

# Macrolide Resistance Mechanisms in Serotypes of *Streptococcus pneumoniae*

#P996

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## Revised Abstract

**Background:** *Streptococcus pneumoniae* is an important pathogen that causes severe life-threatening illnesses in the elderly and children. Increases in macrolide resistance in *S. pneumoniae* clinical isolates have significant clinical implications. The most common mechanisms of macrolide resistance are methylation of the ribosomal target site (encoded by the *ermB* gene) and drug efflux (encoded by the *mefA* or the *mefE* gene). The aim of this study was to characterize these mechanisms in various serotypes of *S. pneumoniae* in order to understand the relationship between macrolide resistance and capsular serotypes. **Methods:** 465 macrolide-resistant (erythromycin MIC  $\geq 1$  mg/L) clinical *S. pneumoniae* isolates, collected through the Tigecycline Evaluation and Surveillance Trial (T.E.S.T.), were evaluated. Detection of genes involved in macrolide resistance (*ermB*, *mefA* and *mefE*) and serotyping were performed by multiplex-PCR. **Results:** 54% of the isolates tested were from North America, 27% from Europe with the remainder (19%) from other continents. Among these 465 strains, the most prevalent pneumococcal macrolide resistance genotypes were *mefE/A* (42%) and *ermB* (37%) followed by *ermB+mefE* (18%) and then other mechanisms (3%). The most prevalent serogroup was 19 (34%) and 6 (17%).

Table 1: Oligonucleotide primers used in this study.

Serotype	Macrolide resistance mechanisms (number of strains)				Total
	<i>ermB</i>	<i>mefE/A</i>	<i>ermB+mefE</i>	other	
19A/F	36	48	71	2	157
6A/B	32	46	1	1	80
Other	105	99	13	11	228
	173	193	85	14	465

Among *ermB+mefE*-positive isolates, 84% were serogroup 19 while only 21% were serogroup 19 among *ermB*-positive isolates. Among *mefE/A*-positive isolates, only 25% were serogroup 19 and the remaining were other serotype. High-level resistance (MLS<sub>B</sub> phenotype) was associated with serogroup 19 while lower-level resistance (M phenotype) was associated with other serotypes. **Conclusions:** This study confirms that certain capsular serotypes are associated with macrolide resistance and confirms also the predominance of high-level macrolide resistance among serogroup 19. These findings emphasize the need for continuous worldwide monitoring of macrolide-resistance and serotypes among *S. pneumoniae*.

## Introduction

- Streptococcus pneumoniae* is a key pathogen of community-acquired respiratory tract infections (RTIs).
- It has been shown that infection with macrolide resistant pneumococci is a notable risk factor for failure of macrolide therapy in RTIs (1, 4).
- Pneumococcal macrolide resistance is usually mediated via one or two major mechanisms (2):
  - Modification of ribosomal macrolide target sites by methylases encoded by the *ermB* gene. This mechanism is globally the **most common** and confers a **high-level of resistance** (MIC<sub>90</sub> values of  $\geq 64$  mg/L) (6).
  - Drug efflux encoded by the *mefA* or *mefE* genes. This mechanism confers a **lower level of resistance** (MIC<sub>90</sub> values of 4 to 8 mg/L).
- Resistance to macrolides in *S. pneumoniae* has increased dramatically during the last decade (5, 10).
- Determining the epidemiology of resistance mechanisms in various serotypes of *S. pneumoniae* may be important to understand the **relationship between macrolide resistance and capsular serotypes**.

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## Materials & Methods

### Bacterial isolates

⇒ **465 macrolide-resistant (Erythromycin MICs  $\geq 1$  mg/L) *S. pneumoniae* clinical isolates** from 2004-2008 collected through the Tigecycline Evaluation and Surveillance Trial (T.E.S.T.) were tested

⇒ **4 control strains for macrolide resistance** were used in this study:

- *ermB*-positive isolate
- *mefE*-positive isolate
- *mefA*-positive isolate
- *ermB+mefE* double positive isolate

⇒ **28 control strains**, representing the serotypes and serogroups targeted by multiplex-PCR, were used in this study.

