In vitro activity of clindamycin and other antimicrobials against gram-positive bacteria and Hemophilus influenzae

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Summary: Seven antimicrobials — clindamycin, penicillin, ampicillin, cloxacillin, erythromycin, lincomycin and cephalexin — were found to have a high degree of activity *in vitro* against 256 isolates of gram-positive bacteria and *Hemophilus influenzae*. Clindamycin was clearly superior against staphylococci and 3.12 μg/ml or less of clindamycin inhibited all 35 isolates of *H. influenzae*. Synergism was not demonstrated when clindamycin was tested in combination with sulfisoxazole or sulfamethoxazole by either the agar dilution or 24-hour growth curve method. This was true for penicillin as well, and the effect was independent of sulfonamide sensitivity. The erythromycin–sulfonamide combination was synergistic against 6 of 10 strains studied by the growth curve method; this effect was not demonstrable by the agar dilution method.

Résumé: *L'activité in vitro de la clindamycine et d'autres antimicrobiens sur les bactéries gram-positives et Hémophilus influenzae*

Nous avons constaté que sept antimicrobiens — clindamycine, pénicilline, ampicilline, cloxacilline, érythromycine, lincomycine et céphalexine — possédaient une forte activité *in vitro* contre 256 isolats de bactéries gram-positives et d'*Hemophilus influenzae*. La clindamycine s'est révélée nettement supérieure contre les staphylocoques et une dose maximum de 3.12 μg/ml de clindamycine a permis d'inhiber la totalité des 35 isolats de *H. influenzae*. Une synergie de clindamycine avec le sulfisoxazole ou le sulfaméthoxazole n'a pu être mise en évidence au cours des essais, ni par la méthode de dilution sur agar, ni par celle de la courbe de pousse en 24 heures. Ceci s'est révélé également vrai pour la pénicilline et l'effet a été indépendant de la sensibilité du sulfonamide. L'association érythromycine–sulfonamide a exercé une action synergique contre 6 des 10 souches étudiées par la méthode de la courbe de pousse, mais n'a pas été démontrée par la méthode de dilution sur agar.

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Previous studies have established the *in vitro* efficacy of clindamycin — 7(S)-chloro-7-deoxylincomycin — against a wide variety of gram-positive and anaerobic bacteria.[1-7] Several successful trials in experimental animals and humans have confirmed this activity *in vivo*[8-13] The purpose of the present study was to evaluate the *in vitro* efficacy of this new antimicrobial against a variety of gram-positive bacteria and *Hemophilus influenzae* isolated from hospitalized children, before introduction of this drug into general clinical use. The activity against *H. influenzae* of clindamycin used alone and in combination with sulfonamides was also evaluated.

Material and methods

Isolates of *Staphylococcus aureus, S. albus, Diplococcus pneumoniae, Streptococcus pyogenes* and *H. influenzae* were obtained from pediatric patients at The Montreal Children's Hospital and identified by standard microbiologic methods.[14] *H. influenzae* was typed by slide agglutination and capsular swelling techniques with commercial antisera (Difco). *S. aureus* was identified by coagulase reaction. Lancefield grouping was performed on all streptococci.

The following drugs were used in sensitivity testing by broth and agar dilution studies: clindamycin (Upjohn 803 An D2), penicillin G (Lilly C6789), ampicillin (Ayerst L-1943-KD), cloxacillin (Ayerst L-1707-RC), erythromycin (Lilly C8045), lincomycin (Upjohn 414 AC D1) and cephalexin (Lilly CO7864). Clindamycin and cephalexin disks were obtained from The Upjohn Company of Canada and Eli Lilly & Company (Canada) Limited, respectively, and the other disks were obtained from Difco. The following drug concentrations were used in the disks: clindamycin, 2 μg; penicillin G, 10 μg; ampicillin, 10 μg; oxacillin, 1μg; erythromycin, 15 μg; lincomycin, 2 μg; and cephalexin, 30 μg.

