

## Susceptibility of *Clostridium perfringens* Isolated from Human Infections to Twenty Antibiotics

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The proper choice of antibiotic for *Clostridium perfringens* infections in patients allergic to penicillin is not clear; the usual recommendations and recent in vitro studies disagree. We tested the susceptibility of 57 strains of *C. perfringens* to eight penicillins, seven cephalosporins, two tetracyclines, clindamycin, chloramphenicol, and rifampin by the agar dilution method. All strains were inhibited by (per milliliter) 4  $\mu\text{g}$  or less of any of the penicillins, chloramphenicol, or clindamycin and 8  $\mu\text{g}$  or less of any of the cephalosporins tested. Penicillin G and amoxicillin inhibited all strains at 0.12  $\mu\text{g}$  or less per ml. Only 54% of the strains were inhibited by 1  $\mu\text{g}$  of tetracycline per ml. Penicillin G remains the drug of first choice for infections with *C. perfringens*; it need not be added to a regimen containing a penicillinase-resistant penicillin given parenterally in high doses. The cephalosporins should be considered as alternative drugs for penicillin-allergic patients. Clindamycin and chloramphenicol are also effective. Tetracyclines cannot be depended upon in clostridial infections without in vitro testing, which is impracticable for initial empirical therapy.

Penicillin G is the antibiotic of first choice for treating cellulitis, sepsis, and myonecrosis caused by *Clostridium perfringens* (1, 2, 8, 11, 18). Although erythromycin and tetracycline are the alternative drugs usually recommended (1, 2, 8, 11, 18), 10 to 30% of *C. perfringens* are resistant to tetracycline, and a few strains are also resistant to erythromycin (6, 8, 9, 13). Cephalosporins and penicillinase-resistant and other semisynthetic penicillins are used for contaminated wounds after severe trauma and for suspected sepsis in patients with cancer, who are also at a higher risk for clostridial infections (3, 4, 14). Because of both scanty information about many of these agents and the need for a safe antibiotic for penicillin-allergic patients, we studied the activity of 20 antibiotics against strains of *C. perfringens* isolated from human infections or feces.

### MATERIALS AND METHODS

**Strains of bacteria.** Fifty-seven isolates of *C. perfringens* type A were collected from the following institutions: University of Colorado Medical Center, 18 strains; Center for Disease Control (CDC), 12 strains; University of Minnesota Hospital, 10 strains; University of Utah Medical Center, 7 strains; University of California-Los Angeles Health Sciences Center, 6 strains; and University of Oregon Health Sciences Center, 4 strains. All strains of *C. perfringens* produced a double zone of hemolysis on sheep-blood agar, were nonmotile,

grew at 46°C, fermented lactose, had no visible spores, produced lecithinase that was inhibited by type A antiserum (Burroughs Wellcome Co., Research Triangle Park, N.C.), and liquefied gelatin. Fifty-five of the strains were isolated from clinical specimens; two were recovered from normal feces.

**Antibiotics.** Antibiotic solutions were prepared in distilled water with standard powders of known potency provided by the following firms: amoxicillin (Hoffmann-LaRoche, Inc., Nutley, N.J.); cloxacillin and dicloxacillin (Bristol Laboratories, Syracuse, N.Y.); methicillin, oxacillin, and ticarcillin (Beecham-Massengill, Inc., Bristol, Tenn.); nafcillin (Wyeth Laboratories, Philadelphia, Pa.); penicillin G, doxycycline, and tetracycline (Pfizer Laboratories, New York, N.Y.); cefamandole, cephalixin, cephaloridine, and cephalothin (Eli Lilly & Co., Indianapolis, Ind.); cefazolin and cephadrine (Smith Kline & French Laboratories, Philadelphia, Pa.); cefoxitin (Merck, Sharp & Dohme, West Point, Pa.); chloramphenicol (Parke, Davis & Co., Detroit, Mich.); clindamycin (The Upjohn Co., Kalamazoo, Mich.); and rifampin (CIBA-Geigy and Co., Summit, N.J.). Stock solutions of antibiotics were prepared fresh or stored at -70°C for a maximum of 4 weeks before use.

