Antibiotic and Sulfonamide Disc Sensitivity Tests of Avian Strains of Escherichia Coli

by R. V. Hemsley, D. A. Barnum and D. G. Ingram

SUMMARY

Avian strains of Escherichia coli (213) were subjected to in vitro antibiotic sensitivity tests with twenty-four different chemotherapeutic agents. Chlorhexidine, polymyxin B, colistin, and nitrofurazone exhibited the greatest inhibitory activities against these strains. Various potencies of antibiotics were compared. The activity of sulfachloropyridazine on various types of media failed to show complete clear zones of inhibition. Serogroup 075 was shown to exhibit an antibiotic sensitivity pattern which was significantly different from other 0 groups.

Introduction

The aetiological significance of E. coli in poultry disease outbreaks has been confirmed (1, 2, 3) and many avian strains have been serologically identified (3, 4, 5). The treatment of diseases associated with E. coli is complicated by the complexity of the "air sac disease" syndrome (6), however, it is expedient that E. coli invasion be effectively controlled. In this respect, research workers (3, 7, 8, 9) have conducted in vitro sensitivity tests in order to assess the effectiveness of various antibiotics and sulfonamides against poultry strains of E. coli. It must be recognized that in vitro sensitivity tests can only be a guide in the choice of an adequate chemotherapeutic agent in the field. There are numerous situations where an in vitro sensitivity test is practical. Provided the sources of error and proper interpretations of the test (10) are recognized, the use of the disc method may be useful and less time consuming than other known methods. In this work the disc was used to assess the activity of various chemotherapeutic agents for avian strains of E. coli.

Materials and Methods

213 strains of E. coli of avian origin were employed. Previously these strains had been subjected to biochemical and serological tests. (11)

All isolates were grown overnight (12 hours) in Tryptic Soy Broth (Difco) and .05 ml inoculated onto 10 cm Petri plates containing exactly 20 ml Bacto-Tryptose Agar (T. A.) (Difco). Sterile pipe cleaners were used to uniformly distribute the inocula over the surface of the agar, then 8-9 commercially prepared sensitivity discs were applied equidistant from each other. Plates were inverted, left at room temperature for 3 hours (26°C.), and incubated at 37°C. for 18 hours, then examined.

Organisms were classified as sensitive when the disc produced a zone of inhibition which was definite and clear regardless of diameter. When no zone was apparent, the organism was recorded as resistant. The presence of some growth, usually fine to barely visible, was used to designate a strain as partially or moderately sensitive.

To assess the activity of sulfa inhibitors (e.g.: p-amino benzoic acid) in T.A. agar, a random sample of isolates was tested on Oxoid Sensitivity Test Media (Granules CM 215) and Bacto Mueller-Hinton Agar. Undiluted, 1:5, 1:10 and 1:20 dilutions of inocula were used. Plates were examined at 10 hours, 18 hours and 3 days. At the end of 18 hours of incubation an attempt was made to recover E. coli from the zones of inhibition on the 1:20 dilution plates. This was accomplished by passing a sterile wire loop around the edge of the disc (not...
Table I. Disc Sensitivity Tests to Chemotherapeutic Agents used on 213 Isolates of Escherichia coli of Avian Origin.

<table>
<thead>
<tr>
<th>Generic Name</th>
<th>Potency</th>
<th>No. Sensitive</th>
<th>No. partially Sensitive</th>
<th>No. Resistant</th>
<th>% Sensitive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorhexidine</td>
<td>250 μg</td>
<td>213</td>
<td>0</td>
<td>0</td>
<td>100.0</td>
</tr>
<tr>
<td>Polymyxin B</td>
<td>50 units</td>
<td>213</td>
<td>0</td>
<td>0</td>
<td>100.0</td>
</tr>
<tr>
<td>Nitrofurazone</td>
<td>100 μg</td>
<td>213</td>
<td>0</td>
<td>0</td>
<td>100.0</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>5 μg</td>
<td>169</td>
<td>39</td>
<td>5</td>
<td>79.3</td>
</tr>
<tr>
<td>Neomycin</td>
<td>5 μg</td>
<td>163</td>
<td>5</td>
<td>45</td>
<td>76.5</td>
</tr>
<tr>
<td>Nalidixic acid</td>
<td>30 μg</td>
<td>155</td>
<td>58</td>
<td>0</td>
<td>72.8</td>
</tr>
<tr>
<td>Dihydrostreptomycin</td>
<td>10 μg</td>
<td>107</td>
<td>3</td>
<td>3</td>
<td>50.2</td>
</tr>
<tr>
<td>Oxytetracycline</td>
<td>5 μg</td>
<td>13</td>
<td>38</td>
<td>162</td>
<td>6.1</td>
</tr>
<tr>
<td>Chlorotetracycline</td>
<td>5 μg</td>
<td>8</td>
<td>51</td>
<td>154</td>
<td>3.8</td>
</tr>
<tr>
<td>Triple Sulfas*</td>
<td>0.25mg</td>
<td>0</td>
<td>22</td>
<td>191</td>
<td>0.0</td>
</tr>
</tbody>
</table>

