In Vitro Characterization of Field Isolants of Pasteurella multocida from Georgia Turkeys

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SUMMARY

Field isolants of Pasteurella multocida from fowl cholera outbreaks in Georgia turkeys were characterized by three sets of criteria: differential biochemical reactions, in vitro drug sensitivity, and serology.

Of the 30 isolants studied, 28 exhibited identical biochemical patterns. These were similar to previously described patterns for turkey isolants of P. multocida. The two exceptions were isolants recovered from the same farm at different times. They differed only in ability to ferment arabinose.

The isolants were generally sensitive to broad-spectrum antibiotics in vitro. The majority were also sensitive to the sulfonamides tested. Variation was sufficient, however, to warrant recommending in vitro sensitivity testing as a guide to selection of the proper therapeutic regimen in individual cases.

Of the 30 isolants tested, 57% were of Heddleston's serotype 3, 3% were of his type 4, and 40% precipitated with antisera against both types 3 and 4. The large proportion of cross-reactors is unique to Georgia isolants. The biochemical patterns, drug sensitivities, and serological types had no apparent relationship to each other.

INTRODUCTION

The biochemical characteristics of avian isolants of Pasteurella multocida (P. aviseptica, P. avicida) have been studied by many workers (3,4,13,15–17,19). Studies limited to turkey isolants were undertaken by Donahue and Olson (7,8,10), who reported on 214 field isolants from Missouri (8). These isolants differed considerably in fermentation patterns from 409 isolants from South Dakota
(11), mostly from chickens. Donahue and Olson (8) concluded that biochemical patterns may reflect differences in geographic and/or host distribution of *P. multocida*.

The problem of drug resistance in strains of *P. multocida* responsible for fowl cholera in turkeys in Missouri was recently investigated by Donahue and Olson (9). In their studies broad-spectrum antibiotics, such as chlortetracycline, were more effective against *P. multocida in vitro* than other antibiotics or sulfonamides. They suggested that the failure of sulfonamides to control fowl cholera epornitics may have been due to developed resistance of the organism to the drugs.

Many efforts have been made to classify strains of *P. multocida* by serologic means. Little and Lyon (22) reported three serotypes which were distinguished by passive immunization of mice and by a rapid slide agglutination test. Roberts (28) devised a classification scheme using capsular antigens and passive immunization tests. Carter (5) and Namioka and Murata (25) respectively described hemagglutination and slide agglutination tests based on antigenic differences in capsular antigens.

Heddleston et al. (20) have adopted classification on the basis of somatic antigens. They recently developed a gel-diffusion precipitin test for serotyping isolants of *P. multocida* (18,19). They described 7 type strains and found that 3 of these (types 1, 3, and 4) as well as isolants reacting with antisera against both types 3 and 4, occurred in domestic turkeys in various parts of North America (18,19).

The present studies were undertaken to determine the biochemical characteristics, *in vitro* drug sensitivities, and serological types of field isolants of *P. multocida* submitted to the Poultry Disease Research Center of the University of Georgia, College of Veterinary Medicine, Athens, Georgia.

**MATERIALS AND METHODS**

**Pasteurella multocida isolants.** Thirty isolants of *P. multocida* were studied. Eleven turkey farms were represented.

**Biochemical characteristics.** Dextrose starch agar (DSA; BBL, Cockeysville, Md.) plates were inoculated from the initial pure cultures and incubated at 37 °C for 24 hours. A single iridescent colony (2) from each culture was selected and used to inoculate brain-heart infusion broth (BHI; BBL), and these were incubated at 37 °C for 24 hours. The media for differential biochemical tests were inoculated from the BHI cultures with sterile Pasteur pipettes.
A) **Fermentation.** The fermentation characteristics of the isolants were tested with the following carbohydrates: arabinose, dextrin, dulcitol, galactose, glucose, glycerol, inositol, inulin, lactose, levulose, maltose, mannitol, raffinose, rhamnose, salicin, sorbitol, sucrose, trehalose, and xylose. Phenol-red broth base (BBL) to which was added 1% of the respective carbohydrate was used to determine the fermentative reactions. All carbohydrate media were filter-sterilized.

B) **Decarboxylation.** The capacity of the *P. multocida* isolants to decarboxylate three amino acids was tested by the method of Moeller (23). The basal medium contained 1% L-lysine dihydrochloride, L-arginine monohydrochloride, or L-ornithine dihydrochloride (BBL).

C) **Other biochemical tests.** Production of indole and H$_2$S was determined with stab cultures on sulfide-indole-motility agar (BBL).

Nitrate reduction, hemolysis, oxidase activity, urease activity, growth on MacConkey's agar, acetylmethylcarbinol production (Voges-Proskauer test), nutrient gelatin liquefaction, and litmus milk changes were all tested in a routine manner (1,27).

