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## The inactivation of chloramphenicol and thiamphenicol by anaerobic *Bacteroides fragilis*

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นราทร ธรรมบุตร, กัญชลี เลิศโกยะสมบัติ, สุดาลักษณ์ จันทรัชดา. ผลกระทบ  
ต่อฤทธิ์ของยาต้านจุลินทรีย์ คลอแรมเฟนิคอล และไทแอมเฟนิคอล โดย  
แอนแอโรบิก แบคทีเรียคลัส ฟราจีลิส. จุฬาลงกรณ์เวชสาร 2527 พฤศจิกายน ;  
28 (11) : 1285-1293

แอนแอโรบิก แบคทีเรียคลัส ฟราจีลิส ทำลายฤทธิ์ของยาต้านจุลินทรีย์ ไทแอม  
เฟนิคอล น้อยกว่าคลอแรมเฟนิคอล การศึกษานี้เห็นได้ชัดว่า กลไกการยับยั้ง  
ทั้ง 2 ถูกทำลายโดยแอนแอโรบัสต์ต่างกัน ทั้ง ๆ ที่ไทแอมเฟนิคอลเป็นอนุพันธ์ของ  
คลอแรมเฟนิคอล

อนึ่ง การศึกษานี้ชี้ให้เห็นว่า ในปัจจุบัน ไทแอมเฟนิคอลเหมาะสมที่จะนำมา  
ใช้รักษาโรคติดเชื้อที่ก่อโดยแอนแอโรบัสต์ที่มีเอ็นซัยม์ เบต้า แล็กตาเมส ทั้ง ๆ ที่  
ไทแอมเฟนิคอลเป็นอนุพันธ์ของคลอแรมเฟนิคอล

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Antibiotics are an important means of managing infections disease, and the suitable choice of which is crucial. In serious infections, the combination of penicillin and an aminoglycoside is often considered but not in the cases of *Bacteroides* or *Chlamydia*. While some anaerobes are sensitive to chloramphenicol, clindamycin and high doses of penicillin, the *chlamydia* and *bacteroides* are more sensitive to thiamphenicol.<sup>(1,2,3)</sup>

Thiamphenicol is a broad-spectrum antibiotic and an analog of chloramphenicol with the substitution of the *p*-nitro-group by a methyl sulfonyl moiety.<sup>(4,5,6)</sup> Its chemical formula is D (=)-threo-2 (*p*-methyl sulfonylphenyl)-dichloroaceto-amino-1, 3-propanediol. Its mechanism of antibacterial action is probably similar to chloramphenicol's which lies in the inhibition of protein synthesis within the bacterial cells.<sup>(7)</sup>

The present paper reports our Comparative Bioassay Study on the inactivation of thiamphenicol and chloramphenicol by *Bacteroides fragilis* from November 1981–May 1982.

## Materials

### *a - Bacterial indices*

a.1 The chosen strain of thiamphenicol – chloramphenicol resistant – *Bacteroides fragilis* was a gift from Dr. Kenneth Goodner, Department of Microbiology, Jefferson Medical College.

Two thiamphenicol – chloramphenicol sensitive strains were isolated from our patients.

a.2 The specific strain of *Aeromonas hydrophila* was supplied by the Aerobic unit, Department of Medical Microbiology, Chulalongkorn Hospital Medical School.

### *b - The synthetic drugs*

The pure thiamphenicol (T P) used was lot. No. 80836/846, with the potency of 1015 mcg./mg. and the expired date of October, 1986. The pure chloramphenicol (C P) used was lot No. 53124 with the potency of 996 mcg./mg., and the expired date of August 1986.

### *c - Equipments*

Equipments at the Anaerobic unit, Department of Medical Microbiology\* included an anaerobic incubator, chamber CO<sub>2</sub> cabinet, jar with the Oxoid gas generator kit with other accessories capable of various biochemical tests according to qualified procedures.<sup>(8,9,10)</sup> The specific *Bacteroides fragilis* strains were therefore easily identified and survived longer at -42°C.<sup>(10,11)</sup>

## Methods<sup>(12,13,13a)</sup>

1. The pure cultures of three *Bacteroides fragilis* strains were anaerobically incubated and grown in fresh thioglycollate broths overnight.

2. With the Mc Farland's turbidity 1.0 standard, the overnight broths were diluted to  $2 \times 10^9$  CFU\*/ml. by fresh sterile trypticase soy broth.

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\* C F U/ml. = Colony forming unit/ml.

3. The diluted broths were then incubated at 37° C for 4 hours.

4. In the mean time (within 4 hours), 2 × 5 ml. of 100 mcg./ml. of each of the chloramphenicol (CP) and thiamphenicol\*\* (TP) were prepared by dilution with sterile, distilled water (deminerized water).