### Regions/Countries:

- \* North America: 54.4%
- \* Europe: 26.9%
- \* Latin America: 7.9%
- \* Asia: 7.3%
- \* Africa: 1.3%
- \* Middle East and South Pacific: 1.1%

### Site Location

- \* Respiratory: 58.5%
- \* Blood: 20.6%
- \* HEENT: 14.6%
- \* Fluids: 4.3%
- \* Other: 2%

### Departments

- \* Medicine: General (34.6%) and ICU (8.3%)
- \* Clinic Office: 14.2%
- \* Emergency Room: 12%
- \* Pediatric: General (8.8%) and ICU (3.2%)
- \* Surgery: General (6.8%) and ICU (2.8%)
- \* Nursing Home/rehab: 0.8%
- \* None Given: 8.5%

### Multiplex-PCR

\* DNA extraction (Fast boiling)

- Subculture on blood agar plates (Tryptic Soy Agar base supplement with 5% sheep blood)
- Incubation overnight at 37°C in 5% CO<sub>2</sub>
- Bacterial suspension in TE buffer (McFarland 1)
- Suspension heated for 5 min at 95°C and immediately frozen at -20°C at least for 5 min.

\* Oligonucleotide primers

### MACROLIDE RESISTANCE

The 5 oligonucleotides (8) used for this study were designed to target the three genes involved in macrolide resistance: *ermB*, *mefE* and *mefA*

Table 1: Oligonucleotide primers used in this study

Target gene	Primers	Sequences (5' to 3')	Product size (bp)	Reference
<i>ermB</i>	EB1 (forward)	GAAAAAGTACTCAACCAATA	639	Monaco et al, 2005, JAC
	EB2 (reverse)	AGTAATGGTACTTAAATTTTAC		
<i>mefE/A</i>	OM10 (forward)	AGCATTGGAACAGCTTTCA	318	
<i>mefA</i>	MefA (reverse)	ATTTGCCGTAGTACAGCC	519	
<i>mefE</i>	MefE (reverse)	TACATGCTTTTCAAGCC		
<i>cpsA</i>	<i>cpsA-f</i>	GCAGTACAGCAGTTTGGTACTGACC	160	Pai et al, 2006, JCM
	<i>cpsA-r</i>	GAATATTTTCATTACAGTCCAGTC		

### SEROTYPING

The 28 oligonucleotide pairs (9) used for this study were designed to target the following serotypes:

**1, 3, 4, 6A/B, 7C/B, 7F/A, 8, 9V/A, 10A, 11A/D, 12F/A, 14, 15A, 15B/C, 16F, 17F, 18ABCF, 19A, 19F, 20, 22F/A, 23F, 31, 33F/A, 34, 35B, 35F, 38.**

A primer pair (*cpsA-f/cpsA-r*) was also included as an internal control targeting the *cpsA* locus found in all pneumococci (7)

\* Multiplex PCRs

- QIAGEN Multiplex PCR kit (15  $\mu$ L-volume reaction)

Components	Description of components	Final Concentration
2X QIAGEN Multiplex PCR Master Mix (6 mM MgCl <sub>2</sub> )	-Hot Start Taq <sup>®</sup> DNA Polymerase -Multiplex PCR buffer -dNTP Mix	1X (3 mM MgCl <sub>2</sub> )
10X Primer Mix (2 $\mu$ M each primer)	-Primers Mix (5 primers + cps)	0.2 $\mu$ M
RNase-free water	-	-
Template DNA	-bacterial suspension	-

### MACROLIDE RESISTANCE

- 1 reaction
- 50°C
- 30 cycles

### SEROTYPING

- 5 reactions
- 55°C
- 30 cycles

## References

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## Results

Figure 1: Global prevalence of macrolide resistance mechanisms among 465 *S. pneumoniae* isolates.