Sensitivity testing was performed by three methods: disk diffusion, broth dilution and agar dilution. For each an inoculum of a 10^{-3} dilution of an overnight broth culture was used. For *Staphylococcus* isolates, disk diffusion studies were performed with Mueller-Hinton agar base according to the method described by Barry, Garcia and Thrupp.[15] Other isolates were tested by a standardized disk diffusion technique (flooding inoculum method).[16] The base was supplemented with 5% sheep blood for the *D. pneumoniae*

and *S. pyogenes* studies. *H. influenzae* was tested on Levinthal's agar. The microtitre broth dilution method was used for *Staphylococcus* and *S. pyogenes* isolates, with Mueller-Hinton broth and Todd-Hewitt broth, respectively. The agar dilution method was used according to the technique of Steers, Foltz and Graves[17] for all isolates. Mueller-Hinton agar was used for *Staphylococcus.*[17] This base was supplemented with 5% sheep blood for *D. pneumoniae* and *S. pyogenes. H. influenzae* was tested on Levinthal's agar. Minimum inhibitory concentration (MIC) was defined as the lowest drug concentration at which no visible turbidity was produced by the microtitre and standard tube dilution methods, and no visible surface growth was observed in the agar dilution and disk diffusion studies.

Combination studies with *H. influenzae* were performed in duplicate by the agar dilution method, with Levinthal's agar containing added DST* base. Ten isolates of this organism (nine type B, one type A) were also studied by a 24-hour growth curve method. A 0.1-ml aliquot of an overnight broth (Levinthal) culture of *H. influenzae* was introduced into 100 ml of Levinthal's broth and incubated in a shaker water bath at 37°C. Each series included a no-drug control, each of the drugs in concentrations equal to one half the MIC as determined by agar dilution sensitivity studies, and a combination of the drugs at the same concentration. Aliquots from each flask were plated out at 0, 2, 4, 6, 8 and 24 hours, using standard logarithmic dilutions on Levinthal agar plates, and were incubated overnight before colony counting. Synergism was defined as at least two measurements of at least a 2-log increase in colony count when a drug combination was used during the 24-hour growth period (as compared with each drug alone).

*Diagnostic sensitivity test (Oxoid).

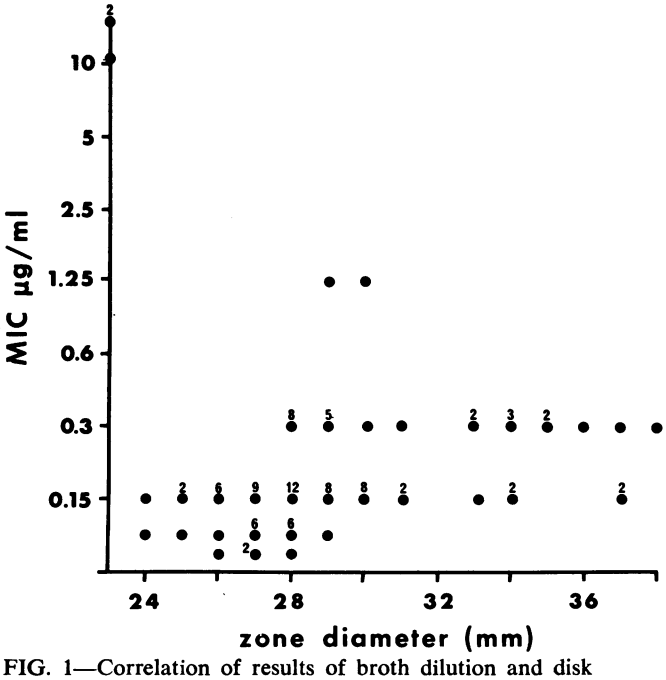

FIG. 1—Correlation of results of broth dilution and disk (2 µg) diffusion sensitivity tests for clindamycin against 103 isolates of *Staphylococcus.*

Results

A total of 256 isolates of bacteria were studied: 103 isolates of *Staphylococcus* sp. (78 *S. aureus,* 25 *S. albus),* 93 *S. pyogenes,* 25 *D. pneumoniae* and 35 *H. influenzae.* Sensitivities of these isolates, as determined by disk diffusion studies, are shown in Table I.

