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Penicillin Sensitivity and Serum Resistance are Independent Attributes of Strains of *Neisseria gonorrhoeae* Causing Disseminated Gonococcal Infection

B. I. EISENSTEIN,* T. J. LEE, AND P. F. SPARLING

Department of Medicine, University of North Carolina School of Medicine, Chapel Hill, North Carolina 27514

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We have determined that isolates of *Neisseria gonorrhoeae* from patients with disseminated gonococcal infection (DGI) are different from randomly collected isolates from patients with uncomplicated (local) disease. Our comparison was based on the six phenotypic properties of: sensitivity to penicillin (Pen^S), erythromycin, and streptomycin; resistance to the bactericidal effects of pooled human sera; requirements for arginine, hypoxanthine, and uracil (AHU^-); and sensitivity to toxic agar. Although the marked association among these traits made analysis difficult, several factors independently related to virulence were defined. The DGI isolates were significanly more Pen^S and resistant to serum, even when the other variables were held constant. An apparent correlation between AHU^- auxotype and virulence was shown to be due to the Pen^S property of most AHU^- isolates. Thus, certain mutations to antibiotic resistance, as well as susceptibility to sera, may result in loss of virulence in the gonococcus, perhaps through alteration of cell envelope structure.

Various phenotypic properties of strains of *Neisseria gonorrhoeae* that caused disseminated gonococcal infection (DGI) have been recently described. These properties, which are much less common in strains causing only uncomplicated local infections, include increased penicillin sensitivity (28), unique nutritional requirements (8, 13), agar susceptibility (8), atypical growth patterns (13), and resistance to the bactericidal action of normal human sera (21). It has not been shown, however, which, if any, of these properties is directly and independently related to virulence or to the potential for bloodstream invasiveness. In particular, the characteristic of requiring arginine, hypoxanthine, and uracil for growth (AHU^- auxotype) has been almost absolutely correlated with susceptibility to penicillin and toxic agar in strains isolated in Seattle.

The present study describes an epidemiological investigation of 502 randomly isolated gonococcal strains causing localized disease and 39 strains recovered from patients with DGI. The phenotypic properties evaluated were: sensitivities to penicillin (Pen^S), erythromycin (Ery^S), and streptomycin (Str^S); nutrient requirements for growth; susceptibility to the bactericidal action of pooled human sera; and sensitivity to toxic agar. An attempt was made to determine not only whether any of these properties was related to virulence but also which properties were correlated with one another, and thus which factors were independently related to virulence. In addition, transformation experiments were performed to analyze the genetic independence of selected factors in isogenic sets of gonococcal strains.

An underlying hypothesis of our investigation was that "wild type" characteristics or lack of mutations to increased antibiotic resistance might be directly related to virulence. Therefore, we defined Pen^S as the property of having a minimal inhibitory concentration (MIC) to penicillin of ≤ 0.03 μg/ml. This breakpoint is based on genetic studies by Sparling et al. (24). Since high-degree streptomycin resistance is a one-step mutation, Str^S was defined as an MIC ≤ 200 μg/ml. Instead of using a simple breakpoint MIC dichotomy for the other antibiotics evaluated, the erythromycin "wild type" (Ery^{WT}) was defined as an intermediate sensitivity pattern with an MIC between 0.25 and 1 μg/ml. This definition is based on genetic and physiological observations made by Sparling and associates (3, 20, 24) which indicate that both erythromycin-resistant (MIC $>$ 1) and -sensitive (MIC $\leq$ 0.12) strains are often genetic mutants. Strains with the intermediate sensitivity pattern most likely have neither mutation.

MATERIALS AND METHODS

Bacterial strains. From March 1974 to October 1976, 39 strains of *N. gonorrhoeae* were collected consecutively from patients with DGI seen either at North Carolina Memorial Hospital (Chapel Hill) or supplied by R. Corey at Duke Hospital (Durham). Eighteen of these were definite DGI strains, being isolated from the skin, joint, or blood of patients with characteristic clinical features (4). A probable DGI strain was one that was isolated from the pharynx, urethra, rectum, or cervix of a patient with characteristic clinical signs together with prompt resolution of systemic symptoms during antimicrobial therapy. In addition, strains of *N. gonorrhoeae* causing uncomplicated or localized infection were obtained at different time periods from the endocervix or urethra of consecutive male and female patients seen at either Durham or Chapel Hill, North Carolina. A total of 347 strains isolated in all months but July and August of 1973 and 155 isolates collected in the first six months of 1976 were analyzed as local strains.

