R FACTORS IN ENTEROBACTERIACEAE CAUSING ASYMPTOMATIC BACTERIURIA OF PREGNANCY

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Escherichia coli carrying resistance-transfer (R) factors caused 6 per cent. of urinary infections contracted outside hospital in 1962–63 and 20 per cent. in 1968–69 (Shaw et al., 1973). Investigations were extended in 1970–71 to find out whether there was an increasing proportion of infections caused by such organisms. Asymptomatic bacteriuria of pregnancy (ASB) is usually acquired by the patient before contact with any hospital environment. This infection was, therefore, studied in patients making their first visit at the antenatal clinics of two hospitals in West London.

Materials and Methods

The surveys. Patients attending the antenatal clinics of Hammersmith Hospital in 1970–71 and the West Middlesex Hospital in 1971 were examined for ASB. A clean mid-stream specimen of urine was obtained from each patient at her first visit to the antenatal clinic. This specimen was examined for bacteriuria by a calibrated standard-loop technique. If a pure growth of more than $10^5$ organisms per ml was present, it was considered significant. Patients with significant bacteriuria were requested to return to the next clinic, and a second mid-stream specimen was obtained and examined. If both specimens showed significant bacteriuria, the patient was considered to have ASB.

Bacteriological techniques. Each isolate was initially Gram stained, its colonial morphology and lactose fermentation were noted and tests were made for indol and urease production and ability to use citrate as a sole carbon source. All organisms which appeared to be either E. coli or Klebsiella sp. were further examined. Sensitivity tests were first performed by placing a standardised inoculum on a nutrient agar plate containing 4 per cent. lysed blood and applying an Oxoid Multi-disc; parallel tests were made with E. coli K12 as control. If the isolate appeared more resistant than the control to any drug it was tested for its ability to grow on Oxoid DST Agar (CM261) plates containing 4 per cent. lysed blood and one of the following drugs incorporated in the agar: ampicillin 25 μg per ml, streptomycin 15 μg per ml, tetracycline 10 μg per ml, chloramphenicol 25 μg per ml, kanamycin 10 μg per ml, sulphadimidine 100 μg per ml, nalidixic acid 25 μg per ml, trimethoprim 2 μg per ml, and gentamicin 8 μg per ml. The plates were inoculated with approximately 10 organisms by means of a multiple-point inoculator and incubated at 37°C overnight. Resistance was recorded if growth was present on any of these plates.

Transfer of resistance was then tested for by direct conjugation with E. coli K12 (Datta, 1968) and, if transfer was not shown, by mobilisation with a depressed R-factor (Anderson, 1965). In the isolates in which resistance was still not transferable, other evidence was sought that it might be plasmid-mediated. An attempt was made to obtain sensitive variants, firstly by growth at 45°C, secondly by ultra-violet irradiation, and thirdly by treatment with acridine.
orange or acriflavine (Novick, 1969). Displacement of the resistance genes by the introduction of R-factors of various compatibility groups was attempted (Datta and Hedges, 1972). Finally, attempts were made to transduce resistance by means of the generalised transducing phage P1 (Arber, 1960).

### TABLE I

**Organisms causing asymptomatic bacteriuria in pregnancy**

<table>
<thead>
<tr>
<th>Organism</th>
<th>Number of strains isolated in Hammersmith Hospital</th>
<th>West Middlesex Hospital</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em></td>
<td>62</td>
<td>51</td>
</tr>
<tr>
<td><em>Klebsiella</em> spp.</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td><em>Proteus</em> spp.</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td><em>Streptococcus faecalis</em></td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td><em>Staphylococcus albus</em></td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>67</strong></td>
<td><strong>58</strong></td>
</tr>
</tbody>
</table>

### RESULTS

During the period under study, 3361 patients attended the antenatal clinic at Hammersmith Hospital for the first time and a mid-stream specimen was obtained from 3330 (99.1 per cent.). Significant bacteriuria was present in 126 of these patients. Of these 126 patients, 103 (82 per cent.) returned as requested for follow-up and ASB was diagnosed in 67 patients, giving an incidence of 2 per cent. At the West Middlesex Hospital there were 58 cases of ASB. The frequency of isolation of different organisms is shown in table I. The most common infecting organism at both hospitals was *E. coli*. At Hammersmith Hospital 24 per cent. of the strains of *E. coli* isolated were resistant to at least one antibiotic, whilst at the West Middlesex only 12 per cent. of *E. coli* were resistant (table II). In both hospitals, resistance to one drug was as common as resistance to more than one drug; resistance to more than two antibiotics