For clindamycin, results of sensitivity testing by disk diffusion and broth dilution against 103 isolates of *Staphylococcus* are illustrated in Fig. 1. All isolates sensitive to 1.25 µg/ml or less exhibited a zone greater than 24 mm in diameter. A 20-mm diameter correlated with susceptibility in the other bacteria studied except for *H. influenzae,* for which disk diffusion results correlated poorly with agar dilution results.

In comparison with the other six antimicrobials, clindamycin was found, by broth dilution and agar dilution methods, to be highly active against staphylococci (Fig. 2). It inhibited 97% of staphylococci at a concentration $\leq$ 0.07 µg/ml. Only penicillin was more active than clindamycin against a few non-penicillinase-producing staphylococci. One of 25 *S. albus* isolates tested was resistant to clindamycin and also to erythromycin and lincomycin. One *Staphylococcus* isolate resistant to lincomycin and eight resistant to erythromycin were sensitive to clindamycin.

Penicillin and ampicillin were active against *S. pyogenes* at very low concentrations (Fig. 3). Clindamycin was also effective and inhibited all 93 isolates at a concentration $\leq$ 0.125 µg/ml. No cross-resistance was found for streptococci to clindamycin, erythromycin or lincomycin.

The 25 isolates of *D. pneumoniae* were uniformly sensitive to all the antimicrobials studied. Although erythromycin and penicillin were more active than clindamycin, all isolates were inhibited by 0.156 µg/ml or less of clindamycin (Fig. 4).

The activity of the seven drugs studied against 35 isolates of *H. influenzae* is illustrated in Fig. 5. An additional 25 isolates were typed and studied by disk diffusion (Table I); 21 were type B, 1 type A, and 3 were not typable. The one strain resistant to ampicillin by disk diffusion studies had tube dilution minimum bactericidal concentrations (MBCs) of 3.125 µg/ml in Mueller-Hinton broth supplemented with Fildes enrichment and 6.25 µg/ml in Levinthal's broth. Clindamycin and erythromycin exhibited the same degree of activity and both were significantly more active than lincomycin and penicillin. All strains of *H. influenzae* were sensitive to ampicillin by the tube dilution technique (MBC $\leq$ 6.25 µg/ml). MICs for clindamycin against *H. influenzae* were often one dilution greater by the agar dilution method as compared with broth dilution studies.

Combination studies carried out with 35 isolates of *H. influenzae* by the agar dilution method, for penicillin, erythromycin or clindamycin together with a sulfonamide

Table I—Results of disk diffusion sensitivity studies in 256 baterial isolates

Bacterium	No. of isolates tested	Clindamycin			Penicillin			Ampicillin			Oloxacillin			Erythromycin			Lincomycin			Cephalexin		
		S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R
S. aureus	78	77		1	15	3	60	15	5	58	76		2	73	1	4	77		1	78		
S. albus	25	24		1	5	9	11	6	10	9	22		3	19	1	5	24		1	25		
S r. pyogenes	93	93			91	2		93			92		1	92	1		93					
D. *pneumoniae*	25	25			25			25			25			25			25			25		
H. influenzae																						
Type B	21	3	8	10	20		1	20		1			21	21					21	20		1
Others	39	1	3	35	33		6	37	2				39	39					39	39		

