Antagonism Between Penicillin and Erythromycin Against *Streptococcus pneumoniae*: Does It Exist?

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ABSTRACT

Background: Penicillin (PEN) or erythromycin (ERY) are commonly used for the treatment of *S. pneumoniae* (SPN) infections and combined as empiric therapy of community-acquired pneumonia. A concern about potential ANTAG between these drugs was raised in a recent report (JAC 2000; 46:973). In response to these findings, a protocol was designed to test the hypothesis in a similar timed kill-curve experiment, with 2 interpretive criteria options.
Methods: 4 clinical isolates of SPN from the US, referred to the SENTRY Program and 1 QC strain (ATCC 49619) were tested. MIC and MBC results were determined using standardized dilution methods (NCCLS). PEN and ERY were tested at clinically relevant concentrations of 10 and 1 μg/ml, respectively (alone and in combination) by timed kill-curve method. As with the earlier cited study, PEN MICs ranged from ≤0.03 - 0.5 μg/ml. ERY results were either susceptible (MIC, ≤ 0.25 μg/ml) or an inducible R phenotype (MIC, > 32 μg/ml). One of the clinical isolates (024-373B) was also tested after induction (ind) with ERY (4 μg/ml). Interpretations were calculated comparing the PEN + ERY effect versus PEN (P) or ERY (E) killing rates alone, where > 0.5 $\log_{10}$ cfu/ml difference was significant (SYN = synergy; INDIF = indifference).
Results:

Organism no.	MIC in μg/ml (category) PEN	ERY	PEN MBC	Drug interactions vs P/E
11-17B	≤0.03 (S)	0.25 (S)	≤0.03	SYN/ANTAG
4-8B	0.06 (S)	≤0.25 (S)	0.12	SYN/ANTAG
ATCC 49619	0.25 (I)	0.25 (S)	0.25	SYN/ANTAG
24-373B	0.25 (I)	>32 (R)	1	ANTAG/INDIF
				ANTAG/SYN (ind)
17-92B	0.5 (I)	≤0.25 (S)	1	SYN/INDIF

Conclusions: There was a consistent bactericidal activity against SPN by each drug alone and combined over the monitored 5 hour period, except for the ERY-R isolate ind by ERY. Drug interactions ranged from SYN to ANTAG, depending on the criteria applied. Practical risks of the macrolide-penicillin combination appeared to be very minimal and was synergy criteria dependent.

INTRODUCTION

Combination therapy with antimicrobials belonging to two different classes has been used to treat infections for decades, with the goal of achieving synergistic effects or producing wider spectrums of coverage. Combination therapy also serves to slow resistant mutations. Penicillin or ß-lactams are the cornerstone of many regimens as they are often combined with other agents with different mechanisms of action such as aminoglycosides, tetracyclines, chloramphenicol, macrolides or quinolones.

ß-lactam and macrolide combination therapy has often been used for empiric treatment of lower respiratory tract infections. This practice maximizes coverage of the three major respiratory bacterial pathogens (*Streptococcus pneumoniae*, *Haemophilus influenzae* and *Moraxella catarrhalis*) as well as atypical pathogens such as *Legionella* spp, *Chlamydia* spp. and *Mycoplasma* spp. Herrell et al (1960), described the favorable use of a formulation called “erythrocillin” which was a mixture of equal amounts of penicillin and erythromycin against resistant strains of *Staphylococcus aureus*. ß-lactams and erythromycin combinations have been widely studied and many different interaction results have been described. Recently a concern about potential antagonism between the two drugs against *S. pneumoniae* was raised by Johansen et al. The present study was undertaken to verify the significance of antagonism in vitro and assess the risk of this decades old combination of antimicrobial classes.

MATERIALS & METHODS

The study protocol followed the one previously described by Johansen et al, in 2000. Four recent clinical isolates and one quality control strain (ATCC 49619) of *S. pneumoniae* were tested. The clinical isolates were from patients with respiratory tract infections, referred to the SENTRY Antimicrobial Surveillance Program monitor in 2000. Penicillin and erythromycin were purchased from Sigma Chemical Co. (St. Louis, Missouri) and Trek Diagnostics Inc. (Westlake, Ohio) supplied MH broth supplemented with 5% lysed horse blood. MICs were determined by the reference broth microdilution method of the NCCLS using commercial panels (Trek Diagnostics). Minimum bactericidal concentration (MBC) for penicillin was determined using subcultures from MIC panels. Contents of broth microdilution test wells were plated on Mueller Hinton (MH) agar plates containing 5% sheep blood. The lowest concentration of penicillin that reduced the initial innoculum by ≥ 99.9% was considered as the MBC.

The timed kill-curves were performed as described in the ASM Clinical Microbiology Procedures' Handbook (1992). The isolates were exposed to clinically relevant concentrations of penicillin (10 μg/ml) and erythromycin (1 μg/ml) alone and in combination as described by Johanson et al. MH broth supplemented with 5% lysed horse blood was used as growth medium. Tubes were incubated at 35ºC in 5% CO_2 for 5 hours. Samples (100 ml) were removed at 0 (T0), 1 (T1), 3 (T3), and 5 (T5) hours of incubation and plated after appropriate dilutions. Colony counts were determined after 24 hour incubation at 35ºC. One clinical isolate (024-373B) was tested with and without induction with erythromycin (4 μg/ml). The inhibitory effect of the drugs was considered synergy or antagonism if there was ≥ 0.5 Log_{10} CFU/ml difference (decrease = synergy, increase = antagonism) between the combination of drugs versus each of the drugs when tested alone. Alternative more strict definitions of ≥ 1 $\log_{10}$ and ≥ 2 $\log_{10}$ CFU/ml differences were also applied.

