Relationship of Complement Resistance and Selected Virulence Factors in Pathogenic Avian Escherichia coli

Richard E. Wooley, University of Georgia
Kathy R. Spears, University of Georgia
John Brown, University of Georgia
Lisa K. Nolan, University of Georgia
Oscar J. Fletcher, North Carolina State University

Available at: http://works.bepress.com/lisa_nolan/70/
Relationship of Complement Resistance and Selected Virulence Factors in Pathogenic Avian Escherichia coli

Author(s): Richard E. Wooley, Kathy R. Spears, John Brown, Lisa K. Nolan and Oscar J. Fletcher

Published by: American Association of Avian Pathologists
Stable URL: http://www.jstor.org/stable/1591764
Accessed: 09/05/2013 17:41
Relationship of Complement Resistance and Selected Virulence Factors in Pathogenic Avian Escherichia coli

Richard E. Wooley,^A Kathy R. Spears, ^ John Brown,^A
Lisa K. Nolan,^A and Oscar J. Fletcher^B

^Department of Medical Microbiology,
College of Veterinary Medicine, University of Georgia, Athens, Georgia 30602
^BCollege of Veterinary Medicine, North Carolina State University,
Raleigh, North Carolina 27606
Received 13 January 1992

SUMMARY. Complement resistance, antibiotic resistance profiles, and virulence profiles of 80 Escherichia coli isolates from the intestines of normal chickens (40 isolates) and chickens diagnosed as having colisepticemia (40 isolates) were compared. Differences were observed between the two groups for antibiotic resistance, siderophore production, presence of type 1 pili, complement resistance, motility, and size of plasmids. The systemic isolates were more likely to have siderophores and type 1 pili, and to be complement-resistant and motile than were the intestinal isolates. No differences between the two groups were observed for colicin production. Further comparison of the 10 most complement-resistant isolates from the systemic group and 10 most complement-sensitive isolates from the intestinal group revealed a correlation between an isolate’s resistance to complement and its ability to kill embryos, express type 1 pili, and be motile. Virulence of avian E. coli strains appears to be correlated with complement resistance and the interaction of this resistance with the ability to produce type 1 pili and be motile.

RESUMEN. Relación de la resistencia al complemento y algunos factores de virulencia sobre la patogenia de Escherichia coli aviar.

Se compararon la resistencia al complemento, la resistencia a los antibióticos, y los perfiles de virulencia de 80 cepas de Escherichia coli aisladas del intestino de pollos normales (40 cepas) y pollos con diagnóstico de colisepticemia (40 cepas). Se observaron diferencias entre los dos grupos en la resistencia a los antibióticos, producción de sideróforos, presencia de pili tipo 1, resistencia al complemento, motilidad y tamaño de los plásmidos. Las cepas aisladas de las aves con colisepticemia tuvieron mayor tendencia que las aisladas del intestino para producir sideróforos y pili tipo 1, ser resistentes al complemento y ser móviles. No se observaron diferencias entre los dos grupos con respecto a la producción de colicina. Una comparación posterior hecha entre las 10 cepas más resistentes al complemento seleccionadas de cada grupo, reveló correlación entre la resistencia al complemento y la capacidad para matar embriones, expresión del pili tipo 1 y la motilidad. La virulencia de las cepas de E. coli aviar parece estar correlacionada con la resistencia al complemento y la interacción de esta resistencia con la capacidad de producir pili tipo 1 y de ser móviles.

Microbial characteristics associated with virulent avian Escherichia coli include antibiotic resistance (5), production of colicins and siderophores (8,13,31,33), type 1 pili (1,8,11,22), plasmids (10,13,28,31), motility (1), hemolytic reaction (31), and embryo lethality (24). Resistance to the lytic action of host complement has also been implicated as a virulence-associated parameter in E. coli isolates from chickens and domestic animals with colisepticemia (9,23,24, 31,33).

The purpose of the present study was to examine the correlation of selected virulence parameters and complement resistance with virulence of avian E. coli by comparing the occurrence of these factors in pathogenic and non-pathogenic strains.
MATERIALS AND METHODS

Test organisms. Forty *E. coli* isolates cultured from chickens with natural cases of colisepticaemia and 40 *E. coli* isolates from the intestinal tract of normal birds were studied. Isolates from infected chickens were obtained from the liver, spleen, bone, or multiple organs of birds diagnosed as having colisepticaemia. Isolates from normal, healthy birds came from the intestinal tracts of broiler chickens at the time of slaughter.

Antibiotic resistance profiles. Antibiotic resistance profiles of the test organisms were determined by the disc diffusion method (4). Antibiotics tested were gentamicin (Gm), streptomycin (St), kanamycin (Kn), ampicillin (Am), chloramphenicol (Cm), polymyxin B (PB), nalidixic acid (Na), tetracycline (Te), and sulfoxsazole (Su).

