ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Dec. 1977, p. 673-677

Vol. 12, No. 6
Printed in U.S.A.

Antibiotic Susceptibility of *Chlamydia trachomatis*

H. JANE BLACKMAN,† CHIEKO YONEDA,[1] CHANDLER R. DAWSON,[1] AND JULIUS SCHACHTER[2]*

The World Health Organization Collaborating Centre for Reference and Research on Trachoma and Other Chlamydial Infections,[1, 2] Francis I. Proctor Foundation for Research in Ophthalmology,[1] and The George Williams Hooper Foundation for Medical Research,[2] University of California San Francisco, San Francisco, California 94143

Received for publication 31 August 1977

The antibiotic susceptibility of *Chlamydia trachomatis* isolates was determined in a tissue culture system. Representatives of all currently recognized serotypes of trachoma-inclusion conjunctivitis agents were tested. Tetracycline and erythromycin yielded similar results, with 1.0 μg/ml preventing chlamydial replication. Rifampin was the most active antibiotic, with 0.25 μg/ml completely suppressing inclusion formation of all strains. Fifty percent end points were usually achieved at one-fourth to one-eighth the 100% suppression level. Penicillin was not as effective, and the assays were often irregular. Antibiotic susceptibility of these chlamydiae was essentially the same, regardless of serotype, anatomic site infected, geographic origin, or antibiotic use in the community.

The agents of trachoma and inclusion conjunctivitis are significant human pathogens. They belong to the species *Chlamydia trachomatis* and not only cause eye disease, but also are major causes of genital tract infections (5). The original isolation procedures for these organisms involved the use of embryonated hens' eggs (10). These chlamydial strains were first recovered less than 20 years ago from ocular disease and later from genital tract infections. Many studies were performed in embryonated hens' eggs on the susceptibility of the organisms to chemotherapeutic agents (6, 11). Since the yolk sac technique was a difficult and time-consuming one, the introduction of the tissue culture (TC) method was a major technical advance for the isolation of these chlamydiae (3). This TC technique was then used for the determination of the antibiotic susceptibility of these organisms (4).

Ridgway et al. (8) have recently published data indicating that a lymphogranuloma venereum chlamydial strain was susceptible to tetracycline and erythromycin, but less so to penicillin, as determined by the TC assay.

The determination of antibiotic susceptibility of chylamydial agents has a number of potentially useful applications, among which are the following: (i) the selection of appropriate antibiotics for individual treatment or large public health programs; (ii) monitoring for the appearance of drug resistance to widely used antibiotics (e.g., in trachoma control programs); and (iii) as a useful tool in epidemiological studies. Indeed, Shiao et al. (9) tested the antibiotic susceptibility of a number of strains in embryonated eggs and found that more isolates from the genital tract were relatively resistant to penicillin. Presumably, resistance developed as a result of penicillin treatment for gonorrhea in patients with overt sexually transmitted disease (9).

For these reasons we studied the antibiotic susceptibility of all major serotypes of the *C. trachomatis* trachoma-inclusion conjunctivitis agents (from immunotype A though immunotype K). The antibiotics that we chose to assay were tetracycline, generally considered to be the drug of choice, and erythromycin, an accepted alternative. Rifampin was also included, as Becker et al. (1) have suggested the theoretic advantages of this antibiotic. Penicillin susceptibility was determined in hopes that this might be a useful epidemiological tool. An effort was made to obtain a broad geographic distribution of the isolates. Several chlamydial isolates, obtained in a village in Tunisia before and after mass treatment campaigns with topical tetracycline, were tested to determine whether antibiotic susceptibility patterns changed and resistance emerged. The same village had also been the site of treatment trials with topical tetracycline, erythromycin, and rifampin (2).