Table 1. Antimicrobial activity (MIC and MBC) for penicillin (PEN) and erythromycin (ERY) tested against five *S. pneumoniae* including antimicrobial interaction categories.[a]

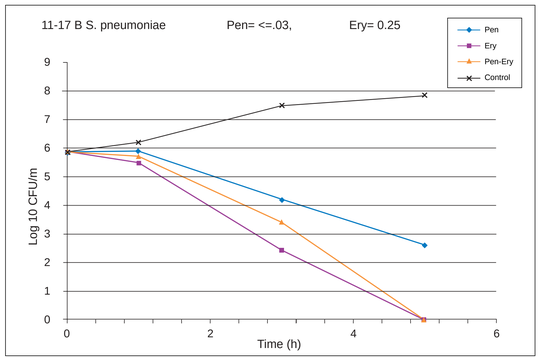
Organism	MIC in μg/ml (susceptibility)[b] PEN	ERY	PEN MBC	Interaction category for PEN/ERY using:[c] ≥ 0.5	≥ 1	≥ 2
11-17B	≤0.03 (S)	0.25 (S)	≤0.03	SYN/IND	SYN/IND	SYN/IND
4-8B	0.06 (S)	≤0.25 (S)	0.12	SYN/ANT	SYN/IND	IND/IND
ATCC 41619	0.25 (I)	0.25 (S)	0.25	SYN/ANT	SYN/ANT	SYN/IND
24-373B	0.25 (I)	>32 (R)	1	ANT/IND	IND/IND	IND/IND
Induced	-	-	-	ANT/SYN	IND/SYN	IND/SYN
17-92B	0.5 (I)	≤0.25 (S)	1	SYN/IND	SYN/IND	IND/IND

a. Interactions were calculated by comparing the killing effect of PEN + ERY with that of PEN or ERY tested alone. Three (3) levels of killing were used to interpret significant drug interactions (synergy or antagonism) at ≥ 0.5 [Johansen et al., 2000], ≥ 1 and ≥ 2 CFU/ml differences between the combination and PEN or ERY.
b. NCCLS [2002] interpretive category, S = susceptible, I = intermediate, and R = resistant.
c. SYN = synergy, IND = indifference, and ANT = antagonism.

RESULTS

- Results of MIC and MBC (penicillin) experiments are summarized in Table 1. Using NCCLS breakpoints, two *S. pneumoniae* isolates were categorized as penicillin-susceptible and three as penicillin-intermediate. Only one isolate (24-373 B) was resistant to erythromycin.
- Penicillin MBC values approximated very closely the MIC values.
- Penicillin exhibited inhibitory activity against all the strains tested which qualitatively correlated to the level of susceptibility of each isolate.
- Erythromycin inhibited all the isolates (not 24-373B, induced) in the kill-curve experiments.
- A wide variation in drug interaction categories was observed depending upon the definitions applied. When comparing the combination kill-curve results to penicillin tested alone, synergistic results were most often encountered (four occurrences using ≥ 0.5 $\log_{10}$ CFU/ml criteria) as well as antagonism (two occurrences). Antagonism was noted for the strain having macrolide resistance when tested with or without induction.
- As the definitions of synergy or antagonism became more strict, antagonism occurrences when compared to penicillin activity alone were less frequent (only one occurrence).
- For interaction interpretations using erythromycin tested alone as the benchmark, antagonism was noted only for the NCCLS control organism and strain 4-8B; this interaction was indifferent as the criteria became more rigorous.

Figure 1: Example timed kill curves for *S.pneumoniae* isolate 11-17B. Penicillin was tested at 10ug/ml, erythromycin at 1 ug/ml and same concentrations were used in the experiment with combination. The combination interpretations compared to penicillin or erythromycin activity tested alone varied from synergy to indifference.



CONCLUSIONS

- The general conclusion of this investigation indicates that variable drug interactions can be observed between penicillin and erythromycin over the initial five hours of a kill-curve experiment.
- Subtle differences between kill-curves and use of varying definitions of synergy or antagonism can markedly change the conclusions of the experiment and resulting clinical implications.
- Overall antagonism was infrequently observed in our experiments and was only associated with the liberal interaction definition of ≥ 0.5 $\log_2$ CFU/ml.
- The composite conclusions of all experiments and all definitions was the clear trend toward indifferent or less commonly "synergistic killing" of *S. pneumoniae* by penicillin and erythromycin.
- With these findings, the clinical risks of poor clinical responses for a ß-lactam with a macrolide seems to be very minimal and further proven by the history of thousands, if not millions, of successfully treated patients. The most recent evidence was published by Martinez et al. (2003) where the analysis of 409 bacteremic cases of pneumococcal pneumonia showed superior clinical results (lower mortality) for ß-lactam plus macrolide therapy.

SELECTED REFERENCES

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