Colicin activity. The colicin test organisms consisted of *E. coli* K-12 (ATCC 23559), a colicin-sensitive control, and the colicin producer *E. coli* K-12 (ATCC 23558). Colicin production was tested by overlaying chloroform-killed colonies of the test organisms with *E. coli* K-12 (ATCC 23559). *E. coli* strain (ATCC 23558) was included on each plate as a positive control (12). Plates were incubated at 37°C for 18 hours and observed for inhibition of growth.

Enterobactin (phenolate siderophore) assay. The siderophore control organisms consisted of *E. coli* AN 193, a non-enterobactin-producing mutant unable to synthesize dihydroxybenzoic acid but able to use enterobactin produced by other organisms (negative control), and *E. coli* AN 194, an enterobactin-producing strain (positive control) (27,29). Enterobactin production was bioassayed in petri dishes (60 × 15 mm) containing 10 ml of low iron agar with 30 μg deferrated ethylenediamine-N,N-diacetic acid (EDDA)/ml. *Salmonella typhimurium* LT-2 enb-7 (10⁴ colony-forming units [CFU/ml]) was used as the indicator organism for enterobactin activity (7). *E. coli* strains AN 193 and AN 194 were included in each test. The test organisms were passed five times in low iron medium, and an overnight culture was concentrated by centrifugation (5000 × g for 10 minutes) and filtration (0.45 μm-pore size filter). The culture filtrate was concentrated 10 times in an evaporator concentrator (Savant model SVC-100H; Savant Instruments, Inc., Farmingdale, N.Y.). Small wells placed in the agar were filled with 50 μl of the culture filtrate. Plates were incubated for 24 and 48 hours at 37°C and examined for growth around the wells (29).

Bacterial resistance to chicken complement. The lytic activity of complement on the test isolates was determined by a quantitative microtiter test method (18). Two-hour cultures grown in Luria-Bertani broth were diluted 1:50 in PG broth (1% peptone plus 1% glucose) (21), and 100 μl (10⁵ CFU) samples were inoculated into 96-well flat-bottomed microtiter plates (Microtest III; Becton, Dickinson & Co., Rutherford, N.J.). Aliquots (100 μl) of chicken complement or heat-inactivated chicken complement were added in twofold dilutions from 50% to 0.8%. Media and culture controls were included. Plates were monitored for growth by an enzyme-linked immunosorbent assay reader (Titertek Multiskan; Flow Laboratories, Inc., McLean, Va.) at 492 nm, absorbance mode, with a range of 1.0. Plates were incubated at 35°C and read hourly from 0 to 6 hours (18).

Type 1 pili: Mannose-sensitive hemagglutination (MSHA) tests. The presence of type 1 pili was detected by the ability of the test isolates to hemagglutinate erythrocytes. The test was done in 96-well round-bottomed microtiter plates with a 3% suspension of guinea pig or chicken erythrocytes in saline solution. Brain-heart infusion cultures of the test organisms were incubated statically in air, with subcultures made every 48 to 72 hours for five transfers before testing. Combinations of 25 μl each of culture, erythrocyte suspension, and either saline solution or 2% d-mannose were mixed and incubated at 37°C for 30 minutes before hemagglutination activity was assessed (15,25).

Plasmid isolation and agarose gel electrophoresis. Plasmid DNA from the test organisms was isolated and purified by the method of Birnboim and Doly (3) from overnight brain-heart infusion broth cultures. *E. coli* strain V 517 was used as a source of reference plasmids (19). Samples (25 μl) of plasmid DNA were loaded into wells of a 0.7% agarose gel (MC Corp., Rockland, Maine) and run at 40 volts for 12 hours on a horizontal electrophoresis apparatus (Model 452; E-C Apparatus Corp., St. Petersburg, Fla.). Gels were stained with ethidium bromide, visualized on an ultraviolet transilluminator, and photographed (29).

Embryo lethality test. To assay embryo lethality, overnight cultures of the isolates were washed twice in phosphate-buffered saline (PBS), resuspended and diluted in PBS, and inoculated into the allantoic cavity of twenty 12-day-old chicken embryos. Twenty-PBS-inoculated control embryos also were included. Eggs were candled daily, and deaths were recorded for 4 days. Twenty eggs were used for each of two inoculum dilutions, approximately 10⁴ and 10⁵ CFU in 0.1 ml of PBS (24).

Motility. Isolates were scored for motility by means of stab inoculation into motility media (Bacto motility test media, Difco Laboratories, Detroit, Mich.). Tubes were incubated at 37°C and examined at 24 and 48 hours for evidence of motility (1,20).