MATERIALS AND METHODS

TC assay. The irradiated McCoy cell technique was used. The cells were grown in Eagle minimal

† Present address: National Eye Institute, Bethesda, MD 20014.

essential medium, with 10% inactivated fetal calf serum, glutamine, and glucose added. No antibiotics or antifungal agents were included in maintenance or testing media, except for those specifically assessed in the experiment. The cells were irradiated with 5,000 rads. They were then suspended with trypsin and replanted on 12-mm glass cover slips in 15-mm-diameter, flat-bottomed glass tubes at approximately 125,000 cells per cover slip. The cells were incubated at 37°C in 5% CO_2 to allow the formation of a monolayer. Before infection, the cells were washed with phosphate-buffered saline. The inoculum was a predetermined dilution of the chlamydial immunotype (titered to yield 100 to 300 inclusions per cover slip) in 1 ml of medium. This inoculum was then centrifuged onto the cells at 2,700 × *g* for 1 h at 27°C. The medium was replaced with growth medium or 1 ml of growth medium containing twofold dilutions of the antibiotic being tested. Each dilution was tested in triplicate. The infected cells were incubated for 60 h, washed with phosphate-buffered saline, fixed with methanol, and stained with iodine. The inclusion counts were determined for the entire cover slip by microscopic examination at ×400.

The stained cover slips were decolorized with methanol and restained by the Giemsa technique to determine whether non-iodine-staining inclusions were produced. However, for counting purposes, only the iodine counts are presented.

Chlamydial isolates. The prototype chlamydial strains were obtained from a collection at the World Health Organization Collaborating Centre for Reference and Research on Trachoma and Other Chlamydial Infections in San Francisco. These prototype strains have been described in the series of publications by Wang and associates (reviewed by Grayston and Wang [5]). Further details on anatomic site and geographic area from which the agents were recovered are included in Table 1. The isolates from Tunisia were made in this laboratory and typed by L. Hanna (University of California). The type B and SEATO isolates are recent isolates and not well-characterized prototypes. All inocula were taken from frozen yolk sac pools because we obtained more reproducible results with frozen yolk sac pools than with frozen TC pools.

Antibiotics. The antibiotics were obtained from the manufacturers as pure powder without preservatives. Solutions were prepared, and portions were frozen at −20°C. Concentration and activity were confirmed by bioassay against standard preparations.

RESULTS

In general, the strains were quite susceptible to the antibiotics tested. For example, chlortetracycline resulted in a complete suppression of chlamydial growth at ≤1.0 μg/ml for all isolates tested. The range of susceptibility was quite narrow, with the most sensitive isolate (SEATO-488) completely suppressed by 0.125 μg of tetracycline per ml. Analogous results were obtained with erythromycin. A complete suppression of inclusion formation was found for all isolates in a range of 0.125 to 1.0 μg of erythromycin per ml. Thus, the in vitro results are consistent with clinical observations. The 50% inhibitory dose was usually one-fourth to one-eighth the 100% suppression level. Results for all strains are presented in Table 1. With most strains, rifampin was approximately 10-fold more active in vitro than was either chlortetracycline or erythromycin. Complete suppression was obtained for all immunotypes at rifampin concentrations of ≤0.25 μg/ml, and 50% suppression was observed at concentrations as low as 0.008 μg/ml. The geometric mean antibiotic concentrations yielding 100 and 50% reduction of inclusion counts, respectively, were: tetracycline, 0.8 and 0.13 μg/ml; erythromycin, 0.5 and 0.09 μg/ml; and rifampin, 0.05 and 0.014 μg/ml. Rifampin was significantly more active than the other antibiotics at both levels of suppression (*t* test, $P < 0.01$). Erythromycin was significantly more active than tetracycline ($P < 0.05$) at 50% inclusion reduction only. The type B isolates from Tunis had similar antibiotic susceptibility patterns although they were isolated before and after a mass treatment campaign with topical tetracycline and clinical trials of topical tetracycline, erythromycin, and rifampin.

Representative titrations for these end points are presented in Table 2. A penicillin assay has been omitted from this representative table because the penicillin assays were either more irregular or less reproducible for most strains than were the results obtained with the other three antibiotics. The results for the other three antibiotics presented in Table 2 are quite similar to those obtained with all other isolates. Penicillin showed a greater range of end points for a complete suppression of inclusion formation (0.25 to 10 U/ml). Most strains were relatively susceptible, with 13/17 being completely suppressed at ≤1.0 U. As with other antibiotics, there was approximately a four- to eightfold differential between the 50% suppressing level and the 100% level. Some bizarre forms and vacuoles with large forms were seen in penicillin-treated cultures. Giemsa-stained monolayers did not yield different end points. The radiation process produces a large number of vacuoles and granules in the cells, making it difficult to count inclusions stained by the Giemsa method.