*Equal amounts of sulfadiazine, sulfamerazine, and sulfamethazine.

exceeding 12 mm diameter) and then inoculating maltose broth. The formation of acid and gas (usually within 12 hours) was considered a positive isolation. (11)

The antimicrobial discs were supplied by two firms1 who distribute these commercially under Food and Drug Regulations, except for two chemotherapeutic agents which were supplied for trial by drug manufacturing companies2.

The chemotherapeutic agents employed along with their potencies are shown in Tables I and II.

The standard deviations and confidence intervals were calculated for relating 0 groups with antibiotic sensitivity patterns according to recommended statistical principles. (12).

Results

Antimicrobial Disc Tests

Ten different antimicrobial discs were tested on all 213 isolates (Table I). Based on the availability of discs, a further fourteen chemotherapeutic agents were assessed as to their activity against E. coli (Table II).

Comparisons of Activity of Antibiotics

Comparisons of various potencies of similar and different antibiotics showed that:

(1) Two levels of oxytetracycline (5 μg and 30 μg) were compared on identical isolates. It was found that most isolates which were resistant at the lower potency were also resistant at the higher potency. Only in 4 of 114 strains (paired) was a resistance observed at the lower level and sensitive at the higher level.

(ii) Comparisons of oxytetracycline, chlorotetracycline, and tetracycline revealed that a high percentage of isolates were resistant to all three antibiotics with only a small proportion revealing differences in their sensitivities. For example, using the 5 μg level, 161 reactions were similar (89.4%) and 19 dissimilar (10.6%).

(iii) With 30 and 2 μg levels of ampicillin, a semisynthetic penicillin, most isolates were resistant or only partially sensitive. There was a wide discrepancy in the results at the two potency levels (eg) 81/83 isolates were sensitive at the 30 μg level, while only 4/83 of these same isolates were sensitive at the 2 μg level. Dissimilar activities occurred for 78 paired tests (94%).

(iv) Since derivatives of neomycin have been shown to be mixtures of various proportions of its isomers (14), framycetin (30 μg and 50 μg) was compared with neomycin (5 μg). Generally, when the isolate was found to be sensitive to neomycin, it was also sensitive to both levels of framycetin (eg) 82/111 at 30 μg and 48/62 at 50 μg. In 12.6% of paired tests were organisms sensitive to framycetin (30 μg) and at the same time resistant to neomycin. At the 50 μg level of framycetin, this was 12.9%.

(v) Antibiotic sensitivity patterns of paired isolates were compared. 83.7% of isolates of E. coli taken from the
Table II. Disc Sensitivity Tests to Fourteen Chemotherapeutic Agents Against Avian Strains of Escherichia coli.

<table>
<thead>
<tr>
<th>Generic Name</th>
<th>Potency</th>
<th>No. Sensitive/ No. Tested</th>
<th>% Sensitive</th>
<th>No. Partially Sensitive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colistin</td>
<td>2 µg</td>
<td>164/165</td>
<td>99.4</td>
<td>1</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>30 µg</td>
<td>123/126</td>
<td>97.6</td>
<td>2</td>
</tr>
<tr>
<td>Framycetin *1</td>
<td>50 µg</td>
<td>59/62</td>
<td>95.2</td>
<td>2</td>
</tr>
<tr>
<td>Framycetin *1</td>
<td>30 µg</td>
<td>97/111</td>
<td>87.4</td>
<td>1</td>
</tr>
<tr>
<td>Furalitadone</td>
<td>50 µg</td>
<td>30/51</td>
<td>58.8</td>
<td>21</td>
</tr>
<tr>
<td>Oxytetracycline</td>
<td>30 µg</td>
<td>23/114</td>
<td>20.2</td>
<td>16</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>5 µg</td>
<td>15/189</td>
<td>7.9</td>
<td>39</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>2 µg</td>
<td>4/114</td>
<td>3.5</td>
<td>57</td>
</tr>
<tr>
<td>Oleandomycin</td>
<td>2 µg</td>
<td>0/164</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Sulfachloropyridazine *2</td>
<td>2 µg</td>
<td>0/160</td>
<td>0</td>
<td>159</td>
</tr>
<tr>
<td>Bacitracin</td>
<td>2 units</td>
<td>0/100</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>2 µg</td>
<td>0/100</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Phenethicillin</td>
<td>2 µg</td>
<td>0/52</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Tylosin</td>
<td>30 µg</td>
<td>0/31</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

*1 Framycetin supplied by Rogar Co. Ltd., St. Hyacinthe, P.Q.  
*2 Sulfachloropyridazine supplied by Ciba Pharmaceutical Co., Three Bridges, New Jersey, U.S.A.

same case but from different birds or lesions, showed the same sensitivity pattern to the antibiotics used in these tests. (13)

Comparisons of Activity of Sulfonamides

(i) On Tryptose Agar

Sulfachloropyridazine failed to exhibit clear zones of inhibition after 18 hours at 37°C against any strains of E. coli. However, all sulfas rarely showed any activity, and on the occasions when they did, inhibition was only partial.