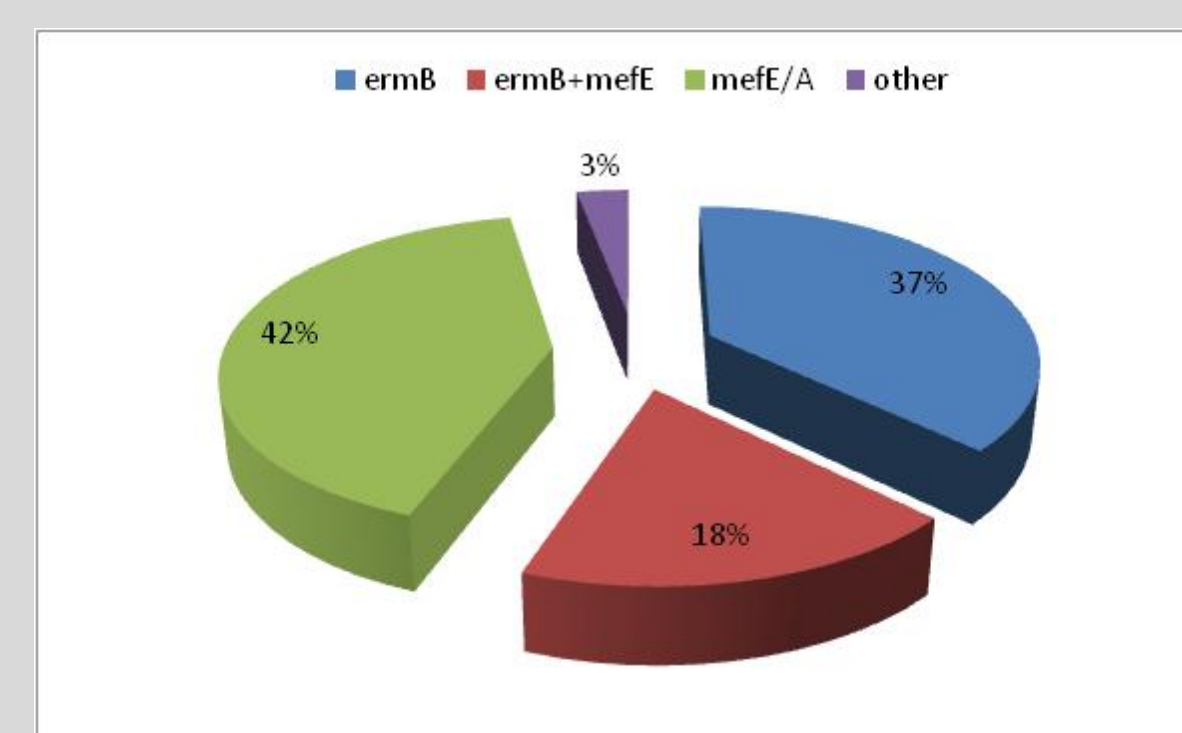


Figure 2: Distribution of the most common serotypes among 465 *S. pneumoniae* isolates.