DISCUSSION

Tetracycline and erythromycin are generally considered the drugs of choice in treating

TABLE 1. *Suppression of inclusions of chlamydia immunotypes*[a]

Immunotype	Strain or isolate	Geographic origin and anatomic site	Suppression							
			μg of antibiotic per ml						U of antibiotic per ml	
			Chlortetracycline		Erythromycin		Rifampicin		Penicillin	
			100%	50%	100%	50%	100%	50%	100%	50%
A	HAR-1	Saudi Arabia–eye	1.0	0.125	0.5	0.25	0.125	0.016	0.5	0.06
B	583-Tunisia	Tunisia–eye	0.5	0.125	0.25	0.125	0.03	0.008	≥1.0	–[b]
	864-Tunisia	Tunisia–eye	1.0	0.125	1.0	0.125	0.06	0.008	≥1.0	ND[c]
	809-Tunisia	Tunisia–eye	1.0	0.25	0.5	0.06	0.25	0.016	0.5	0.06
C	TW-3	Taiwan–eye	1.0	0.5	0.5	0.06	0.25	0.03	0.5	0.06
D	IC Cal-8	California–eye	1.0	0.25	0.5	0.06	0.06	.016	<10	–[b]
E	Bour	California–eye	1.0	0.125	1.0	0.25	0.125	<.06	≤1.0	0.1
	707	California–genital	1.0	<0.25	≤1.0	ND	ND	ND	≤1.0	<0.1
	797	California–genital	1.0	0.125	0.5	0.06	ND	ND	0.5	0.25
F	IC Cal-3	California–eye	1.0	0.125	0.5	0.125	0.016	0.004	0.25	0.03[b]
G	392	California–eye	1.0	0.125	0.25	0.06	0.06	0.016	0.25	0.06
H	471	California–genital	1.0	0.125	0.5	0.06	0.06	0.03	0.25	0.03
I	523	California–eye	1.0	0.06	ND	ND	ND	ND	1.0	0.10
J	475	California–genital	0.5	0.06	1.0	0.125	0.06	0.016	≥1.0	ND
K	UW 31	Seattle–genital	1.0	0.5	0.5	0.125	0.03	0.008	0.25	0.06
SEATO-68		Bangkok–genital	0.5	0.06	0.125	0.06	0.016	0.008	0.5	0.125
SEATO-488		Bangkok–genital	0.125	0.03	0.5	0.06	ND	ND	0.25	0.06

[a] Iodine staining.
[b] Irregular result.
[c] ND, Not done.

TABLE 2. *Antibiotic susceptibility of C. trachomatis (example, Tunisian isolate 864)*

μg of antibiotic per ml	Suppression of inclusions (%)		
	Chlortetracycline	Erythromycin	Rifampin
1.00	100[a]	100	
0.50	95	97	
0.25	82	86	
0.125	66	57	
0.063	14	38	100
0.031		17	98
0.016			85
0.008			49
0.004			14

[a] Average inclusion count was 226 in antibiotic-free cells.

chlamydial infections. The replication of all strains tested was completely suppressed at readily achievable levels (≤1.0 μg/ml).

The results of this study indicate that there is a marked similarity in antibiotic susceptibility of all *C. trachomatis* isolates, regardless of anatomic site involved in the infection, immunotype recovered, or area of residence of the infected individual. Isolates obtained from Asia, Africa, or North America all had similar antibiotic susceptibility patterns.

Several of the isolates tested were derived from the genital tract. They did not show any specifically increased resistance to penicillin. One of the isolates (392-type G) was isolated from a patient after penicillin treatment for gonococcal infection, and the penicillin susceptibility of this strain was quite similar to the others. Penicillin assays were often irregular, and some of the end points were difficult to determine accurately. A similar problem was described by Ridgway et al. (8).

