susceptible and erythromycin- resistant *S.pneumoniae* isolates to Telithromycin and azithromycin .

S. pneumoniae n= 45		Drug	Number of strains having MIC(µg /ml) equal to:														
			≥64	32	16	8	4	2	1	0.5	0.25	0.12	0.06	0.03	0.15	0.007	≤0.003
Ery-S n=33		TEL											3	5	4	4	17
		AZM						4	4	2	3	5	3	2	3	7	
Ery-R n=12	cMLS n=7	TEL	4	2	1												
		AZM	7														
	M n=4	TEL						2	2								
		AZM		1	3												
	iMLS n=1	TEL										1					
		AZM	1														

Ery S = erythromycin –susceptible ,Ery – R=erythromycin –resistant cMLS= constitutive resistance; M= M resistance;iMLS= inducible resistance
TEL = telithromycin AZM=azithromycin

Table (2) MBCs of erythromycin-susceptible and erythromycin- resistant *S.pneumoniae* isolates to Telithromycin and azithromycin .

S. pneumoniae n= 45		Drug	Number of strains having MBC(µg /ml) equal to:														
			≥64	32	16	8	4	2	1	0.5	0.25	0.12	0.06	0.03	0.15	0.007	≤0.003
Ery-S n=33		TEL						2	5	4	6	3	3	10			
		AZM				10	5	7	3	5	3						
Ery-R n=12	cMLS n=7	TEL	4	3													
		AZM	7														
	M n=4	TEL				3	1										
		AZM	2	1	1												
	iMLS n=1	TEL								1							
		AZM	1														

Ery S = erythromycin –susceptible ,Ery – R=erythromycin –resistant cMLS= constitutive resistance; M= M resistance;iMLS= inducible resistance
TEL = telithromycin AZM = azithromycin