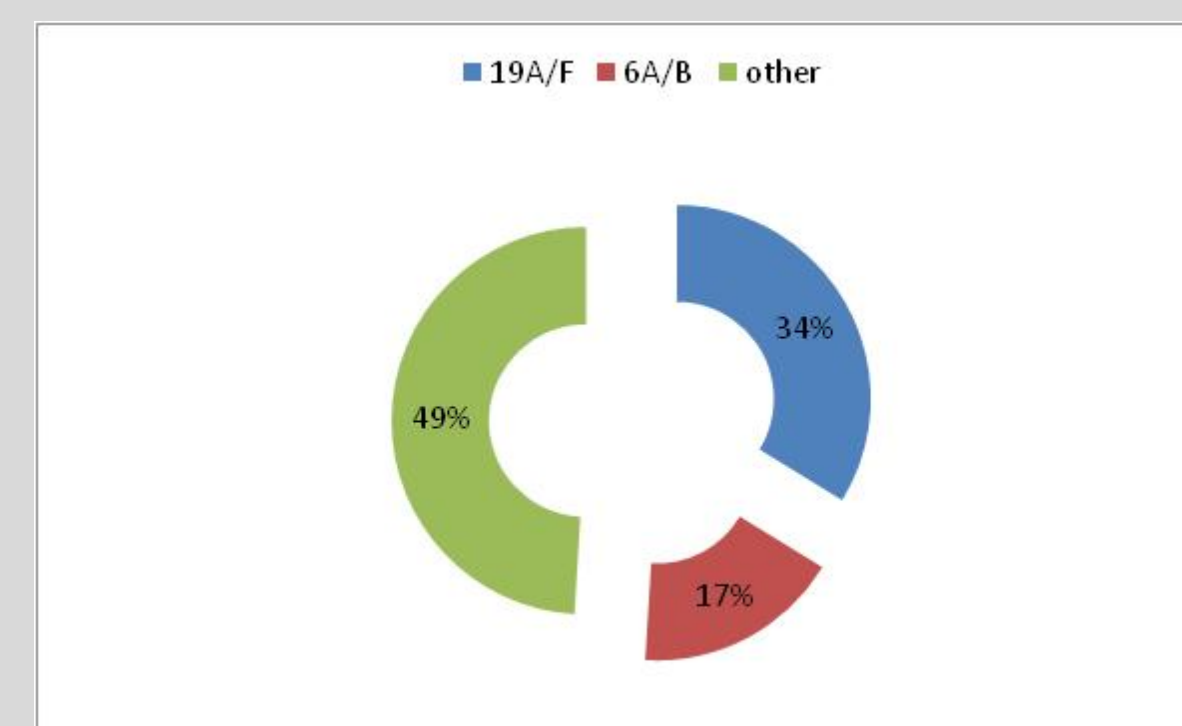


Figure 3: Geographical differences in macrolide resistance among serogroup 19.

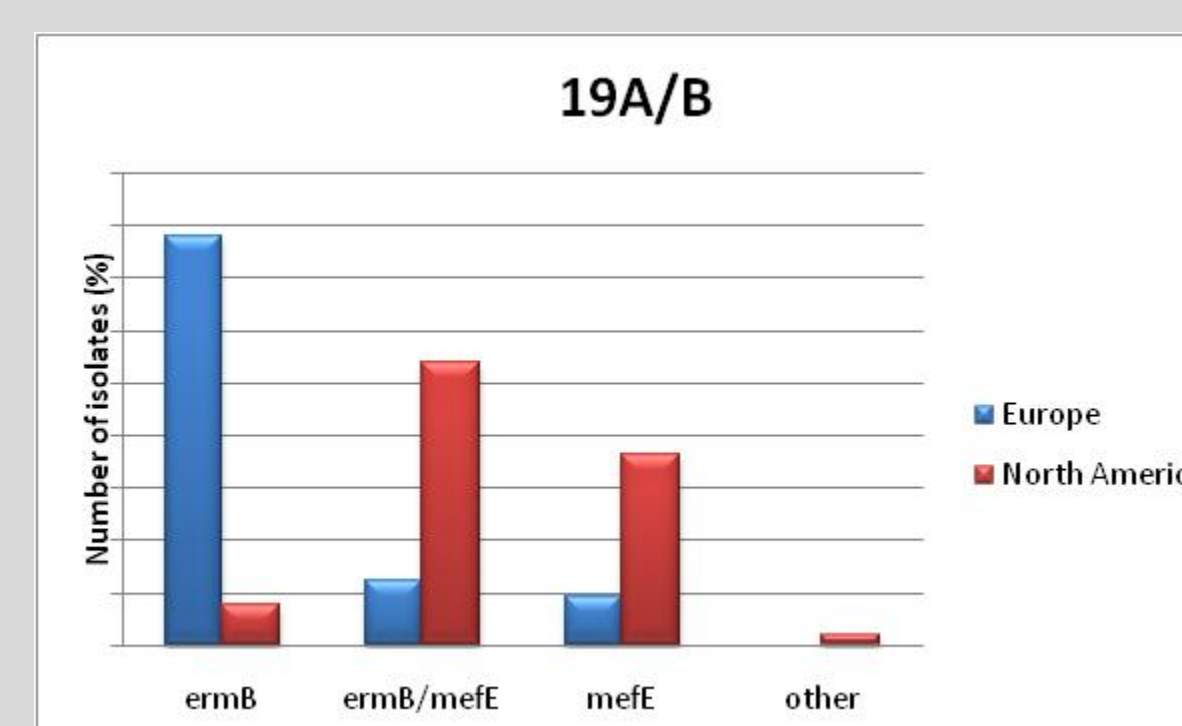


Figure 4: Distribution of the most common serotypes among *ermB+mefE*-positive isolates.