5. Fresh sterile trypticase soy blood agars with the "well" in the blood media were prepared as described by Nara-thorn et al.<sup>(18,18a)</sup>

6. The standard inhibition zones (diameters) (SIZ) of chloramphenicol and thiamphenicol against aerobic *Aeromonas hydrophila* in the wells were performed and measured (control).

Next, each antibiotic was inactivated with the *Bact. fragilis* broths (No. 3) and anaerobically incubated for 3 or 6 hours before their residual activities were determined by bioassay with the *A. hydrophila* aerobically. After a re-incubation, the inhibition zones were measured and compared with the standard zones.

### Results

1. The given strain of resistant-CP-TP-*Bact. fragilis* and 2-local sensitive-CP and TP-*Bact. fragilis* strains were used.

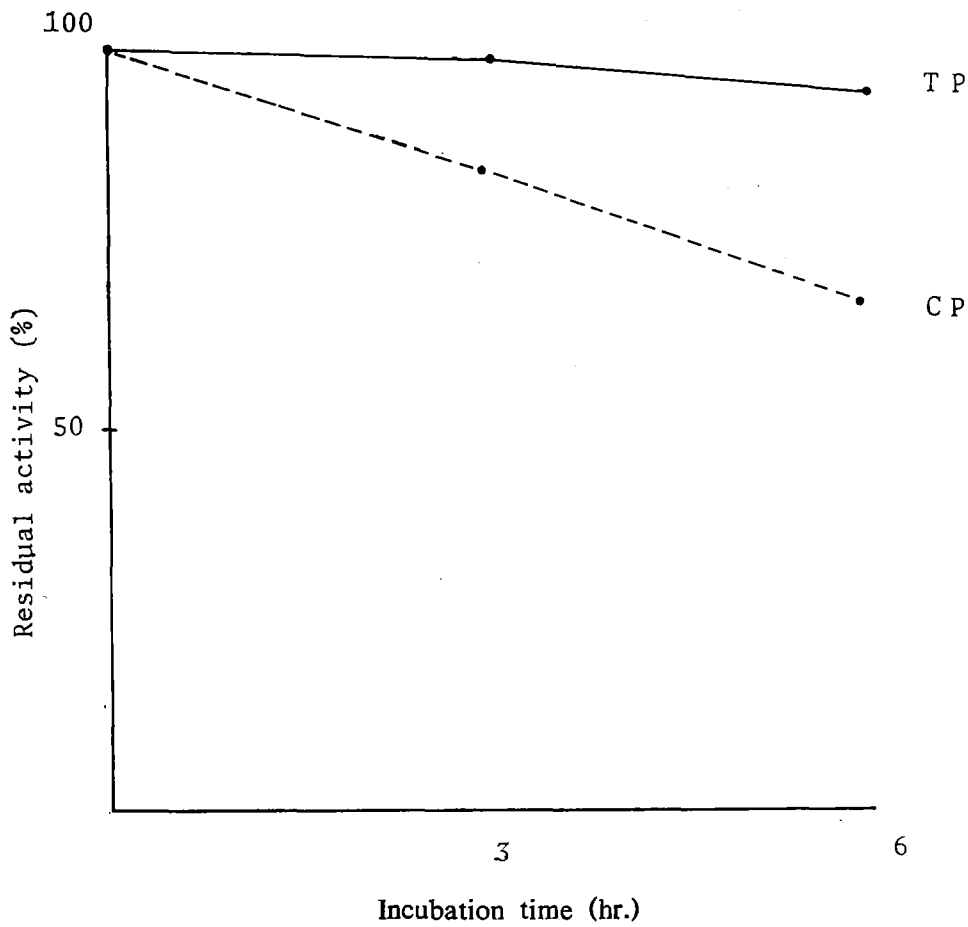
2. The SIZ for CP is 26 mm., while the SIZ for TP is 24 mm. in diameters.<sup>(12,18,18a)</sup>

**Table 1** Showing the residual activities (after inactivation) of chloramphenicol (CP) and Thiamphenicol (TP) by bioassay with *A. hydrophila*.