Transformation and sera experiments were performed with strains F62, FA1035, FA19, FA48, and FA5. Strain F62 was kindly supplied by D. S. Kellogg, Jr., Atlanta, Ga. Strain FA1035 was obtained from a patient with DGI from Seattle, Wash., and kindly supplied by K. K. Holmes. The other strains have been described in detail previously (19, 24). All strains were confirmed as *N. gonorrhoeae* by colonial morphology, the Gram stain, oxidase reaction, and, in most cases, metabolism of glucose but not maltose. Strains were stored at −70°C in Trypticase soy broth containing 20% glycerol and subcultured from the frozen state. Most strains were primarily of colonial type 3 or 4, except for transformation experiments, when colonial type 1 or 2 strains were used (7).

Media and growth. Determination of colonial morphology and routine subculture were on GC base agar (Difco) containing 1% defined supplements 1 and 2 of Kellogg et al. (7). Broth cultures were in GC base broth, which was made identical to GC base agar containing 1% defined supplements 1 and 2 except for the omission of starch and agar. All cultures were incubated at 37°C in a humidified 5% CO_2 incubator.

Antibiotic sensitivity and auxotyping. Approximately 10^3 colony-forming units were inoculated with a Steers replica-inoculator device onto GC base agar containing 1% defined supplements 1 and 2 and doubling dilutions of antibiotic. The ranges of antibiotic concnetrations studied were 0.008 to 1 μg of penicillin per ml and 0.015 to 4 μg of erythromycin per ml. Auxotyping media, including the complete defined (NEDA) medium, were prepared as described by Catlin (1). Purified agar (100 g) was washed once with water and twice with ethanol, followed by four additional methanol washes. Each wash was performed by 5 min of manual stirring of the agar in 1 liter of methanol followed by vacuum-assisted filtration. The damp agar was dried at 37°C overnight before use. Cultures were incubated for 40 h at 37°C in an atmosphere of 5% CO_2. For antibiotic testing, the MIC was the least concentration that prevented visible growth. Results were reproducible within one dilution on replicate determinations. Control strains of known sensitivity were included in each test. Media for antibiotic sensitivity testing were always used within 48 h of preparation.

Toxic agar sensitivity. Two different agar grades of NEDA were made to test for toxic agar sensitivity. The standard grade utilized water-ethanol-methanol-washed agar, as used for auxotyping. The toxic grade agar was prepared by water-ethanol washing only, omitting the methanol wash step. Sensitivity to toxic agar was determined by inoculating cells onto NEDA prepared with either standard or toxic grade agar. Decreased or absent growth on the toxic agar with growth on the standard agar was defined as toxic agar sensitivity. Equal growth was defined as toxic agar resistance. The same lot of purified agar was used in all determinations.

Serum bactericidal assay. Pooled human sera from eight individuals without history of previous gonococcal infection were stored at −70°C and thawed only once at room temperature. A modification of the micromethod described by Tramont et al. (26) was used to test strains for resistance to serum bactericidal activity. Strains were subcultured onto GC base agar containing 1% defined supplements 1 and 2 of Kellogg, and after 20 h of growth cells were suspended in sterile phosphate-buffered solution (minimal broth Davis, Difco) to give approximately 10^5 colony-forming units/ml. Equal amounts (0.05 ml) of this suspension were added to microtiter plate wells containing either 0.05 ml of minimal broth with 0.5% bovine albumen (control well) or 0.025 ml of minimal broth with 0.5% bovine albumen and 0.25 ml of pooled human sera (test well). As an additional control, pooled human sera were heated at 56°C for 30 min to inactivate complement. The microtiter plate with test wells was incubated for 30 min at 37°C with 5% CO_2, after which time 10-μl amounts from each well were streaked onto GC base agar containing 1% defined supplements 1 and 2 of Kellogg and incubated at 37°C for 40 h with 5% CO_2. Strains that exhibited ≥80% decrease in survival after incubation with serum compared to incubation in control wells were considered serum sensitive. A known serum-sensitive strain (F62) was used as a control strain to check the activity of the pooled sera used. As an external control of this method, about half of the strains tested in this way were also harvested from the exponential phase of growth in GC base broth with defined supplements 1 and 2 prior to the test for serum sensitivity. Selected strains were also studied as colonial types 1 or 4 and as newly subcultured or serially passed (10 to 14 daily subcultures) cells. Results were highly reproducible and unchanged by growth, colonial type, or number of serial passes.