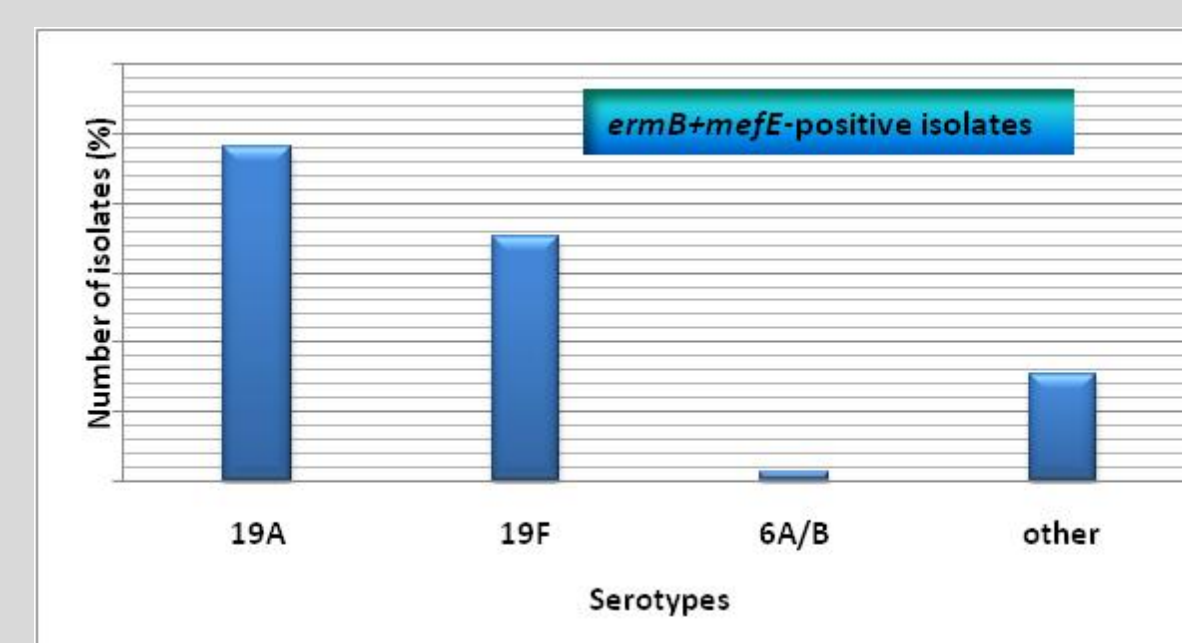


Figure 5: Distribution of the most common serotypes among *mefE/A*-positive isolates.