**Susceptibility testing.** Agar dilution tests were done by the method of Sutter and Washington (15). Antibiotics were diluted in twofold steps above and below 1  $\mu\text{g}/\text{ml}$  in Brucella agar (Pfizer) supplemented with 5% laked sheep blood and vitamin K<sub>1</sub> (10  $\mu\text{g}/\text{ml}$ ). The inoculum was adjusted to a no. 1 MacFarland turbidity standard ( $3 \times 10^8$  colony-forming units) and then diluted with pre-reduced Carey-Blair buffer, so that each spot delivered by

the multipoint inoculator contained between  $10^4$  and  $10^8$  organisms. The viable counts were confirmed by the method of Miles and Misra (10). Tests were done in triplicate, and a control strain of *C. perfringens* (CDC no. 15443) was included with each experiment. Growth was defined as more than one discrete colony on at least two out of three spots after incubation at 35°C in an anaerobic glove box in an atmosphere of 85% N<sub>2</sub>, 5% CO<sub>2</sub>, and 10% H<sub>2</sub> for 24 and 48 h.

## RESULTS

End points at 24 and 48 h were similar, except that 12% of the minimal inhibitory concentrations (MIC) at 48 h were one dilution step higher. The MIC of the control strain of *C. perfringens* was always within one twofold-dilution step of the MIC reported by the CDC or our earlier results for antibiotics not tested by the CDC. The results shown in Table 1 are those obtained at 24 h.

Penicillin G and amoxicillin had the lowest MIC. One hundred percent of the strains were inhibited by 0.12 µg of either antibiotic per ml. All the semisynthetic penicillins and cephalosporins tested were active against *C. perfringens* at concentrations readily achieved in human serum with usual doses. Chloramphenicol and clindamycin were also active over a narrow range against all strains. In contrast, 19% of strains were resistant to 4 µg of tetracycline per ml. Rifampin inhibited all but one strain at

0.015 µg/ml; the remaining strain was 500-fold more resistant.

## DISCUSSION

Our results for penicillin G agree with those of others who used similar methods (6, 8, 9, 13). The semisynthetic penicillins tested were also active against *C. perfringens*. Any of the penicillins we tested should constitute effective therapy against *C. perfringens*. Our findings with cephalosporins agree with published results for cefamandole, cephalexin, cephaloridine, and cephalothin (16, 17). Cefazolin, cefoxitin, and cephadrine showed activity comparable to the other cephalosporins. Our results with chloramphenicol and clindamycin were similar to those of others (6, 8, 9, 13). We also found resistance to tetracyclines among strains of *C. perfringens* (6, 8, 9, 13). About half of our strains were inhibited by 1 µg of tetracycline per ml, but 64 µg/ml was required to inhibit 100% of strains; 21% of strains required more than 1 µg of doxycycline per ml.

Tetracyclines may be chemically inactivated in environments having low oxidation-reduction potentials (7). Erythromycin is much less active, if at all, at a low redox potential and an acid pH (12). The acidosis and anaerobic environment in myonecrosis caused by *C. perfringens* (19) would contraindicate the use of these drugs on theoretical grounds. Penicillin, ampicillin, and cephalothin are not appreciably affected by different pH conditions in media (13).

In conclusion, our data support the efficacy of all the penicillins tested against *C. perfringens*. Neither tetracycline nor erythromycin should be considered as an alternative drug for infection with *C. perfringens*, unless susceptibility can be demonstrated by appropriate testing. In most penicillin-allergic patients, a cephalosporin would be both safe and effective treatment for *C. perfringens* (5, 18); clindamycin and chloramphenicol would be alternative drugs.

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TABLE 1. Antibiotic susceptibility of *C. perfringens* (57 strains)

Class and antibiotic	Cumulative % susceptible to indicated concn (µg/ml)				
	0.5	1	2	4	8
<b>Penicillins</b>					
Amoxicillin	100				
Penicillin G	100				
Ticarcillin	91	100			
Methicillin	61	100			
Nafcillin	95	98	100		
Oxacillin	98		100		
Cloxacillin	86	96	98	100	
Dicloxacillin	81	95	96	100	
<b>Cephalosporins</b>					
Cefamandole	100				
Cephaloridine	98	100			
Cefoxitin	39	91	100		
Cephalothin	14	67	98	100	
Cefazolin	74	84	89	96	100
Cephalexin	32	70	82	93	100
Cephadrine	42	67	82	93	100
<b>Miscellaneous</b>					
Chloramphenicol	0	11	100		
Clindamycin	75	85	98	100	
Doxycycline	82	89	96	98	100
Rifampin	98				100
Tetracycline	46	56	74	81	91

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