S = sensitive, I = intermediate, R = resistant

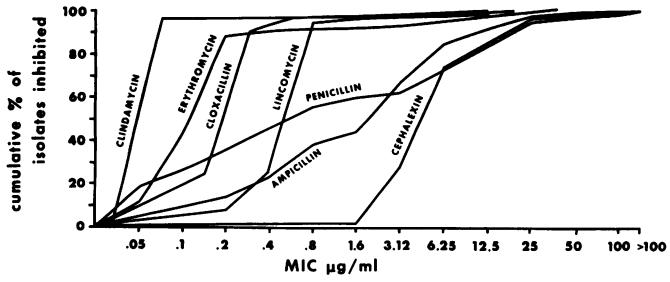

FIG. 2—Susceptibility of 103 isolates of *Staphylococcus* sp. to seven antimicrobials (agar dilution, inoculum replicating method, and broth dilution method).

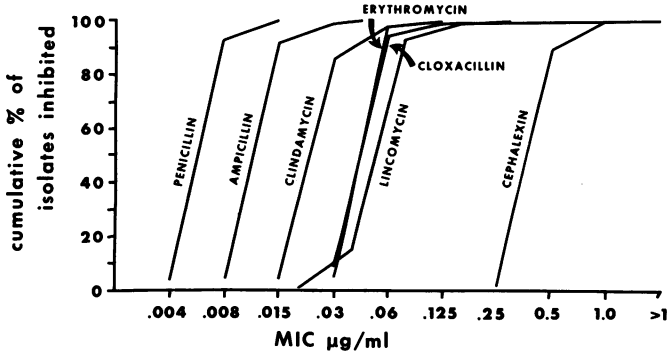

FIG. 3—Susceptibility of 93 isolates of *S. pyogenes* to seven antimicrobials (method as in Fig. 2).

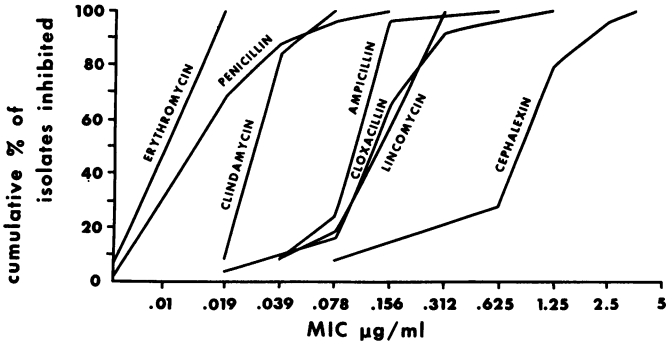

FIG. 4—Susceptibility of 25 isolates of *D. pneumoniae* to seven antimicrobials (method as in Fig. 2).

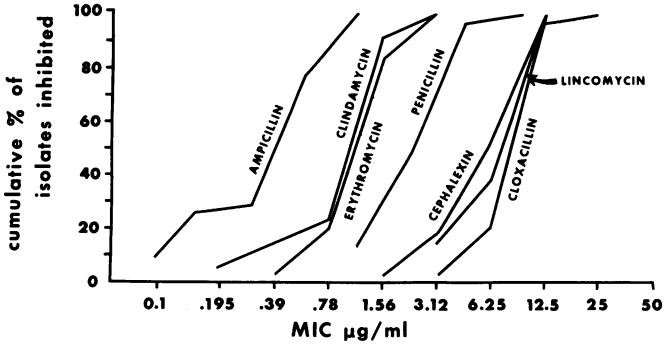

FIG. 5—Susceptibility of 35 isolates of *H. influenzae* to seven antimicrobials (method as in Fig. 2).