Hemolysis. Hemolytic activity was detected by stab-inoculation of the test organisms into blood agar plates containing 5% bovine erythrocytes. Plates were incubated at 37°C and observed at 24 and 48 hours.

Biostatistics. The quantitative microtiter test results were analyzed by calculating the regression co-
Table 1. Comparison of percent resistance to antimicrobial agents and antimicrobial resistance profiles of systemic and intestinal avian *E. coli* isolates.

<table>
<thead>
<tr>
<th>Antimicrobials</th>
<th>% Resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Systemic</td>
</tr>
<tr>
<td>Am</td>
<td>17.5&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Cm</td>
<td>0</td>
</tr>
<tr>
<td>Gm</td>
<td>27.5&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Kn</td>
<td>35.0</td>
</tr>
<tr>
<td>Na</td>
<td>5.0</td>
</tr>
<tr>
<td>PB</td>
<td>0</td>
</tr>
<tr>
<td>St</td>
<td>70.0&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Su</td>
<td>95.0&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Te</td>
<td>80.0&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Combinations
- St-Su-Te: 17.5 (Systemic) 15.0 (Intestinal)
- Gm-Kn-St-Su-Te: 15.0 (Systemic) 5.0 (Intestinal)
- Kn-St-Su-Te: 10.0 (Systemic) 17.5 (Intestinal)
- St-Te: 2.5<sup>b</sup> (Systemic) 15.0<sup>a</sup> (Intestinal)
- Gm-St-Te: 0<sup>b</sup> (Systemic) 15.0<sup>a</sup> (Intestinal)
- Gm-St-Su-Te: 10.0 (Systemic) 10.0 (Intestinal)
- Su: 10.0<sup>b</sup> (Systemic) 0<sup>b</sup> (Intestinal)
- Su-Te: 7.5 (Systemic) 0 (Intestinal)

*<sup>a</sup>Z test. Within a row, values followed by different lower-case superscripts are significantly different (*P* < 0.05).

<sup>b</sup>Abbreviations: Am = ampicillin, Cm = chloramphenicol, Gm = gentamicin, Kn = kanamycin, Na = nalidixic acid, PB = polymyxin-B, St = streptomycin, Su = sulfisoxazole, Te = tetracycline.

The results show the differences in resistance patterns between systemic and intestinal *E. coli* isolates. The systemic isolates differ in their resistance to individual antimicrobial agents compared to the intestinal isolates. The systemic isolates had significantly greater resistance to ampicillin and sulfisoxazole, whereas the intestinal isolates had greater resistance to streptomycin and tetracycline. Resistance to groups of antibiotics also differed between the two groups for the anti-biotic resistance profiles of St-Te, Gm-St-Te, and Su (Table 1).

No difference was seen between the two groups of isolates in their ability to produce colicins (Table 2). Systemic isolates had a significantly greater ability to produce siderophores and type 1 pili and were more likely to be non-hemolytic and motile (Table 2); they also had a greater ability to resist the lytic effect of chicken complement (Table 2). Systemic and intestinal isolates often contained both small and large plasmids, but no systemic isolates contained only small plasmids (Table 2).

When the 10 systemic isolates with the greatest resistance to complement were compared with the 10 intestinal isolates with the greatest sensitivity to complement, no significant differences were observed for plasmid distribution, antibiotic resistance profiles, type of hemolysis, and the production of colicins and siderophores (Table 3). The complement-resistant systemic organisms were more likely to be mo-

Table 2. Comparison of percent positive reaction of systemic and intestinal avian *E. coli* to selected tests.

<table>
<thead>
<tr>
<th>Test</th>
<th>% Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Systemic</td>
</tr>
<tr>
<td>Colicin</td>
<td>65.0</td>
</tr>
<tr>
<td>Siderophore</td>
<td>95.0&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Type 1 pili</td>
<td>100&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Motility</td>
<td>100&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Hemolysis</td>
<td></td>
</tr>
<tr>
<td>Beta</td>
<td>7.5&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Alpha</td>
<td>50.0</td>
</tr>
<tr>
<td>None</td>
<td>42.5&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Complement group
- Sensitive: 0<sup>b</sup> (Systemic) 27.5<sup>a</sup> (Intestinal)
- Intermediate: 32.5<sup>b</sup> (Systemic) 60.0<sup>b</sup> (Intestinal)
- Resistant: 67.5<sup>a</sup> (Systemic) 12.5<sup>b</sup> (Intestinal)

Plasmids
- Large only (>15 megadaltons): 55.0 (Systemic) 35.0 (Intestinal)
- Small only (<15 megadaltons): 0<sup>b</sup> (Systemic) 22.5<sup>a</sup> (Intestinal)
- Both large and small: 45.0 (Systemic) 40.0 (Intestinal)
- None: 0 (Systemic) 2.5 (Intestinal)

<sup>a</sup>Z test. Within a row, values followed by different lower-case superscripts are significantly different (*P* < 0.05).
tile and have type 1 pili than the complement-sensitive intestinal isolates, and they killed more embryos in the chicken embryo lethality test (Table 3).