**Drug sensitivity.** Drug-sensitivity plates were inoculated from the BHI subcultures with sterile swabs. The isolants were tested for sensitivity to 8 antibiotics, 2 nitrofurans, and 8 sulfonamides, as listed in Table 1. Of particular interest were three sulfonamides which have frequently been used to treat outbreaks of fowl cholera. These were agribon (sulfadimethoxine; Hoffman-LaRoche, Nutley, N.J.), Esb$_3$ (sulfachlorpyridine; Squibb, Princeton, N.J.), and S.Q. (sulfaquinoxaline; Merck, Rahway, N.J.).

Antibiotic and nitrofuran sensitivities were determined on DSA and/or blood agar (BA; BBL). Mueller-Hinton agar (MHA, BBL) plates were used for sulfonamide sensitivity tests. The inoculated DSA and BA plates were incubated at 37 C for 18–20 hours. Growth was slower on MHA, and these plates were incubated for 24–48 hours. An isolant was regarded as sensitive to a given drug if there was a visible clear zone surrounding the disc.

**Serology.** Antisera. Specific immune sera were provided by K. L. Heddleston, National Animal Disease Laboratory, Ames, Iowa. The type strains used in these tests were: Type 1, X-73, Type 3, P-1059, and Type 4, P-1662 (18).

Antigens. Antigens were prepared by the procedure of Heddleston *et al.* (18).
Table 1. *In vitro* sensitivities of 30 *Pasteurella multocida* isolants from turkeys to selected chemotherapeutic agents.

<table>
<thead>
<tr>
<th>Generic name</th>
<th>Brand name</th>
<th>Amount per disc</th>
<th>Percent of Georgia isolants sensitive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlortetracycline</td>
<td>Aureomycin</td>
<td>30 μg</td>
<td>100</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>Lincomycin</td>
<td>15 μg</td>
<td>100</td>
</tr>
<tr>
<td>Lincomycin</td>
<td>Albamycin</td>
<td>2 μg</td>
<td>0</td>
</tr>
<tr>
<td>Novobiocin</td>
<td>Terramycin</td>
<td>30 μg</td>
<td>100</td>
</tr>
<tr>
<td>Oxytetracycline</td>
<td>10 IU</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Penicillin</td>
<td>10 μg</td>
<td>97</td>
<td></td>
</tr>
<tr>
<td>Streptomycin</td>
<td>30 μg</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Tetracycline</td>
<td>30 μg</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Nitrofurazone</td>
<td>Furacin</td>
<td>100 μg</td>
<td>100</td>
</tr>
<tr>
<td>Furazolidone</td>
<td>Furozone</td>
<td>100 μg</td>
<td>100</td>
</tr>
<tr>
<td>Sulfadiazine</td>
<td>1 mg</td>
<td>87</td>
<td></td>
</tr>
<tr>
<td>Sulfamerazine</td>
<td>1 mg</td>
<td>83</td>
<td></td>
</tr>
<tr>
<td>Triple sulfa</td>
<td>0.25 mg</td>
<td>79</td>
<td></td>
</tr>
<tr>
<td>Sulfamethizole</td>
<td>Thiosulfil</td>
<td>0.25 mg</td>
<td>83</td>
</tr>
<tr>
<td>Sulfathiazole</td>
<td>50 μg</td>
<td>83</td>
<td></td>
</tr>
<tr>
<td>Sulfadimethoxine</td>
<td>Agribbon</td>
<td>1 mg</td>
<td>87</td>
</tr>
<tr>
<td>Sulfachlorpyridine</td>
<td>Esb³</td>
<td>2 mg</td>
<td>97</td>
</tr>
<tr>
<td>Sulfquinocaine³</td>
<td>S.Q.</td>
<td>1 mg</td>
<td>90</td>
</tr>
</tbody>
</table>

*ADisc, 9 mm in diameter.  
BDisc, 13 mm in diameter.

**Gel-diffusion precipitin tests.** The gel-diffusion precipitin tests were performed by the method of Heddleston *et al.* (18) with minor modifications. Agar was poured to a depth of 2 mm in petri dishes (no. 1008, Falcon Plastics, Oxnard, Calif.). The seven wells were 5 mm in diameter with centers 7 mm apart. The agar gel medium consisted of 8.0% NaCl, 0.7% Ionagar no. 2 (Colab, Chicago Heights, Ill.), 0.1M phosphate-buffered saline (pH 7.2) at 10 ml per 100 ml, and 1% thiomersol at 1 ml per 100 ml.

**RESULTS**

**Biochemical characterization.** **A) Fermentation.** Acid, but no gas, was produced in all positive reactions. The 30 field isolants consistently fermented the following carbohydrates: galactose, glucose, glycerol, levulose, mannitol, sorbitol, sucrose, and xylose. None of them fermented dextrin, dulcitol, inositol, inulin, lactose, maltose, raffinose, rhamnose, salicin, or trehalose. Only two field isolants fermented arabinose. These were from a single farm but from different outbreaks. They were the only isolants studied from that farm.