Transformation procedure. Transformation procedure, preparation of transforming deoxyribonucleic acid, and other methods were essentially as

described previously (19, 22, 24).

Antibiotics. Antibiotics were from sources previously described (19). Other chemicals were of the highest available purity.

Statistical methods. Both Fisher's exact test and, when applicable, chi-square tests were used to determine the significance of relationships of phenotypic characteristics between groups of strains (25). Statistical evaluation of the data was aided by a computer program written by the Biostatistics Department of the University of North Carolina School of Public Health.

RESULTS

Changing epidemiology of phenotypic characteristics with year of isolation. Our primary goal was to find if certain phenotypic properties correlated more with DGI strains as compared with local isolates. However, since our DGI strains were isolated over a 3-year span of time (1974–1976), we felt that it was necessary to first ascertain the phenotypic stability of our denominator population of local isolates over a similar period of time (1973 versus 1976).

Results showed that a significant change had occurred in the local isolates between 1973 and 1976 towards increased penicillin sensitivity ($P < 0.05$), streptomycin sensitivity ($P < 0.001$), and incidence of the $Arg^- Hyx^- Ura^-$ (AHU^-) auxotype ($P < 0.005$) (Table 1). No change was apparent for serum, erythromycin, or toxic agar sensitivity. Comparing the old (1974) and new (1975–1976) DGI strains for the same characteristics (Table 2), the only significant change had been in the increased incidence of the AHU^- auxotype. An additional trend towards increased Pen^S ($P = 0.08$) had also occurred. No difference between definite and probable DGI strains was detected.

Phenotypic comparisons between DGI and local isolates. It was felt that the most conservative comparison between DGI and local strains would use the subset of local strains that was least different from the total group of DGI isolates. Therefore, all the comparisons of phenotypic frequencies used the recent (1976) local strain subset as the basis for comparison. Since the DGI isolates had not changed over time for most properties studied, they were grouped together in most comparisons against the local isolates. Because of the fluctuation over time of the Pen^S and AHU^- frequencies, only the 1975–1976 DGI strains were used in

TABLE 1. *Frequency of phenotypic properties among genitourinary isolates from patients with uncomplicated gonorrhea*

Phenotype[a]	No. of isolates with phenotype/total no. of isolates		*P* value[b]
	1973	1976	
Pen^S	104/347 (28%)	61/155 (39%)	<0.05
Str^S	222/347 (64%)	130/155 (84%)	<0.001
Ery^{WT}	227/337 (67%)	110/155 (71%)	NS
$Serum^R$	17/25 (68%)	71/114 (62%)	NS
$Arg^-Hyx^-Ura^-$ auxotype (AHU^-)[c]	5/78 (6.4%)	32/153 (21%)	<0.005
Toxic $agar^S$	8/30 (27%)	33/94 (35%)	NS
Total no. of isolates	347	155	

[a] Pen^S was defined as MIC ≤ 0.03 μg/ml. Str^S was defined as MIC ≤ 200 μg/ml. Ery^{WT} was defined as having an MIC of 0.25 to 1 μg/ml.
[b] Chi-square test. NS, Not significant.
[c] Includes $Pro^-Arg^-Hyx^-Ura^-$ strains.