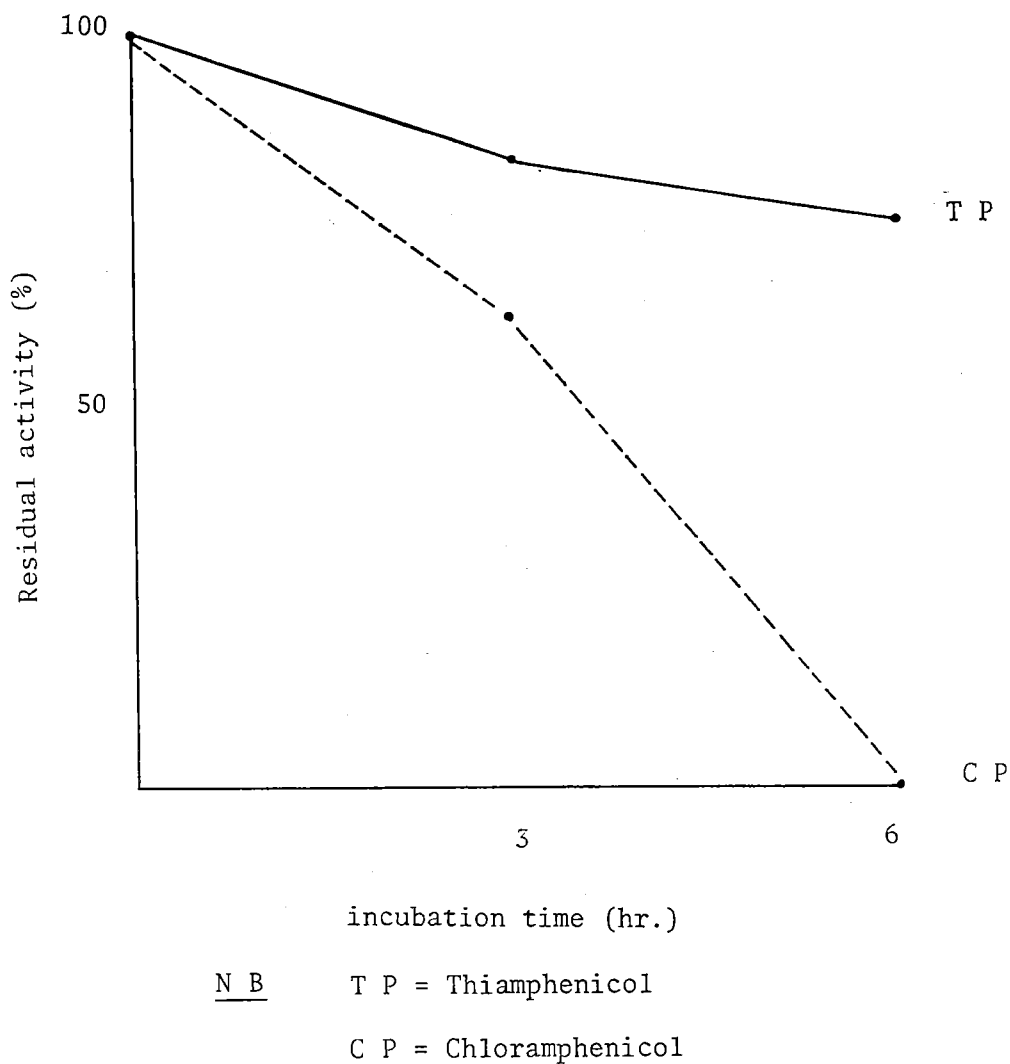
Regimens	CP	TP
	SIZ = 26	SIZ = 24
1- The two sensitive strains of <i>Bact. fragilis</i>		
1.1 After 3 hours incubation	22 (84.6%)*	23.6 (98.3%)*
1.2 After 6 hours incubation	18 (69.2%)*	23 (95.8%)*
2- The resistant strain of <i>Bact. fragilis</i>		
2.1 After 3 hours incubation	16 (61.5%)	20 (83.3%)
2.2 After 6 hours incubation	0 (0%)	18 (75%)

\* mean residual activity of the antibiotics.

\*\* Methyl alcohol is the only primary diluent of pure chemical CP and TP drugs.

