Bactericidal Effect of Combinations of Nalidixic Acid and Various Antibiotics on
Enterobacteriaceae

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The activity of nalidixic acid combined with each of 10 other antimicrobial agents on 95 strains of Enterobacteriaceae was studied. Synergy was found less often than antagonism, and the commonest outcome was indifference. Combinations of nalidixic acid with kanamycin, gentamicin, or colistin were more often synergistic than antagonistic, whereas with the other antibiotics the combination was more often antagonistic than synergistic. Synergistic effects were more common with Shigella than with other genera. In vitro examination for synergy or antagonism appears to be advisable before nalidixic acid is used therapeutically in combination with other antimicrobial agents.

Nalidixic acid is a synthetic antimicrobial drug (25) which is active against gram-negative bacteria and is used mainly in the therapy of urinary tract infections (1, 20, 29, 31). After 10 years of usage, it remains effective against most gram-negative bacilli (7, 13, 26, 32), in spite of the general increasing resistance of these bacteria to other antimicrobial agents (21). Early reports on nalidixic acid (8, 17) indicated the ready development of resistance of the single-step type both in vitro and in vivo. Most recent studies have confirmed that resistance to this drug develops rapidly during treatment, irrespective of the dose used (1). Furthermore, the emerging resistant organisms are extremely stable, retain their biochemical and serological properties, and persist after cessation of therapy (1, 20, 29). No transfer of nalidixic acid resistance has been demonstrated (29, 33). To prevent or delay the emergence of resistance, it has been suggested that nalidixic acid always be used in combination with another antimicrobial agents (1) Relatively few reports (2-4, 6, 17, 18, 28, 30) about combinations of nalidixic acid with other antibacterial agents and none about the interaction of this drug in combination with a wide range of other agents have appeared. For these reasons, we decided to study the bactericidal effect of combinations of nalidixic acid with other antimicrobial agents active against gram-negative bacteria. Since it is well-known that nitrofurantoin often produces an antagonistic effect with nalidixic acid, especially on Proteus strains (30), this combination was not studied.

MATERIALS AND METHODS

Ninety-five strains of Enterobacteriaceae isolated from infected wounds, sputum, stools, and blood cultures during 1971 in the diagnostic laboratory of the Department of Clinical Microbiology, Hadassah University Hospital were studied. These strains were identified according to Ewing (19) as Escherichia coli (15 strains), Shigella (15 strains), Citrobacter (3 strains), Salmonella (12 strains), Proteus (14 strains), Providence (6 strains), Klebsiella (11 strains), Enterobacter (9 strains), and Serratia (10 strains).

The cellophane transfer technique of Chabbert (9) as modified by Cluzel et al. (16) was used. As the principles and techniques of this method were recently described and illustrated in detail (14, 15, 22), only a brief resume is given here. Strips of Canson blotting paper (200 g/m²) were dried, after immersion in antibiotic solutions in the concentrations shown in Table 1. The dried strips were placed on the surface of a plate containing Oxoid DST agar. The strips were arranged in the form of a cross around a central square hole cut in the agar (Fig. 1); the object of the hole is to avoid diffusion towards the center of the plate. Four different antimicrobial agents can be tested on one plate. Diffusion of the antimicrobial agents was allowed to continue overnight at 37 C. Tambours were prepared by stretching commercially available cellophane (PO 300, 0.019 thickness) over a Pyrex glass ring 12 cm in diameter. After the period of diffusion, the blotting-paper strips were removed. The tambour
positions of can strips that have been removed after the first period of nistic effect. These results that transfer technique.

\begin{center}
\begin{tabular}{|c|c|}
\hline
Antibiotic & Concentration (\mu g/ml) \\
\hline
Ampicillin & 500 \\
Carbenicillin & 2,000 \\
Cephaloridine & 500 \\
Streptomycin & 500 \\
Kanamycin & 500 \\
Gentamicin & 500 \\
Chloramphenicol & 1,000 \\
Tetracycline & 200 \\
Colistin & 650 \\
Rifampin & 1,000 \\
Nalidixic acid & 500 \\
\hline
\end{tabular}
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TABLE 1. Concentrations of antibiotic solutions used for filter-paper impregnation

![Diagram showing the interpretation of the results that can be obtained with the cellophane transfer technique.](image)

was placed on the plate, and the inner surface was flooded with a suspension containing approximately \(10^6\) organisms/ml of the culture to be tested. This suspension was obtained by appropriate dilution of an overnight culture in tryptic soy broth (Difco). Surplus suspension was removed with a Pasteur pipette. The plate with the tambour in place was incubated overnight at 37 C. The tambour was then transferred to an antibiotic-free plate of Oxoid DST agar and was again incubated overnight. Care was taken to avoid “carry-over” as described by Chabbert and Waterworth (12).

Results were interpreted according to Chabbert and Patte (14), Garrod and O’Grady (22), and Garrod and Waterworth (23), and were classified as synergy, indifference, or antagonism. This method cannot differentiate between an additive effect and a true synergistic bactericidal effect (10).

The criteria used were as follows. Synergism: the bactericidal effect of the two drugs together was greater than that of each alone; the edge of the bacterial growth at the junction of two adjacent strips formed an obtuse angle (Fig. 1A). Antagonism: the bactericidal effect of one drug was reduced by the presence of the other; the edge of the bacterial growth at the junction of two adjacent strips formed an acute angle (Fig. 1C). Indifference: the action of the two drugs together was no greater than that of each alone; the edge of the bacterial growth at the junction of adjacent strips formed a right angle (Fig. 1B).