TABLE 2. *Frequency of phenotypic properties of DGI isolates by year of isolation*

Phenotype	No. of isolates with phenotype/total no. of isolates			*P* value[a] (1974 vs 1975–76)
	1974	1975	1976	
Pen^S	7/12 (58%)	7/8 (88%)	17/19 (89%)	NS
Str^S	11/12 (92%)	7/8 (88%)	18/19 (95%)	NS
Ery^{WT}	11/12 (92%)	8/8 (100%)	17/19 (89%)	NS
$Serum^R$	2/2 (100%)	7/8 (88%)	19/19 (100%)	NS
AHU^-	1/10 (10%)	3/7 (43%)	13/19 (64%)	0.008
Toxic $agar^S$	4/9 (44%)	4/8 (50%)	10/16 (63%)	NS
Total no. of isolates	12	8	19	

[a] Fisher's exact test. NS, Not significant.

comparison against the 1976 local strains. The latter represented a midpoint time of collection relative to the DGI isolates used for those comparisons.

Significant differences between DGI and local strains were apparent for the properties of penicillin sensitivity, the erythromycin "wild type," the AHU$^-$ auxotype, and serum resistance (serum^R; Table 3). No statistically significant differences were seen for streptomycin, or toxic agar sensitivity, though certain trends emerged. There was a tendency towards increased toxic agar sensitivity among the DGI isolates. The lack of a difference in Str^S between local and DGI isolates may have been due to the comparison of DGI isolates with only recent (1976) predominantly Str^S local isolates. However, comparison of 1974 DGI isolates with 1973 local isolates showed that 11 of the 12 former isolates (92%) and only 222 of the latter 347 isolates (64%) were Str^S ($P < 0.05$), indicating that Str^S may be an additional characteristic of DGI isolates.

Correlations among phenotypic characteristics. Since previous studies (8, 13) have shown a very high correlation between the properties Pen^S and AHU$^-$, we reexamined this correlation, as well as those among the other phenotypic properties, in the recent random (1976 local) isolates. DGI isolates were not included in this analysis, since they represented a set of nonrandom strains selected for the property of bloodstream invasiveness. These virulent strains might, therefore, have possessed a biased congruence of certain phenotypes.

We found striking correlations between AHU$^-$ and Pen^S, between Ery^{WT} and Pen^S, and between Str^S and Pen^S (Table 4). The correlations among the antibiotics were similar to those previously found in this laboratory (9, 23). Notably only one AHU$^-$ strain was found to be penicillin resistant (Pen^R). Although AHU$^-$ was also associated with Ery^{WT} and Str^S, these associations disappeared after adjusting them for the striking AHU$^-$-Pen^S association. In addition, we found no significant association between serum^R and either Pen^S or AHU$^-$ (Table 5).

Adjusted strain comparisons by year of isolation. Because of the marked associations found between Pen^S and AHU$^-$, we reexamined the epidemiology of Pen^S and AHU$^-$ frequencies over time after adjusting the data for this striking correlation. Although the adjusted AHU$^-$ frequency markedly increased from 1973 to 1976 (20 to 51% among Pen^S strains only), the adjusted Pen^S frequency remained relatively stable (Table 6). A similar pattern emerged for the 1974 and 1975–1976 DGI strains (data not shown). Since all 17 AHU$^-$ DGI strains and 36 of 37 AHU$^-$ local strains were Pen^S, the increased frequency of Pen^S seen among recent isolates was probably secondary to a more marked increase in AHU$^-$ strains. Among the AHU$^+$ strains only, there was a small change (31 to 25%) towards decreased penicillin sensitivity.

TABLE 4. *Associations between Pen^S and other phenotypes in random (1976 local) isolates*

Phenotype	No. of isolates with phenotype/ total no. of isolates		P value[a]
	Pen^S	Pen^R	
AHU$^-$	31/61 (51%)	1/92 (1%)	<0.001
Ery^{WT}	51/61 (84%)	60/94 (64%)	<0.01
Str^S	59/61 (97%)	71/94 (76%)	<0.001

[a] Chi-square test.

TABLE 5. *Associations between serum^R and other phenotypes among random (1976 local) isolates*

Phenotype	No. of isolates with phenotype/ total no. of isolates		P value[a]
	Serum^R	Serum^S	
Pen^S	32/71 (45%)	14/43 (33%)	NS
AHU$^-$	15/71 (21%)	7/43 (16%)	NS

[a] Chi-square test. NS, Not significant.

