

Tetracycline Resistance of *Corynebacterium diphtheriae* Isolated from Diphtheria Patients in Jakarta, Indonesia

ROBERT C. ROCKHILL,^{1*} SUMARMO,² HILDA HADIPUTRANTO,¹ SJAWITRI P. SIREGAR,² AND BURHANUDIN MUSLIHUN²

U.S. Naval Medical Research Unit No. 2, Jakarta Detachment, APO San Francisco, California 96356,¹ and Department of Child Health, Cipto Mangunkusumo Hospital, Jakarta, Indonesia²

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Of 133 *Corynebacterium diphtheriae* isolates from diphtheria patients in Jakarta, Indonesia, 86% were resistant to ≥ 32 μg of tetracycline per ml. All isolates were sensitive to ampicillin, cephalothin, chloramphenicol, clindamycin, penicillin, erythromycin, and kanamycin. The general resistance of *C. diphtheriae* to tetracycline in this part of Indonesia appears to be unique compared with resistance reported in studies done in other parts of the world.

Diphtheria is a major cause of morbidity and mortality in Indonesia. One estimate is that of every 5,000,000 children born in this country, approximately 29,000 (580 per 100,000) will develop the faucial form of the disease, and more than 5,000 (100 per 100,000) will die, giving an infection-to-death ratio of approximately 6:1 (12). In Western countries, vaccination measures have reduced the yearly incidence to less than 0.07 per 100,000 and the infection-to-death ratio to 10:1 (3, 8).

The major factor influencing infection and mortality rates is vaccination, and Indonesian public health authorities are attempting to lower the prevalence of the disease by including diphtheria vaccination in the Expanded Program on Immunization. Today, treatment is required for a large number of patients with this disease. Included in the treatment regimen is appropriate antimicrobial therapy to render *Corynebacterium diphtheriae* nonviable and thus stop toxin production that leads to associated complications. Erythromycin, tetracycline, clindamycin, and penicillin are considered the drugs of choice (9, 13); however, the emergence of *C. diphtheriae* resistant to erythromycin, clindamycin, and lincomycin has been reported (2, 6). We found that a significant number of *C. diphtheriae* isolated from hospitalized diphtheria patients in Jakarta are resistant to tetracycline. Since this is a commonly used antibiotic in lieu of penicillin, clinicians should be aware that it may not be effective in treating diphtheria.

Nasopharyngeal-alginate swab specimens were collected from 181 suspected diphtheria patients in Cipto Mangunkusumo Hospital, Jakarta, Indonesia from October 1979 through June 1980. Cystine tellurite blood agar medium (14) was inoculated with the specimen and incubated 18 to 72 h at 37°C. Suspect *C. diphtheriae*

colonies were subcultured to brain heart infusion agar, and the cultures were incubated 18 to 20 h at 37°C. Clones were tested for biochemical activity and biotype according to Herman and Bickham (5) and toxin production by a modified Elek procedure (1) with a diphtheria antitoxin (Bio Farma, Bandung, Indonesia).

The disk diffusion method (7) with Mueller-Hinton agar (BBL Microbiology Systems, Cockeysville, Md.) supplemented with 5% sheep blood was used for susceptibility testing. The disk (BBL) antimicrobials included ampicillin (10 μg), cephalothin (30 μg), chloramphenicol (30 μg), erythromycin (15 μg), kanamycin (30 μg), clindamycin (2 μg), penicillin (10 U), and tetracycline (30 μg). An agar dilution method (15) was used to determine the minimal inhibitory concentration of tetracycline (0.25 to 128 $\mu\text{g}/\text{ml}$) in Mueller-Hinton agar (BBL) containing 5% sheep blood. A Steers (11) replicator was used to inoculate the agar dilution plates with about 0.001 ml of an inoculum prepared by incubation of a culture of *C. diphtheriae* in brain heart infusion broth (BBL) for 20 to 24 h at 37°C and dilution with Mueller-Hinton broth (BBL) to match a McFarland no. 0.5 standard. This provided about 10^4 colony-forming units per ml to the surface of the agar dilution plates. The tetracycline minimal inhibitory concentration for 107 of 133 isolates was confirmed by C. H. Jellard, Provincial Laboratory of Public Health, Edmonton, Alberta, Canada.

Toxigenic, biotype mitis, *C. diphtheriae* was isolated from 133 of the 181 patients within the 0- to 1- (17%), 2- to 3- (35%), 4- to 5- (20%), 6- to 7- (8%), 8- to 9- (8%), and 10- to 20- (12%) year-old age groups. All 133 *C. diphtheriae* isolates were sensitive to ampicillin, cephalothin, chloramphenicol, clindamycin, penicillin, erythromycin, and kanamycin. However, only 19 of 133 (14%)

were sensitive to tetracycline by this method. The tetracycline minimal inhibitory concentration was 0.5 $\mu\text{g/ml}$ for the same 19 *C. diphtheriae* isolates. The 114 remaining isolates were inhibited by 32 $\mu\text{g/ml}$ (32%), 64 $\mu\text{g/ml}$ (79%), or ≥ 128 $\mu\text{g/ml}$ (3%). The 19 sensitive strains were isolated from persons between 1 and 9 years old.

The tetracycline-resistant *C. diphtheriae* isolates (≥ 32 $\mu\text{g/ml}$) were interesting because of the reported sensitivity of *C. diphtheriae* to this antibiotic in other studies. Gordon et al. (4) tested 14 *C. diphtheriae* isolates and found all were sensitive to 0.8 μg of tetracycline per ml. Likewise, Jellard and Lipinski (6) tested 950 strains isolated from persons in one area of Canada, and all of the strains were sensitive to this antimicrobial agent with the disk diffusion method. However, two strains were highly resistant to erythromycin and lincomycin. Rare ($<0.07\%$) tetracycline-resistant isolates have been cultured from other persons in Canada (C. H. Jellard, personal communication). No other reports of studies could be found that showed such a tetracycline resistance pattern as did the isolates we studied. The reason for tetracycline resistance in Jakarta is not known. Tetracycline is regularly used empirically by clinicians in Indonesia to treat various bacterial-like infections in adults and children. Therefore, its common usage might have provided a selective mechanism leading to the present resistance.

The finding that only 19 of the 133 strains were sensitive to 0.5 μg of the antimicrobial agent per ml could suggest plasmid-mediated resistance since the minimal inhibitory concentration then became ≥ 32 $\mu\text{g/ml}$ for the remaining 90 (84%) isolates. Plasmid-mediated resistance to other antibiotics, erythromycin, and clindamycin, has been suggested by Coyle et al. (2). Since their report, Schiller et al. (10) have isolated plasmids from *C. diphtheriae* and diphtheroids that were shown to code for erythromycin resistance.

Whether the resistance of our Indonesian isolates is due to plasmid or cellular involvement remains to be determined. The fact remains that a significant number of the isolates from children and adults were resistant to clinically unachievable levels of tetracycline, and this antimicrobial agent should no longer be considered as an alternative for the treatment of diphtheria in Jakarta. Fortunately, the bacterium appears to remain sensitive to penicillin and erythromycin. Further study and continued surveillance seems warranted to determine the type of resistance

and whether *C. diphtheriae* develops resistance to these two antimicrobial agents in the future.

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