**B) Decarboxylation.** All of the isolants decarboxylated ornithine; none decarboxylated either of the other two amino acids.

**C) Other biochemical tests.** All isolants produced indole and none produced $H_2S$. All reduced nitrate to nitrite (without gas) within 24 hours. The isolants were uniformly negative for hemolysis, growth on MacConkey's agar, production of acetyl-
Table 2. Distribution of *P. multocida* serotypes among 11 Georgia turkey farms.

<table>
<thead>
<tr>
<th>Farm</th>
<th>Type 3</th>
<th>Type 4</th>
<th>Types 3/4A</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1</td>
<td></td>
<td>3</td>
</tr>
<tr>
<td>B</td>
<td>4</td>
<td></td>
<td>3</td>
</tr>
<tr>
<td>C</td>
<td>4</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>D</td>
<td>1</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>E</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>1</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>J</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>K</td>
<td>1</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Totals</td>
<td>17</td>
<td>1</td>
<td>12</td>
</tr>
</tbody>
</table>

*These isolants were precipitated by antisera to both types 3 and 4.*

methyl-carbinol, and liquefaction of nutrient gelatin. There was little or no growth on urea agar. All exhibited oxidase activity. Five strains produced acid on litmus milk.

**Drug sensitivity.** Table 1 shows the drug sensitivity. In most cases, isolants that were resistant to one drug were resistant to several; that was especially true of the sulfonamides.

**Serology.** Seventeen of the 30 isolants reacted with antiserum to type 3 only, one with type 4 only, and thirteen were precipitated by antisera of both types 3 and 4. That individual antigens precipitate with immune sera of both types 3 and 4 has been reported by Heddleston *et al.* (19), and confirmed by Derieux (6).

Table 2 shows distribution of the serotypes among the submissions from the 11 farms. Each farm from which a cross-reactive isolant was recovered also yielded at least one conventional serotype from another submission.

**DISCUSSION**

The turkey isolants of *P. multocida* from Georgia were quite similar in fermentative characteristics to those from Missouri (8). The pattern of fermentation exhibited by 93% of our isolants was identical to that of 80% of the Missouri isolants.

On the other hand, significant differences were noted between the fermentative actions of isolants from turkeys and those from South Dakota chickens (11). Of the latter group, only about 16% displayed the same characteristics as were most common among turkey isolants. These results strongly suggest that the fermentation patterns of isolants of *P. multocida* correspond more closely to host species than to geographical location.
This study did not use the "sensitive/slightly sensitive" drug-sensitivity recording system of Donahue and Olson (9), based on clear zones around discs of high and low drug concentrations. That system was viewed as somewhat artificial with respect to a therapeutic choice because of the many variables in in vitro drug sensitivity tests, well reviewed by Froe and Williams (14).

The results of this study of in vitro drug sensitivity correlated closely with those of Donahue and Olson (9) with respect to antibiotic and nitrofuran sensitivities. However, a greater percentage of our isolants were sensitive to sulfonamides. This may reflect the higher concentrations of drugs used in the present study. Because the P. multocida isolants were variable in sensitivities to the commonly used sulfonamides, it is suggested that in vitro tests be done routinely as a guide to selection of a therapeutic agent to treat individual outbreaks of fowl cholera.

Two previous studies have included serotyping of P. multocida isolants from field cases of fowl cholera. Langpap and Matisheck (21) reported on the serotypes of 56 turkey isolants from 12 states. They found that 9% were of type 1, 57% were of type 3, and 34% were of type 4. Heddleston et al. (18,19) reported the serotypes of 18 turkey isolants from various geographical locations. Of these, 11% were type 1, 67% were type 3, 17% were type 4, and 6% (a single isolant from New York) precipitated antisera to both types 3 and 4.

It is evident that Georgia isolants react with antisera to both types 3 and 4 more frequently than isolants reported from other parts of the continent. This finding has important implications for the control of fowl cholera by vaccination procedures. The cross-reacting strains were isolated from multiple submissions from several farms. However, in no case was this the only serotype isolated from those farms (Table 2). It appears that cross-reacting strains are widely distributed.

Furthermore, the fact that repeated fowl cholera outbreaks on a farm were caused by isolants of different serotypes suggests that it is not a single resident population of P. multocida which may be responsible for recurrent problems.

Dorsey (12) concluded that there was no correlation between biochemical reactions and serological types. The minimal variation in biochemical patterns, moderate variation in drug sensitivities,
and marked variation in serotypes presented in these studies lead to the conclusion that there are no absolute correlations among the three in vitro criteria.

REFERENCES