TABLE 3. *Unadjusted frequency of phenotypic properties: 1976 local versus DGI isolates*

Phenotype[a]	No. of isolates with phenotype/total no. of isolates		P value[b]
	DGI isolates	Local isolates	
Pen^S	24/27 (89%)	61/155 (39%)	<0.001
Str^s	36/39 (92%)	130/155 (84%)	NS
Ery^{WT}	36/39 (92%)	110/155 (71%)	0.006
Serum^R	28/29 (97%)	71/114 (62%)	<0.001
AHU$^-$	16/26 (62%)	32/153 (21%)	<0.001
Toxic agar^S	18/33 (55%)	33/94 (35%)	NS
Total no. of isolates	39	155	

[a] Only 1975–1976 DGI strains were examined for the Pen^S and AHU$^-$ DGI isolates.
[b] Fisher's exact test. NS, Not significant.

Factors independently associated with DGI isolates. We found that DGI strains were characteristically Pen^S, Ery^{WT}, AHU^-, and $serum^R$ (Table 2). To determine which factors were independently associated with virulent infection, we reexamined each variable after adjusting for significant correlations among the variables noted above. Thus, the Pen^S frequency was corrected for its high association with AHU^- and Ery^{WT}, and AHU^- and Ery^{WT} were adjusted for Pen^S (Table 7). Since neither the Ery^{WT} nor the AHU^- adjusted Pen^S frequencies changed with time, all years were used in these comparisons. The other comparisons were limited to the recent isolates.

Although the adjusted Pen^S frequencies remained much higher in the DGI group, the adjusted AHU^- and Ery^{WT} frequencies did not. Thus, our earlier finding of high AHU^- and Ery^{WT} frequencies in DGI strains (Table 3) was due to the marked AHU^--Pen^S and Ery^{WT}-Pen^S associations. Of these three phenotypic properties, only Pen^S appeared strongly and independently related to virulence. However, trends towards increased AHU^- (70 versus 52%) and Ery^{WT} frequencies (97 versus 84%, $P = 0.09$) did emerge among the Pen^s strains. Because $serum^R$ was not correlated with any other phenotypic property, no adjustment was made for $serum^R$ frequency. Hence, $serum^R$ was another phenotypic property independently related to virulence.

The properties of both Pen^S and $serum^R$ were present concurrently in only 28% (32/114) of the 1976 local strains but in 85% (23/27) of recent DGI strains ($P < 0.001$). Of the four DGI strains that were not Pen^S $serum^R$, three were Pen^R $serum^R$, and one was Pen^S $serum^S$. Table 8 presents data comparing the frequencies of AHU^-, Str^S, Ery^{WT}, and agar sensitivity ($agar^S$) among only the Pen^S $serum^R$ strains isolated recently. The trends towards increased AHU^- and Ery^{WT} frequencies in the DGI strains (Table 7) were statistically significant ($P < 0.05$) when this smaller cluster of strains was analyzed. Thus, AHU^- and Ery^{WT} may amplify virulence in strains already containing the main virulence-associated characteristics (Pen^S se-

TABLE 6. *Adjusted local strain comparisons by year*

Phenotype	No. of isolates with phenotype/total no. of isolates (%)		*P* value[a]
	1973	1976	
Pen^S			
Among AHU^- strains only	5/5 (100%)	31/32 (97%)	NS
Among AHU^+ strains only	20/64 (31%)	30/121 (25%)	NS
AHU^-			
Among Pen^S strains only	5/25 (20%)	31/61 (51%)	0.009
Among Pen^R strains only	0/44 (0%)	1/92 (1%)	NS

[a] Fisher's exact test. NS, Not significant.