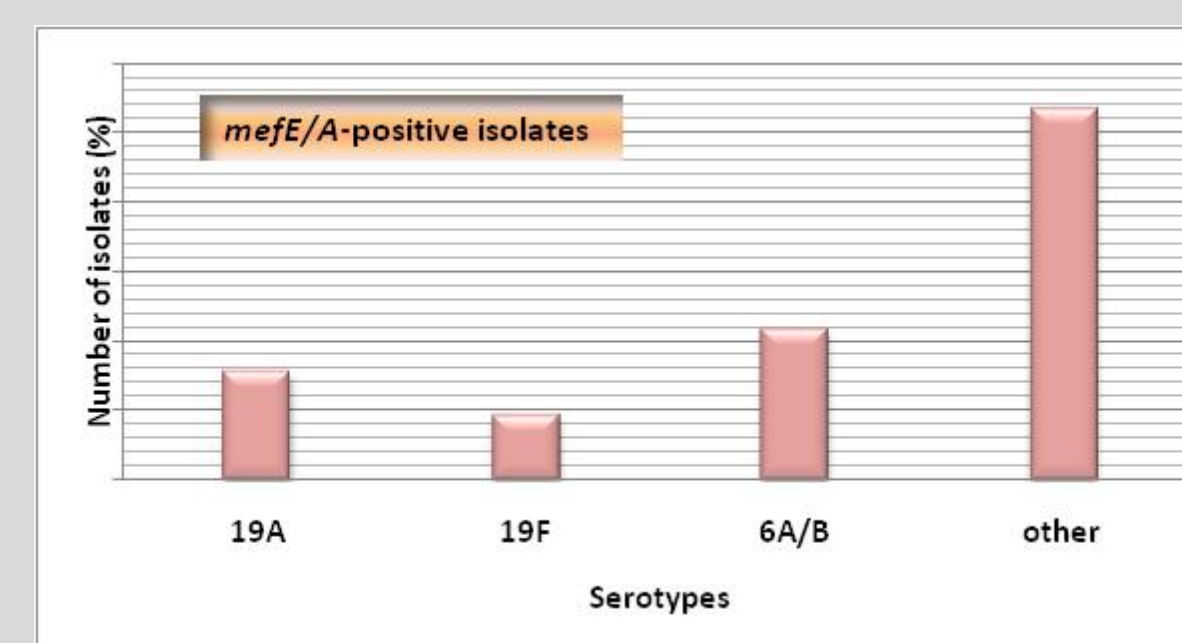


Figure 6: Distribution of Erythromycin MICs among 19A/F isolates.