**DISCUSSION**

No statistical differences were observed between the two groups for the production of colicins (Table 2). In two previous studies, colicin production was reported to be more prevalent in sick birds than in healthy birds, but a positive reaction was found in more than a third of all *E. coli* isolates tested in these two studies, including samples from both sick and healthy birds (13,14). Another study indicated that a colicin/complement-resistance interaction is important in chick virulence (2). Data from the present study indicate that colicin acting alone is not important in virulence, as it is produced by almost half of the normal intestinal *E. coli* isolates.

Significant differences were seen between the two groups of *E. coli* isolates for Te, St, and Su, three antibiotics to which more than 50% of the tested isolates were resistant (Table 1). The resistance to Am was significantly greater in the systemic isolates than in the intestinal isolates, a finding similar to those of previous studies (6,11,13,16,17). The most common antibiotic resistance profile seen was St-Su-Te, but percent resistance differed significantly between the two groups for the profiles of St-Te, Gm-St-Te, and Su. The profile of St Su-Te has been reported to have a high incidence in poultry and be plasmid-mediated (14,16,17). In a previous study, greater antibiotic resistance was found in sick birds than in healthy birds (5).

Both *E. coli* groups had the ability to produce siderophores (Table 2). Although the systemic isolates had a significantly higher positive rate (95.0%) than the intestinal isolates (75.0%), siderophore production alone does not appear to correlate with virulence. Other studies have also indicated that isolates from sick birds have a high probability of producing siderophores (31,33).

A large sampling of avian *E. coli* isolates have type 1 pili to facilitate attachment (11,30), and such pili have been correlated with other factors in virulence (1,8,22). The present study also indicated that possession of type 1 pili contributes to virulence, as 100% of the systemic isolates and only 57.5% of the intestinal isolates had type 1 pili (Table 2).

Motility has been reported in 48% of *E. coli* isolates from birds with colisepticaemia (32). Another study indicated that a greater proportion of motile isolates come from sick birds than from healthy birds (1). Others have suggested that motility has no association with pathogenicity (26). The present study shows a strong association between motility and virulence. All of the systemic isolates were motile, but only 55.0% of the intestinal isolates were motile (Table 2).

Statistically significant differences between the two groups of isolates were observed in the type of hemolytic reactions on blood agar plates. The systemic isolates had a significantly greater percentage of non-hemolytic reactions (Table 2). Other studies have also indicated the absence of hemolytic reactions in virulent *E. coli* isolates (14,31).

Both systemic and intestinal isolates possessed either large plasmids or both large and small plasmids. None of the systemic isolates contained only small plasmids, as did 22.5% of the intestinal isolates, but systemic isolates contain small plasmids in association with large plasmids (Table 2). The correlation between systemic isolates and possession of large plasmids is consistent with results of previous reports that indicate that large plasmids are associated with virulence (31). Such plasmids may
encode siderophore production and complement resistance (33).

The factor that may be the most important contributor to virulence is an organism’s ability to resist the lytic action of host complement (23,24). A significantly greater percentage of systemic isolates than intestinal isolates was classified as resistant to complement (67.5% vs. 12.5%) (Table 2). Perhaps most important to note was the fact that all disease-associated isolates exhibited some degree of complement resistance, whereas 27.5% of the intestinal isolates were sensitive to complement action. Other studies have indicated that virulent organisms are often complement-resistant, but these studies did not compare the virulent isolates with isolates from normal flora, nor were the virulent isolates ranked in regard to complement resistance (9,33). Recent reports indicate that a complement resistance gene associated with a small plasmid and/or the organism’s chromosome (23) is necessary for an avian E. coli isolate to be virulent and that large conjugative R-plasmids of virulent avian isolates do not encode for complement resistance or other virulence factors (34). Because complement resistance appears to be an important factor in the virulence of avian E. coli, the 10 most complement-resistant E. coli isolates from the systemic group were compared with the 10 most complement-sensitive isolates from the intestinal group for their possession of selected phenotypic characteristics. The complement-resistant isolates were the more virulent in the embryo lethality assay (Table 3), lending support to the hypothesis that complement resistance is an important contributor to the development of avian septicemia. Motility and the presence of type 1 pili were also characteristic of these resistant isolates, which suggests that these factors contribute to virulence.

REFERENCES