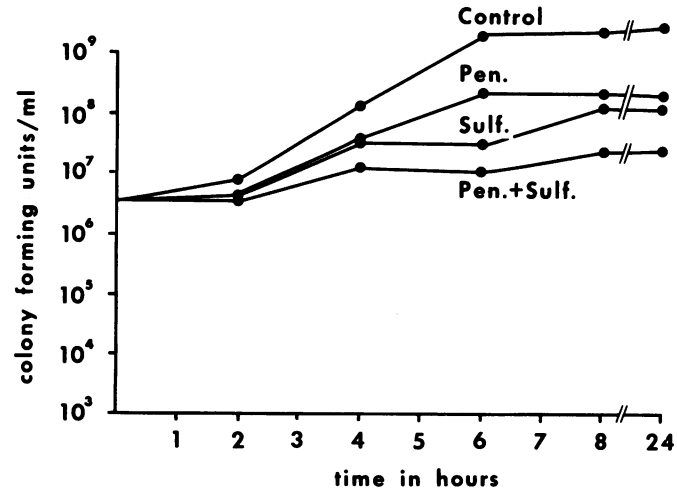

FIG. 6—Composite growth curves showing effects of penicillin and sulfamethoxazole (concentrations equal to one half the MIC by agar dilution studies) alone and in combination against 10 isolates of *H. influenzae*.

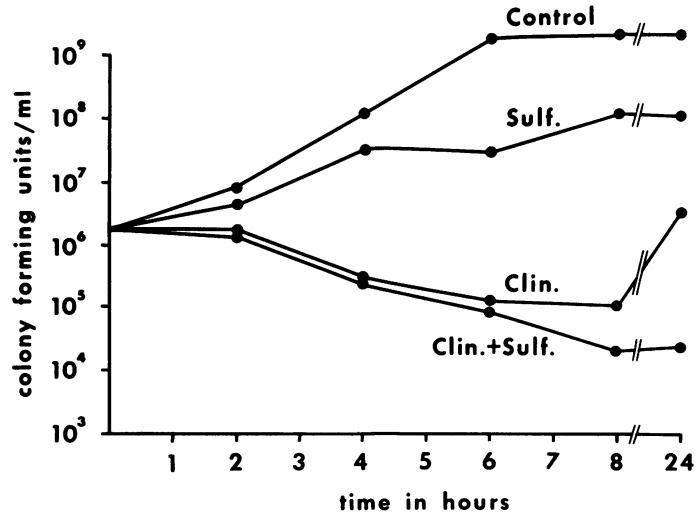

FIG. 7—Composite growth curves showing effects of clindamycin and sulfamethoxazole (concentrations equal to one half the MIC by agar dilution studies) alone and in combination against 10 isolates of *H. influenzae*.

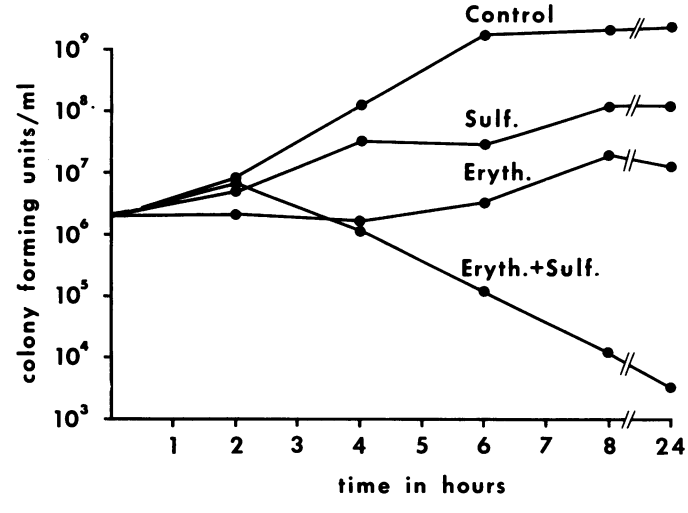

FIG. 8—Composite growth curves showing effects of erythromycin and sulfamethoxazole (concentrations equal to one half the MIC by agar dilution studies) alone and in combination against 10 isolates of *H. influenzae*.