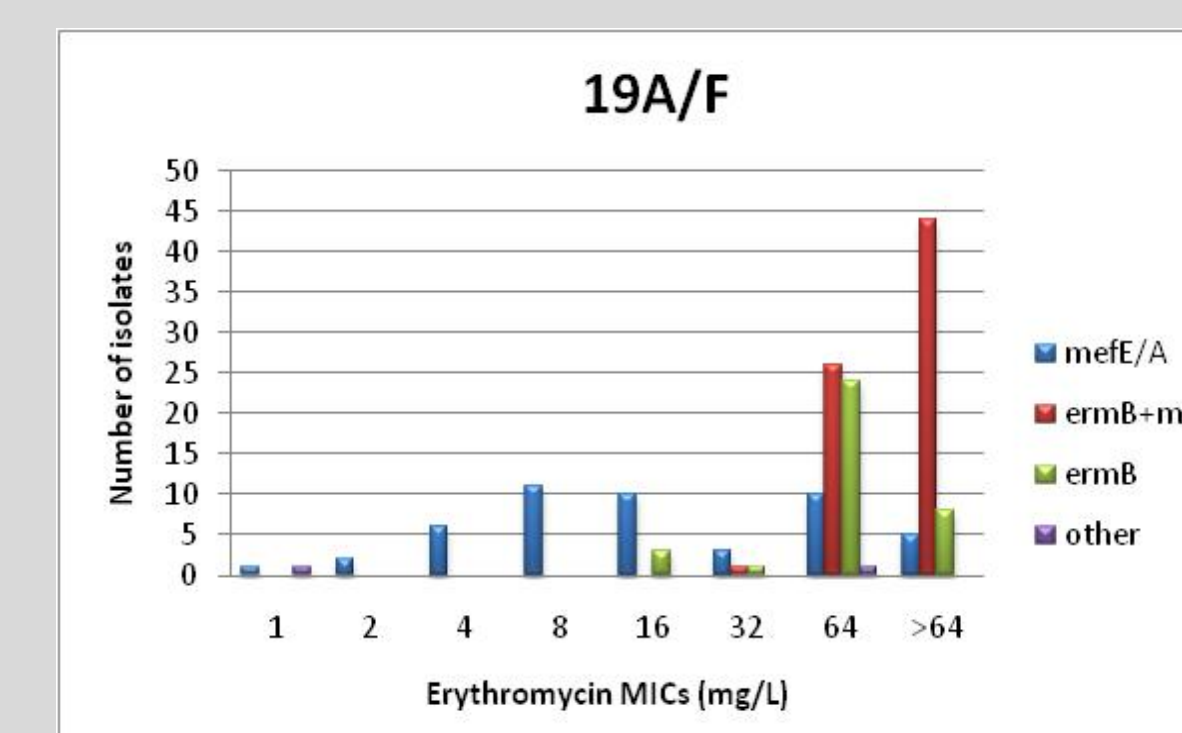
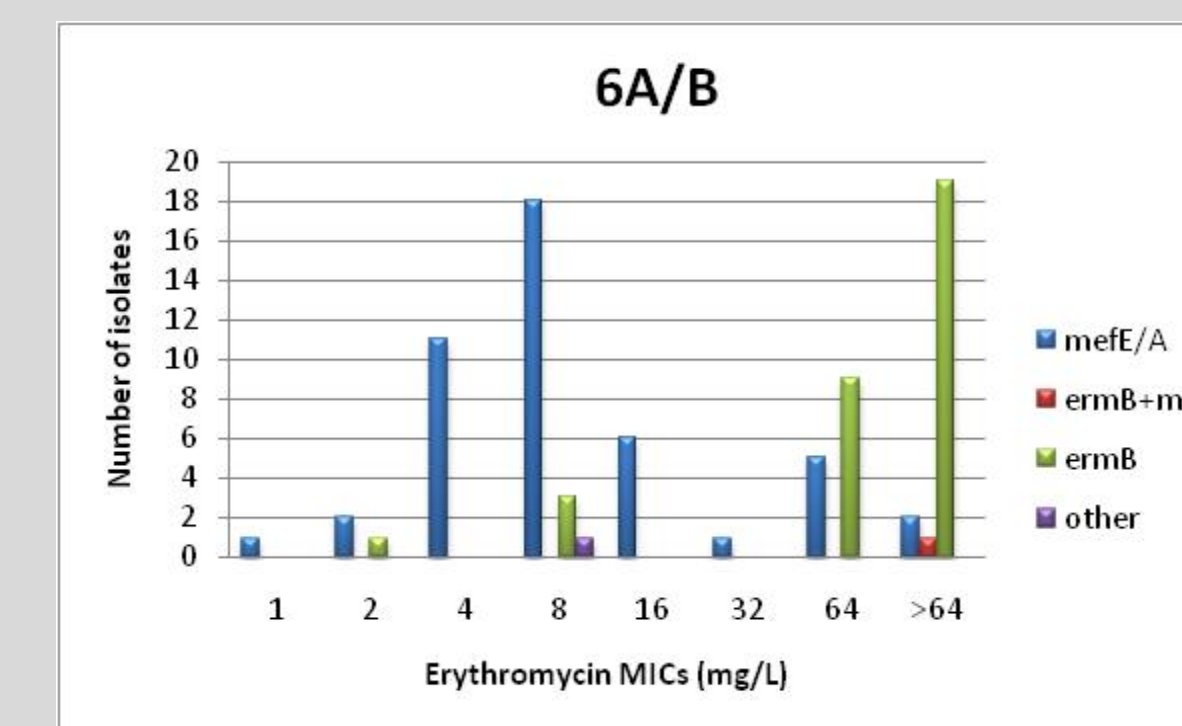


Figure 7: Distribution of Erythromycin MICs among 6A/B isolates.



## Conclusions

- In this study, lower-level efflux *mefE/A*-mediated resistance is as frequent as high-level *ermB*-mediated resistance, but it is important to highlight the prevalence of *ermB+mefE*-positive isolates.
- The **most prevalent serotypes** among these macrolide-resistant isolates were:
  - 19A/F (33.8%)
  - 6A/B (17.2%)
- Serogroup 19 is associated with *ermB* in Europe but with *ermB+mefE* and also *mefE* in North America
- A high proportion of *ermB+mefE* isolates are expressing **serogroup 19**:
  - 19A (48.2%)
  - 19F (35.3%) while *mefE/A* isolates largely express serotypes other than serogroup 19.
 Serogroup 19 is highly related to *ermB+mefE* genes as has already been described (3).
- The **serogroup 19A/F is clearly associated with high-level erythromycin resistance (*ermB+mefE*)** while the serogroup 6A/B can be associated with *ermB* but also with *mefE/A*.
- Continued **monitoring of macrolide resistance and serotypes** is crucial.