### TABLE II

**Antibiotic resistance of enterobacteriaceae causing asymptomatic bacteriuria**

<table>
<thead>
<tr>
<th>Antibiotic sensitivity</th>
<th>Number (and percentage) of organisms isolated in Hammersmith Hospital</th>
<th>West Middlesex Hospital</th>
<th>both hospitals</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>E. coli</em></td>
<td><em>Klebsiella</em> spp.</td>
<td>Total</td>
</tr>
<tr>
<td>Fully sensitive</td>
<td>47 (24)</td>
<td>0</td>
<td>47</td>
</tr>
</tbody>
</table>
| Resistant              | 15 (24)                  | 3                  | 18 (28) | 6 (12)   | 3                  | 9 (16) | 21 (18)   | 6                  | 27 (22)
occurred in only one instance in each group (table III). Five strains of Klebsiella spp. were resistant to ampicillin alone and one strain to ampicillin and streptomycin.

Transfer of resistance was demonstrated from six strains of E. coli and one strain of Klebsiella sp. Sensitive variants were obtained from a further two isolates of E. coli and in a third strain the resistance markers could be displaced by another R factor. This indicated that resistance was plasmid borne in at least 9 of the 21 (43 per cent.) strains of E. coli. Ampicillin resistance was transferable from one of the six strains of Klebsiella spp. The transducing phage P1 could not be grown in any of the strains in which transfer could not otherwise be demonstrated. The total frequency of resistance of E. coli and Klebsiella

<table>
<thead>
<tr>
<th>Hospital</th>
<th>Number of strains resistant to</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fully sensitive</td>
</tr>
<tr>
<td>Hammersmith</td>
<td>47</td>
</tr>
<tr>
<td>West Middlesex</td>
<td>45</td>
</tr>
</tbody>
</table>


spp. was 18 of 65 (28 per cent.) cases at Hammersmith Hospital and 9 of 56 (16 per cent.) at the West Middlesex Hospital.

DISCUSSION

Since the discovery of antibiotic resistance determined by R factors there has been considerable discussion as to the possible rate of spread of these among bacteria (Report, 1969). Bacteria carrying R factors are widely distributed among the aerobic Gram-negative flora of the human intestine (Datta, 1969), and in infections caused by Salmonella spp. (Anderson, 1968) and Shigella spp. (Davies, Farrant and Uttley, 1970). Many strains of enterobacteria other than Salmonella spp. and Shigella spp. (hereafter called “coliforms”) that cause infection in hospitals also carry R factors and these limit the choice of antibiotic suitable for therapy (Anderson, Datta and Shaw, 1972).

The frequency of R factors in “coliforms” causing disease outside hospital is therefore of interest. Urinary-tract infections were selected for study because they are the commonest coliform infections in the general community. Pregnant women at their first antenatal attendance represent a sample directly from the community without recent exposure to hospital environment. This study showed that approximately one-fifth of strains of E. coli and Klebsiella spp. causing asymptomatic bacteriuria of pregnancy were resistant to at least one chemotherapeutic agent and this was, in one-third of these strains, shown to be...
transferable. Multiple resistance, i.e., resistance to more than two agents, was rare and a suitable antibiotic was available for treatment in every case. There was no significant increase in frequency of resistant organisms or in the frequency of resistance to any particular agent when this series was compared with the symptomatic infections studied in 1968–69 and reported in the previous paper (Shaw et al., 1973), though both studies show an increase compared with figures obtained in 1962–63. Gillespie and his colleagues (1971) examined a similar population of pregnant women in the Bristol area and found a slightly higher frequency of resistance than we observed in London. Williams and Leigh (1966) found organisms causing ASB to be more frequently resistant to sulphonamides than organisms causing symptomatic urinary infection. If this finding is valid for our population, it suggests that although resistant organisms are present in the general community, they are not causing infection to an overwhelming degree, nor is multiple resistance posing a chemotherapeutic problem.

**SUMMARY**

The frequency of antibiotic resistance in coliform bacilli causing asymptomatic bacteriuria of pregnancy in two hospitals in West London was studied. Approximately one-fifth of the strains were resistant to at least one antibiotic. No increase in resistance was shown when these figures were compared with a previous study of symptomatic bacteriuria made in 1968–69, although both studies show an increase compared with figures obtained in 1962–63. In one-third of strains, resistance was shown to be mediated by R factors.

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**REFERENCES**