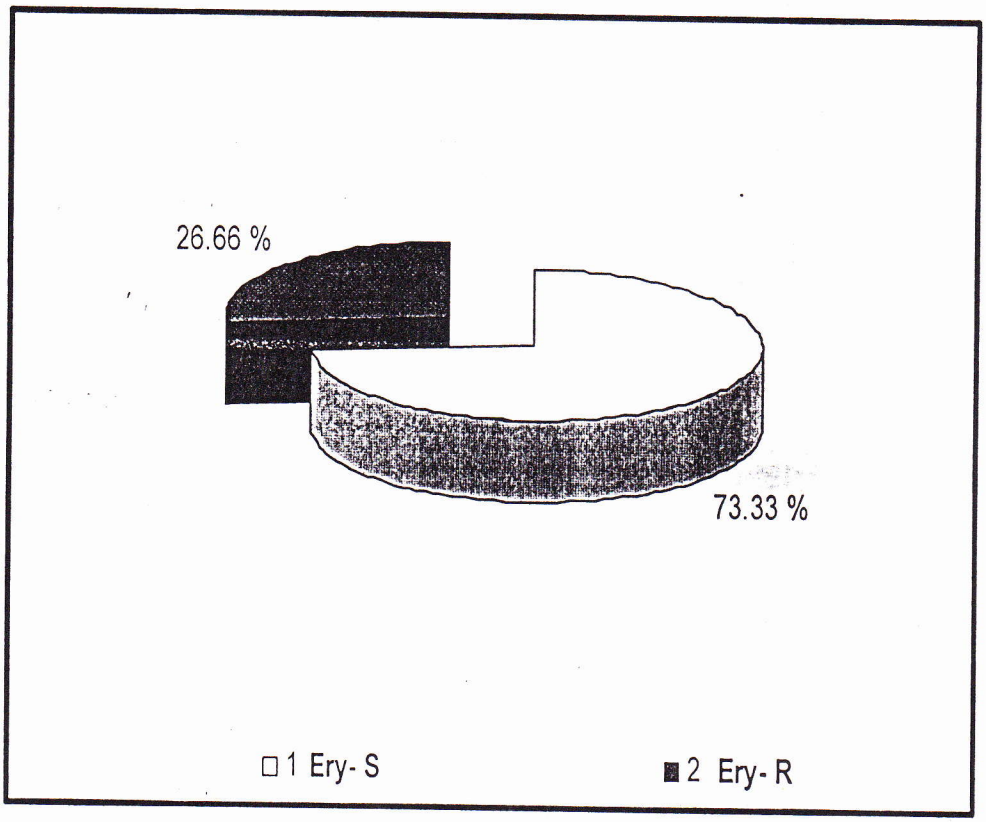

Figure (1) Percentage of erythromycin –susceptible and erythromycin – resistant clinical isolates of *Streptococcus pneumonia.*

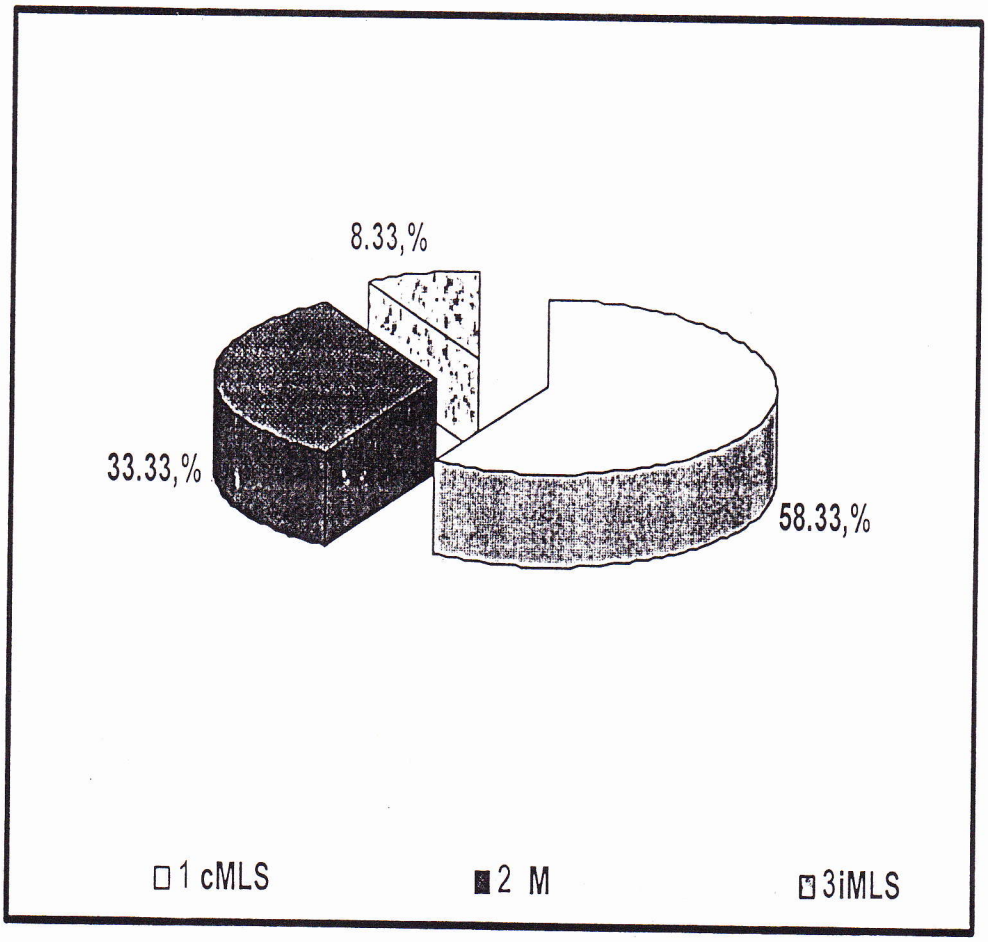

Figure (2) Distribution (%) of erythromycin –resistance phenotypes in isolates of erythromycin –resistant *Streptococcus pneumonia.*

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:2µg) on the right and josamycin(JOS:30µg) on the left;penicillin (P:10 Units) is on the bottom of the plate.

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

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