TABLE 7. *Adjusted comparisons of DGI and local strains*

Phenotype	No. of isolates with phenotype/total no. of isolates		*P* value[a]
	DGI	Local	
Pen^S			
Among AHU^- only	17/17 (100%)	36/37 (97%)	NS
Among AHU^+ only	12/19 (63%)	50/185 (27%)	<0.001
Among Ery^{WT} only[b]	23/24 (96%)	51/110 (46%)	<0.001
Among $Ery^{non\text{-}WT}$ only[b]	1/2 (50%)	10/45 (22%)	NS
AHU^-			
Among Pen^S only[b]	16/23 (70%)	31/60 (52%)	NS
Among Pen^R only[b]	0/3 (0%)	1/92 (1%)	NS
Ery^{WT}			
Among Pen^S only	30/31 (97%)	51/61 (84%)	NS
Among Pen^R only	6/8 (75%)	59/94 (63%)	NS

[a] Fisher's exact test. NS, Not significant.
[b] Only recent strains were examined.

TABLE 8. *Frequency of phenotypic properties among recent Pen^S serum^R strains only*

Phenotype	No. of isolates with phenotype/total no. of isolates		P value[a]
	DGI	Local	
AHU$^-$	16/22 (73%)	14/32 (44%)	<0.05
Str^S	22/23 (96%)	32/32 (100%)	NS
Ery^{WT}	22/23 (96%)	24/32 (75%)	<0.05
Agar^S	13/20 (65%)	9/20 (45%)	NS

[a] Chi-square test. NS, Not significant.

rum^R). The disciminatory effect of adding all four factors was marked: 16 of the 26 (62%) recent DGI isolates but only 12 of the 114 (11%) 1976 local strains were Pen^S serum^R Ery^{WT} AHU$^-$ ($P < 0.001$).

Genetic manipulation of virulence-associated factors. Proof of independence of these virulence-associated properties required genetic studies in order to eliminate any subtle and unnoticed selection bias present in the strains studied. In transformation experiments identical to that described by Sparling et al. (24), the three genes for penicillin resistance, *penA, ery,* and *penB,* were serially donated from FA48 (prototrophic, Pen^R) to DGI strain FA1035, a Pen^S AHU$^-$ recipient. Whereas there was a fourfold increase in resistance to penicillin with each gene introduced (so that the recipient Pen MIC went from 0.015 to 1 μg/ml), there was no change in the AHU$^-$ property in the Pen^R transformants. The donation of gene *str* had no effect on these phenotypic properties either. Similar experiments were also performed using FA5 (Pen^R serum^S) as donor and FA19 (Pen^S serum^R) as recipient. All Pen^R transformants tested remained serum^R. Thus, the properties of AHU$^-$, Pen^S, serum^R, and Str^S were derived from separate genes in the gonococcus.

DISCUSSION

Our results indicate that virulence in the gonococcus is highly correlated with at least two phenotypic properties: penicillin sensitivity and resistance to the bactericidal effect of pooled human sera. More importantly, these two properties are independent of each other and of other characteristics previously associated with gonococcal virulence. In addition these properties, AHU$^-$ and Ery^{WT}, may have a smaller independent association with virulence among certain subsets. It is doubtful that a single invasive strain of *N. gonorrhoeae* is the cause of most cases of DGI because of the heterogeneity of nutritional requirements of our DGI strains and the observation by Johnston et al. (6) that their 16 DGI strains could be categorized into four different serotypes on the basis of envelope protein structure.

Weisner et al. (28) reported that DGI strains in Seattle were significantly more penicillin, tetracycline, and streptomycin sensitive than random isolates. In a more recent study from Seattle, however, Knapp and Holmes (8) showed that DGI isolates were not only usually Pen^S, but most (89%) had nutritional requirements for arginine, hypoxanthine, and uracil and most were inhibited by unknown substances in toxic agar. It was suggested that regional differences in the prevalence of the DGI syndrome might be related to differences in prevalence of AHU$^-$ strain(s). Since all of their highly penicillin-sensitive isolates were AHU$^-$, it was not possible to discriminate between AHU$^-$ and Pen^S as virulence factors.

Morello et al. (13) also found that DGI isolates in Chicago were usually Pen^S AHU$^-$ and exhibited "atypical" growth patterns. (In our studies, isolates that were AHU$^-$ and toxic agar^S also had slow "atypical" growth on GC base agar with 1% defined supplements 1 and 2.) Of their DGI isolates, 72% were Pen^S and 36% were AHU$^-$, whereas only 13% of their local isolates were Pen^S and about 5% were AHU$^-$. However, by controlling Pen^S and AHU$^-$ for the individual variables, further analysis shows that their data are in agreement with ours. Thus, among their Pen^S isolates only, the difference in AHU$^-$ between the DGI and local isolates was not significant, whereas the difference in Pen^S among non-AHU$^-$ isolates was significant (about 50% of the DGI isolates versus about 10% of the locals).