RESULTS

The results of the 950 combinations tested are summarized in Tables 2 and 3. Most of the combinations showed indifference; the overall

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\begin{tabular}{|c|c|c|c|}
\hline
Nalidixic acid combined with & Percentage of strains showing \\
& Synergism & Indifference & Antagonism \\
\hline
Ampicillin & 2 & 72 & 26 \\
Carbenicillin & 1 & 76 & 23 \\
Cephaloridine & 5 & 83 & 12 \\
Streptomycin & 5 & 80 & 15 \\
Kanamycin & 25 & 63 & 12 \\
Gentamicin & 38 & 52 & 10 \\
Chloramphenicol & 3 & 41 & 56 \\
Tetracycline & 2 & 50 & 48 \\
Colistin & 24 & 64 & 12 \\
Rifampin & 12 & 57 & 31 \\
\hline
Total & 12 & 64 & 24 \\
\hline
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TABLE 2. Effects of nalidixic acid combined with other antimicrobial agents on 95 strains of Enterobacteriaceae

\begin{center}
\begin{tabular}{|c|c|c|c|}
\hline
Species & Percentage of strains showing \\
& Synergism & Indifference & Antagonism \\
\hline
E. coli & 14 & 64 & 22 \\
Shigella & 23 & 51 & 26 \\
Citrobacter & 17 & 50 & 33 \\
Salmonella & 12 & 63 & 25 \\
Proteus & 9 & 35 & 56 \\
Providence & 7 & 56 & 37 \\
Klebsiella & 9 & 71 & 20 \\
Enterobacter & 9 & 74 & 17 \\
Serratia & 4 & 81 & 15 \\
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TABLE 3. Effects of combinations of nalidixic acid and 10 other antimicrobial agents on 95 strains of Enterobacteriaceae
The frequency of antagonism was fairly high (24%) and synergism was unusual (12%).

Table 2 shows that there were important differences between the different combinations. With nalidixic acid in combination with the beta lactams, the incidence of synergism was very low, particularly with carbenicillin (1%). With cephaloridine, the rate of antagonism was also low (12%). The lowest incidence of antagonism was seen with aminoglycosides (10% for gentamicin). On the other hand, with kanamycin and gentamicin, the incidence of synergism was quite high. Among the aminoglycosides, the rate of antagonism was greater than that of synergism only with streptomycin.

Combinations of nalidixic acid with chloramphenicol or tetracycline showed a strikingly high rate of antagonism (56 and 48%, respectively), whereas a synergistic effect was rare (2 and 3%, respectively). Combinations with colistin were quite often synergistic (24%), and with rifampin, antagonism was relatively common (31%). Antagonism was thus more frequent than synergism with all of the combinations except for nalidixic acid and gentamicin, kanamycin, or colistin. There was no correlation between the result of a specific combination and the susceptibility of the strain to either of the two drugs. For example, with Proteus strains which are resistant to colistin, synergism was found on three occasions and antagonism on three.

Table 3 shows that different results were obtained with different species. Synergism was much more frequent with Shigella than with any other genus. On the other hand, antagonism occurred with more than half the Proteus strains. The same combination of two drugs gave different results on different genera. The combination of nalidixic acid with kanamycin or gentamicin was synergistic on only 1 of 30 strains of the Klebsiella-Enterobacter-Serratia group. On the other hand, the combination with kanamycin was synergistic on 10 and that with gentamicin on 11 strains of Shigella.

**DISCUSSION**

The antimicrobial action of nalidixic acid is due to inhibition of deoxyribonucleic acid (DNA) synthesis, although the exact mode of action of the drug on DNA replication is unknown (5). The bactericidal effect, however, does seem to be related to active bacterial metabolism. There is good correlation between the results presented here and the general rules about combinations of bactericidal and bacteriostatic drugs (24, 27).

The high incidence of antagonism that we found between nalidixic acid and the bacteriostatic drugs chloramphenicol and tetracycline conforms to these schemes. The few other published reports of in vitro studies of these combinations show similar results. Both Deitz et al. (17) and Dominici et al. (18) failed to demonstrate bacteriostatic synergism. Mouton (28) reported that tetracycline and chloramphenicol antagonized the bactericidal effect of nalidixic acid, especially on strains of Proteus. More recently, Brisou (6) found antagonism with a nalidixic acid-chloramphenicol combination on 30% of Serratia strains. The antagonism produced by these combinations is probably due to the bacteriostatic action of chloramphenicol or tetracycline, which diminishes bacterial metabolism and interferes with the bactericidal activity of nalidixic acid.

There are only isolated reports in the literature about other unfavorable combinations with nalidixic acid. Brisou (6) found antagonism with ampicillin in 20% of Serratia strains. Mouton (28) reported antagonism between streptomycin and nalidixic acid as a rare event. Baudens and Chabbert (3) reported a high rate of antagonism between rifampin and nalidixic acid.

The synergism that we found between nalidixic acid and gentamicin, kanamycin, and colistin not only conforms to the general rules of Jawetz (24) and Manten and Wise (27), but also to those reported in the literature. Brisou (6) reported synergism on 20% of Serratia strains with a nalidixic acid-gentamicin combination and also reported synergism between nalidixic acid and gentamicin. Baudens (4) showed a strong synergistic effect between nalidixic acid and polymyxin B in Serratia strains.

Although the use of nalidixic acid in combination with other drugs to avoid the selection of resistant mutants appears to be desirable, the results reported here indicate that most other antimicrobial agents quite frequently produce an antagonistic effect when combined with nalidixic acid. It is therefore advisable to examine the effect of the different potential combinations in vitro before using them clinically. The only combinations in which we found a strong synergistic bactericidal effect with a low risk of antagonism were either of two aminoglycosides, gentamicin and kanamycin, or with colistin.

**LITERATURE CITED**