(ii) On Oxoid Sensitivity Media

Sulfachloropyridazine gave zone diameters 2-6 mm larger than on T.A. As dilutions of inocula were increased from 1:5 to 1:20, an increase in zone diameter of 1-5 mm was observed. Examination at 10 hours, 18 hours and three days showed an increased zone diameter with increasing time, from 0 to 3 mm.

Triple sulfas usually gave larger and clearer zones on oxoid sensitivity media than on tryptose agar, however all were still partially clear. A number of isolates which had exhibited complete resistance on T.A. exhibited visible zones of inhibition on this media. Zone diameters increased 1-5 mm between 10 hours and 18 hours when dilutions of 1:5 and 1:10 were compared.

(iii) On Mueller-Hinton Agar

Sulfachloropyridazine, using undiluted inocula, gave diameters ranging from 20-25 mm. At dilutions of 1:10 these increased to 27-33 mm, and at 1:20 to 31-36 mm. No completely clear zones of inhibition were observed. E. coli were recovered from within these zones and demonstrated by fermentation tests.

Triple sulfas gave zone diameters which were generally larger and of better quality on M-H agar than on T.A. media. The zone diameters increased as the dilutions of inocula increased from 1:10 to 1:20. Some isolates which were resistant on T.A. were moderately sensitive on Mueller-Hinton Agar. No isolates showed complete zones of inhibition.

Antibiotic discs used as controls showed similar results on all three types of media.

Relationship of O Serogroups to Antibiotic Sensitivity Patterns

The sensitivity patterns of dihydrostreptomycin, (DS) chloramphenicol, (C) and the tetracyclines (T) were coded and then related to the various O groups identified. The standard deviations and confidence intervals were calculated in order to estimate the significance of this relationship at the .05 level. (12). Generally antibiotic sensitivity patterns were well distributed over the 23 O groups identified. It was shown that the incidence of 078 in the pattern DS-C + T — was significantly higher than any other O group. No other relationships were significant.
Discussion

The antibiotic sensitivity patterns of strains of *E. coli* were similar to those reported by Glantz (7) who used the tube dilution method.

The in vitro activity of certain chemo- therapeutic agents used against strains of *E. coli* has diminished since their initial use. Sojka and Carnaghan in Great Britain (3) showed an increase in the incidence of tetracycline resistant strains from 3.5% in 1957 to 63.2% in 1960. They failed to find strains resistant to chloramphenicol. The increase in resistance to tetracyclines was attributed to the rapid development of the broiler industry and the widespread use of these agents in the feed. Likewise in the U.S.A. (7) resistance was shown to be 63.4% to chlortetracycline, 64.5% to oxytetracycline, and 19.9% to chloramphenicol in 1961. Tables I and II indicate that 94.1% of strains isolated in 1964-65, mainly from Ontario, were resistant to low levels of tetracyclines, 0.9% to nitrofurazone, and 20.7% to chloramphenicol.

Transferable drug resistance may be incriminated in changes of antibiotic sensitivity of enteric bacteria (15). The development of drug resistance has also been blamed on the feeding of antibiotics and sulphonamides to livestock and the mechanisms by which this resistance increases has been reviewed (16, 17). Results, obtained using three different media with discs of sulphasalicydrizadine, failed to confirm the observations of other Canadian workers (18, 19) that the growth of *E. coli* strains was completely inhibited by this sulphonamide. In the present study clear zones of inhibition could not be shown with sulfachloropyridazine nor triple sulphas even when dilutions of the inocula were increased and the time of examination of the tests varied. The size of the zone of inhibition around these sulphas in vitro was increased, however, by using media free of PABA and/or other sulfa-inhibitors.

Although 078 exhibited an antibiotic sensitivity pattern which was statistically significantly different from other O groups, it is doubtful whether this pattern would be maintained. Sensitivities have been shown to change due to time and geographical factors. It was shown by Glantz (7) that 078 was particularly sensitive to chloramphenicol and not to chlortetracycline. The work described here was similar.

It must be realized that clinical trials are necessary to provide reliable chemo- therapeutic test data, however, disc sensitivity in vitro tests are useful to establish the possible efficacy of drugs prior to their administration in the field.

REFERENCES