We conclude that AHU$^-$ strains are more virulent, not because of any major inherent direct effect of mutations to Arg$^-$ Hyx$^-$ or Ura$^-$ on virulence but rather because the AHU$^-$ strains are usually penicillin sensitive. This distinction is important both to future studies of the biochemical genetics of virulence and to understanding regional differences in prevalence of bacteremic gonococcal infection (8).

Schoolnik et al. (21) also showed that most DGI isolates were serum resistant. The correlation of serum resistance and antibiotic sensitivity with virulence is not unique to the gonococcus. Enteric bacteria that cause bacteremia are more serum resistant than those isolated from stool or urine samples (16, 27). In addition, isogenic strains of *Salmonella* that were made more resistant to penicillin became avirulent regardless of changes in serum sensitivity (17). The suggestion that this relationship may be true as well in *Neisseria meningitidis* was made by Miller and Bohnhoff in 1945 (12). One of seven strains which became highly Pen^R lost

all virulence for mice. Unlike other investigators, who found a close genetic relationship between antibiotic sensitivity and serum resistance (11, 17) in isogenic derivatives of *Salmonella, Shigella,* and *Escherichia coli,* we could find none.

Although the structural nature of virulence in the gonococcus is presently unknown, it is likely that it is related to the bacterial surface (18). Studies by various groups of investigators (2, 26) have shown that the serum bactericidal reaction in the gonococcus involves surface proteins and lipopolysaccharides. In addition, studies in *Salmonella, E. coli,* and *Shigella* (10, 14) indicate that changes in the lipopolysaccharide structure of bacterial envelopes markedly affects animal virulence. The association between cell surface and antibiotic resistance has also been firmly established in studies of envelope permeability in the gonococcus (3) and envelope biochemistry in the strains of *Salmonella* (14). In addition, experiments in this laboratory indicate that mutations that affect sensitivity to antibiotics and susceptibility to sera alter outer envelope proteins in isogenic strains of the gonococcus (unpublished data).

Although 85% of DGI strains but only 28% of local strains were both serum^R and Pen^S, our findings do not explain why some patients with presumed virulent (Pen^S serum^R) organisms do not develop DGI. Hence, these properties are, in general, necessary but not sufficient for organisms to disseminate. It is possible that there are additional bacterial virulence factors, such as AHU$^-$, Ery^{WT}, and others, as yet unidentified. Moreover, it is highly likely that host factors may help determine which potentially virulent organisms disseminate. For instance, Petersen et al. (15) have shown that human deficiency of the eighth component of complement may lead to susceptibility to DGI. Multivariate analysis on larger populations may add to a better understanding of the interactions among virulence-associated factors.

An aspect of this investigation that remains somewhat obscure is the recent increased prevalence of AHU$^-$ isolates from patients with uncomplicated disease. One possible reason for this change is the presence of some selective pressure in our treatment modality that would permit a greater failure rate among patients with AHU$^-$ strains than those with AHU$^+$ ones. Since AHU$^-$ strains generally are slow atypical growers (13), these strains may persist in patients treated with a high-dose, short-acting antibiotic that kills fast-growing organisms better than dormant ones (i.e., penicillin). Paradoxically, penicillin therapy may then allow more Pen^S strains to persist in the community because of the high association between AHU$^-$ and Pen^S. This scheme of events may explain the surprising observation by the National Monitoring Study (5) that a tendency towards a greater failure rate exists among patients harboring Pen^S strains compared to those with strains of intermediate penicillin resistance. This hypothesis might explain how the property Pen^S, or the property AHU$^-$, can be either an independent variable or merely an associated one, depending on whether the selective determinant is virulence or persistence. Pen^S strains, which are secondarily AHU$^-$, tend to produce DGI, whereas slowly growing AHU$^-$ strains, which are generally Pen^S, tend to persist in the community under present treatment regimen pressure.

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