As expected from the results of Becker et al. (1), rifampin had a greater activity in vitro than did either tetracycline or erythromycin. However, this in vitro superiority has not been corroborated in clinical situations, where therapeutic results are essentially equivalent with tetracycline or erythromycin (2). Keshishyan et al. (7) have shown that rifampin resistance may be easily induced in vitro. However, resistance did not emerge in isolates obtained from trachomatous individuals in a community where rifampin, chlortetracycline, and erythromycin had been used.

Thus, the results obtained in this study indicate that antibiotic susceptibility patterns determined in the TC system are generally analogous to those observed in the clinical situation. But the results with penicillin, which is active in vitro but not clinically useful, caution against overly enthusiastic extrapolation from laboratory results. Clinical trials of candidate antimicrobial agents will be required to prove efficiency.

The complicated growth cycle of the *Chlamydia* allows for different levels of antibiotic susceptibility at differing developmental stages. For example, penicillin is active during the stage of the cycle when cell wall synthesis and binary fission take place. Penicillin is ineffective in later stages when "condensation" occurs. Our assay system was based on adding the antibiotics shortly after infection. Markedly different results would have been obtained had some of the antibiotics been added at different stages of the developmental cycle. However, it seems reasonable to assess the role of antibiotics in preventing replication by treatment at the time of infection.

The susceptibility patterns determined by TC are similar for chlamydiae, regardless of anatomic site infected or geographic area involved, suggesting that antibiotic susceptibility will not be a useful epidemiological tool. Resistance has not been shown to develop in isolates obtained in communities before and after chemotherapy campaigns and trials. The TC system offers a simple and relatively sensitive method for determining the antibiotic susceptibility of *C. trachomatis* and offers the potential for routine monitoring to determine whether resistance would develop in the clinical milieu and for testing new chemotherapeutic agents.

ACKNOWLEDGMENTS

This work was supported by Public Health Service grants 5 TO1 EY-00094 and EY-004427 from the National Eye Institute and AI-10193 from the National Institute of Allergy and Infectious Diseases.

LITERATURE CITED

1. Becker, Y., Y. Asher, N. Himmel, and Z. Zakay-Rones. 1969. Rifampicin inhibition of trachoma agent in vivo. Nature (London) 224:33–34.
2. Dawson, C. R., I. Hoshiwara, T. Daghfous, M. Messadi, D. W. Vastine, and J. Schachter. 1975. Topical tetracycline and rifampicin therapy of endemic trachoma in Tunisia. Am. J. Ophthalmol. 79:803–811.
3. Gordon, F. B., and A. L. Quan. 1965. Isolation of the trachoma agent in cell culture. Proc. Soc. Exp. Biol. Med. 118:354–359.
4. Gordon, F. B., and A. L. Quan. 1972. Susceptibility of *Chlamydia* to antibacterial drugs: tests in cell cultures. Antimicrob. Agents Chemother. 2:242–244.
5. Grayston, J. T., and S.-P. Wang. 1975. New knowledge of chlamydiae and the diseases they cause. J. Infect. Dis. 132:87–105.
6. Jawetz, E. 1969. Chemotherapy of chlamydial infections, p. 253–282. *In* S. Garattini, A. Goldin, F. Hawking, and I. J. Kopin (ed.), Advances in pharmacology and chemotherapy, vol. 7. Academic Press Inc., New York.

7. **Keshishyan, H., L. Hanna, and E. Jawetz.** 1973. Emergence of rifampin-resistance in *Chlamydia trachomatis*. Nature (London) 244:173–174.
8. **Ridgway, G. L., J. M. Owen, and J. D. Oriel.** 1976. . A method for testing the antibiotic susceptibility of *Chamydia trachomatis* in a cell culture system. J. Antimicrob. Chemother. 2:71–76.
9. **Shiao, L. C., S.-P. Wang, and J. T. Grayston.** 1967. Sensitivity and resistance of TRIC agents to penicillin, tetracycline, and sulfa drugs. Am. J. Ophthalmol. 63:1558–1568.
10. **T'ang, F.-F., H.-L. Chang, Y.-T. Huang, and K.-C. Wang.** 1957. Trachoma virus in chick embryo. Natl. Med. J. China 43:81–86.
11. **Tarizzo, M. L., and R. Nataf.** 1970. The treatment of trachoma/Le traitement du trachome. Rev. Int. Trach. 47:7–48/49–87.