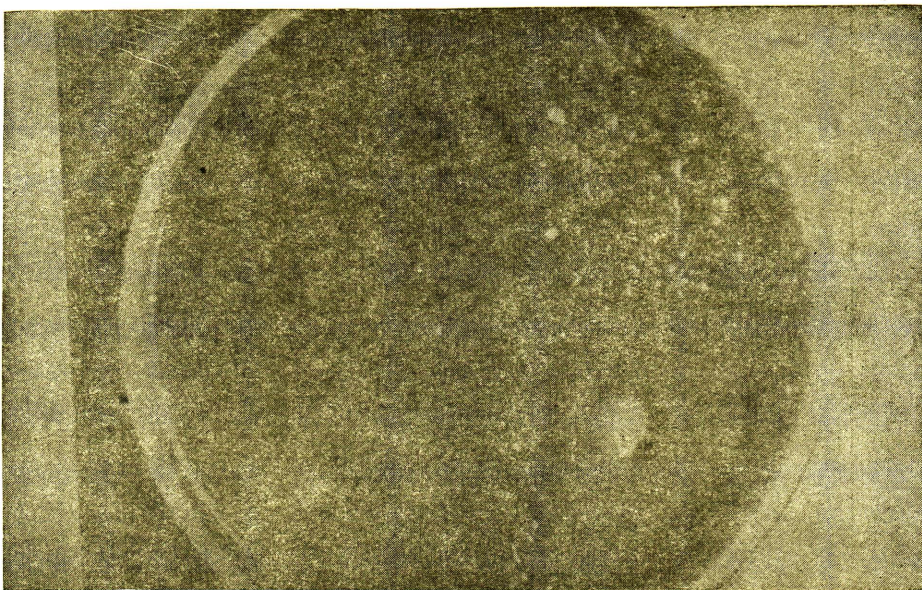


**Figure 1** Mean residual activities of thiamphenicol (T P) and chloramphenicol (C P) incubated with the sensitive strains of *Bact. fragilis*



**Figure 2** Residual activity of thiamphenicol (T P) and chloramphenicol (C P) incubated with the resistance strain of *Bact. fragilis*.

**Figure 3** Showing the inhibition zone of *A. hydrophila* by an antibiotic in the "well".



### Discussion

Chloramphenicol, a substance originally isolated from a culture of *Streptomyces venezuelae*, is now manufactured synthetically. Chloramphenicol is potent inhibitor of protein synthesis in microorganisms. It blocks the attachment of amino-acids to the nascent peptide chain on the 50S unit of ribosomes by interfering with the action of peptidyl transferase.<sup>(7)</sup>

The mechanism of thiamphenicol, the analog of chloramphenicol with substitution of the p-nitrogroup by a methylsulfonyl moiety, is probably the same, or more advantageous.

Although chloramphenicol can be inactivated by chloramphenicol acetyl-

transferase<sup>(14,15)</sup>, chloramphenicol hydrolase<sup>(16)</sup> and nitroreductase<sup>(17,18)</sup> in specific pathogens, chloramphenicol resistance is mostly due to the drug's destruction by the acetyltransferase under a plasmid control.<sup>(7)</sup>

From Table 1 and Figure 1 of the sensitive-*Bact. fragilis* strains, one can interpret that C P is inactivated more than T P.

Our findings also support the work of Britz et al in 1978 that the major active product of *Bact. fragilis* in chloramphenicol inactivation is the acetyl transferase.<sup>(15)</sup>

In Table 1 and Figure 2, thiamphenicol retained more activity 83.3%,

75 % at 3 and 6 hrs. respectively towards the anaerobic resistant *B. fragilis* strain than chloramphenicol.

In these events, the study shows that the modes of inactivation of thiamphenicol are different to chloramphenicol.

In this study of anaerobic *Bact. fragilis*, the inactivation of chloramphenicol is by acetyl transferase<sup>(15)</sup> while thiamphenicol by other means.

The fact is that thiamphenicol has no p-nitrogroup.\* The bacterial nitro reductase is probably non-significant to thiamphenicol as it is to chloramphenicol.<sup>(17,18)</sup> Therefore, the chlo-ramphe-nicol resistant strain of *Bact. fragilis* may be sensitive to thiam-phenicol.

Not only do many anaerobic bacteria possess the Beta-lactamase to destroy Betalactam antibiotics,<sup>(2)</sup> but these drugs are too expensive\*\* for our patients and the general practitioners. Neither metronidazole nor thiamphenicol is approved by F D A,\*\*\* but they are widely used by many clinicians.

Although chloramphenicol infrequently causes gastrointestinal upsets, prolonged administrations of more than 3 gm. daily to adults regularly result in abnormalities of the early forms of red blood cells, the elevation of serum

iron and anemia. These changes are reversible upon discontinuance of the drug. However, very rare individuals exhibit apparent idiosyncrasy to chloramphenicol and develop severe or fatal depression of bone marrow function. The mechanism of this aplastic anemia is not understood but is distinct from the dose-related reversible effect described above.<sup>(7)</sup>

In premature and newborn infants, chloramphenicol can induce collapse (Gray's syndrome) because its normal mechanism of detoxification (glucuronide conjugation in the liver) is not yet developed. However, the mentioned side effects are not seen clinically in thiamphenicol.<sup>(5,6,7)</sup>

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\* substitution of nitrogroup by a methyl sulfonyl group.

\*\* Natimycin of Schering-Essex Pharmaceutical Co. and Ceftriaxone, a new cephalosporin of Roche Pharmaceutical Co.

\*\*\* Food and Drug Administration, U.S.A.



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