(either sulfisoxazole or sulfamethoxazole), failed to demonstrate any synergism between any of these drugs by this method and isobologram analysis.[24]

None of the 10 isolates studied by the 24-hour growth curve method demonstrated synergism for either penicillin or clindamycin in combination with a sulfonamide. Penicillin and the penicillin–sulfamethoxazole combination demonstrated the least difference between use of individual drugs, a combination and the control (Fig. 6). Clindamycin tended to have more activity both alone and in combination with sulfamethoxazole when compared with sulfamethoxazole alone and the control alone, but the effect was simply additive (Fig. 7). Erythromycin and sulfamethoxazole were synergistic for 6 of the 10 strains (5 type B and 1 type A) (Fig. 8). This effect was most obvious beginning at approximately 6 to 8 hours after the incubation of these drugs with bacteria. The erythromycin–sulfamethoxazole combination was bactericidal for one strain of *H. influenzae* as judged by complete killing by the combination at 24 hours. None of the other combinations of drugs was bactericidal, although erythromycin and clindamycin used alone and in combination had the most significant effect on the reduction of bacterial counts. Antagonism was not demonstrated for any of the combinations, and the effects of the individual drugs and combinations were independent of the individual MICs of any of the drugs and independent of the bacteria type.

Discussion

This investigation has confirmed the high degree of activity of clindamycin *in vitro* against isolates of gram-positive bacteria and *H. influenzae*. Previous investigators have reported similar findings using isolates from a wide variety of clinical sources.[2-7] In general, clindamycin was more active than lincomycin and erythromycin, especially against isolates of *Staphylococcus*. This observation and the susceptibility of *H. influenzae* to clindamycin confirm previously reported findings.[2,3,5,7]

These results differ slightly from some of those previously reported. Most staphylococci were highly sensitive to clindamycin in all of our studies, which included 78 isolates of *S. aureus* and 25 isolates of *S. albus*. Marsik and Parisi[4] reported a small number of *S. aureus* and a larger number (20 of 63 isolates studied) of *S. epidermidis* resistant to clindamycin. The timing of the present study — before the introduction of this antibiotic into general use in the hospital — may explain this disparity. McGehee, Barrett and Finland[1] have reported the development of resistance by staphylococci to lincomycin and clindamycin in clinical and *in vitro* situations. Cross-resistance was not complete between lincomycin and clindamycin, in contrast to findings from previous studies.[1]

H. influenzae, type B is an important bacterial pathogen in children. The combination of penicillin and a sulfonamide is often recommended as a substitute for ampicillin or chloramphenicol in the treatment of *H. influenzae* infections such as otitis media.[18-22] This recommendation is based on considerations of the price difference between these drugs, of penicillin hypersensitivity and of the toxicity of chloramphenicol. There are insufficient data at this time to evaluate the efficacy of clindamycin in *H. influenzae* otitis media. Earlier reports claiming more favourable therapeutic results in *H. influenzae* meningitis with the combination of penicillin and sulfonamides than with sulfonamides alone seemed to provide a rationale for this recommendation.[23] We have been unable to confirm this activity *in vitro* by the agar dilution method or by growth curve analysis for either penicillin or clindamycin. The combination of erythromycin with sulfamethoxazole was synergistic against 6 of 10 strains of *H. influenzae* tested by the growth curve method. This was not obvious by the agar dilution method and these results indicate the technical difficulties often encountered in studies of synergism. These difficulties are compounded by the lack of clinical studies confirming the importance of drug synergism in treating *H. influenzae* infections. Nevertheless, the present study demonstrates no advantage in adding sulfonamides to either penicillin or clindamycin *in vitro* in inhibiting the growth of *H. influenzae*. Although synergism was demonstrated for a significant number of isolates exposed to the combination of erythromycin and a sulfonamide, this effect was usually not obvious before at least 6 hours of incubation of the drug–bacteria mixture. Further studies are obviously needed to document these effects in a variety of *in vitro* and *in vivo* test systems before their significance can be better determined. The practice of using various combinations of antimicrobials for the treatment of *H. influenzae* infections needs to be reassessed in the light of these findings.

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