Growth on Congo Red Agar: Possible Means of Identifying Penicillin-Resistant Non-Penicillinase-Producing Gonococci

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Exceptional strains of gonococci that grow on Congo red agar were found to be penicillin-resistant non-penicillinase producers.

In an earlier study (3), it was noted that an agar medium containing Congo red dye could be used to differentiate *Neisseria gonorrhoeae* and *Neisseria meningitidis*. The gonococci tested failed to grow in the presence of this dye, whereas meningococci grew and produced red colonies. In the present, more extensive, survey of *Neisseria* strains, some gonococcal isolates were observed to grow on Congo red agar. These strains were found to be more resistant to penicillin than other gonococci.

All strains were plated on GC medium base (Difco Laboratories, Detroit, Mich.) containing 1% defined supplement (1) and 0.01% Congo red (Harleco, Philadelphia, Pa.). Plates were incubated at 37°C in 10% CO₂ for 24 h. Penicillin minimal inhibitory concentrations (MICs) were determined for each of the gonococcal isolates by dropping 10 µl, containing 10³ gonococci, on plating medium containing twofold dilutions of penicillin G (E. R. Squibb & Sons, Princeton, N.J.) (2) without Congo red. At the MIC, no growth was observed at 48 h.

Strains that failed to grow on Congo red agar were relatively susceptible to penicillin; i.e., MICs were less than 0.5 µg/ml (Table 1). In contrast, those strains that did grow on Congo red were more resistant to penicillin; MICs ranged from 0.5 to 2.0 µg/ml. These strains produced red colonies like the meningococci tested, although the gonococcal colonies were considerably smaller than the meningococcal colonies.

Gonococci resistant to penicillin by virtue of penicillinase production, however, did not grow on Congo red agar (Table 1). This suggests that the ability of certain gonococcal strains to grow in the presence of Congo red is related to an alteration in the cell envelope that also endows the cells with increased resistance to penicillin. This may be related to the similar findings of Sparling et al. (2, 4, and 5) that increased resistance to penicillin was frequently associated with

TABLE 1. Growth of *Neisseria* strains on Congo red agar

Strains tested ^a	No. of strains	Growth on Congo red agar	
		No.	%
<i>N. gonorrhoeae</i> :			
Penicillin susceptible (MIC < 0.5 µg/ml)	27	0	0
Penicillinase producers	5	0	0
Penicillin resistant ^b (MIC ≥ 0.5 µg/ml)	16	16	100
<i>N. meningitidis</i> :			
Groups A, B, C, X, Z, and nontypable	9	9	100

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^b See reference 2.

increased resistance to other antibiotics, surfactants, and dyes. The reason why meningococci, which are susceptible to penicillin, grow in the presence of Congo red is not clear. Both meningococci and gonococci bind similar quantities of the dye in liquid (3). The Congo red-sensitive gonococci may additionally transport dye into the cell.

Congo red agar thus provides a relatively simple means of distinguishing most gonococci from meningococci by their inability to grow on this medium. Those strains that do grow in the presence of this dye are penicillin-resistant, non-penicillinase producers. These observations may find application in predicting, rapidly and simply, those cases of gonorrhea most likely to be "treatment failures," although the inability to recognize penicillinase producers by this method places obvious limitations on the procedure. However, among known penicillin-resistant strains, the non-penicillinase-producing strains

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can easily be differentiated from penicillinase producers by their growth on Congo red agar.

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