THE ADHESINS AND FIMBRIAE OF PROTEUS MIRABILIS STRAINS ASSOCIATED WITH HIGH AND LOW AFFINITY FOR THE URINARY TRACT

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SUMMARY. In strains of Proteus mirabilis of urinary origin, no correlation was found between proticine types, reflecting relative affinity for the urinary tract, and the production of haemagglutinins and presence of fimbriae, a measure of adhesiveness.

INTRODUCTION

Strains of Proteus mirabilis of diverse origin grown under a variety of conditions produce one or more of three kinds of haemagglutinin. Each haemagglutinin was associated with the presence of fimbriae of a distinct kind (Old and Adegbola, 1982). The mannose-resistant klebsiella-like (MR/K) and the mannose-resistant proteus-like (MR/P) haemagglutinins (HAs) were produced commonly; the mannose-sensitive (MS) HA was rarely detected (Old and Adegbola, 1982).

A reliable method of typing strains of P. mirabilis by proticine production and sensitivity (P/S types) has established that certain strains of particular P/S types have a special affinity for the urinary tract in man (Senior, 1977; 1979). It has not yet been established, however, why some strains are more virulent for the upper urinary tract than others.

The aim of this study was to examine the HAs and fimbriae produced by strains of P. mirabilis of different P/S types in order to establish if there was any relationship between haemagglutinins and P/S types.

MATERIALS AND METHODS

Bacteria. Forty eight strains of P. mirabilis, characterised by the methods of McKell and Jones (1976), were studied; (i) 23 strains from Dundee patients—six male (aged 2–74 years) and 17 female (aged 34–86 years) with symptoms of upper urinary tract infection—were of P/S types associated with high affinity for the urinary tract; eight strains each of types 3/1,8 and 3/1,13 and seven strains of type 3/1,8,13; (ii) the other 25 strains, from Dr R. Maskell, Public Health Laboratory, Portsmouth, were from boys with significant bacteriuria (>10⁵ bacteria/ml of freshly voided urine), and were of diverse P/S types not associated with high affinity for the urinary tract: two isolates each of types 0/4,10 and 2/7 and one isolate each of types 0/0, 0/4, 0/5, 0/7, 1/0, 1/4, 2/0, 2/1, 2/12, 3/1, 3/7, 12, 4/10, 5/4, 5/7, 5/10, 7/0, 7/1, 7/5, 8/0, 10/1 and 12/7.

Cultures were stored on nutrient agar slants at 4°C until tested.

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Media. Test strains were grown in Nutrient Broth No. 2 (Oxoid) and on phosphate-buffered agar (PBA)—Nutrient Agar CM3 (Oxoid) with KH₂PO₄ 0.36% (w/v) and Na₂HPO₄ 0.64% (w/v) pH 7.0. Media were prepared and dispensed as previously described (Old and Adegbola, 1982).

Growth conditions. Each strain was serially subcultured in nutrient broth incubated statically in air. The following series of broth subcultures were made for each strain: (i) at 37°C, subcultured eight times at 3-day intervals; (ii) at 30°C, subcultured six times at 4-day intervals; and (iii) at ambient temperature (c. 20°C), subcultured six times at 7-day intervals. Cultures of each strain were also made on PBA: (i) at 37°C for 24 h, and (ii) at 20°C for 48 h.

Haemagglutination tests. The broth cultures were centrifuged (2000 g for 25 min) and the bacterial cells resuspended to a density of c. 5 x 10⁹ bacteria/ml in saline. Bacteria from the PBA cultures were harvested by scraping from the agar and resuspended in saline to a density of c. 10¹ⁱ bacteria/ml. The resultant suspensions were tested in rocked-tile tests for haemagglutination in the presence and absence of D-mannose using fresh erythrocytes from fowl (F), guinea pig (G), horse (H), man (group O) (M) and sheep (S) (Old and Adegbola, 1982) and ox erythrocytes treated with tannic acid (tanned) by the method of Duguid (1959). Activity due to the following kinds of HAS was judged to be present by the following criteria: (i) MS-HA (mannose-sensitive) when fresh erythrocytes of fowl, guinea pig or horse were agglutinated in the absence but not in the presence of D-mannose; (ii) MR/P-HA (mannose-resistant, proteus-like) when fresh erythrocytes of any species were agglutinated in the presence and absence of D-mannose; (iii) MR/K-HA (mannose-resistant, klebsiella-like) when tanned (but not fresh) ox erythrocytes were agglutinated. These HAS were described by Duguid and Old (1980) and their separate identification in haemagglutinating strains by Old and Adegbola (1982) and Adegbola and Old (1982).

The haemagglutinating power (HP) of haemagglutinating cultures was determined by the method of Duguid (1959).

Electronmicroscopy. Portions of bacterial cultures taken before suspensions were made for HA testing, were mixed with bacitracin solution (100 µg/ml) in clean tubes and a drop of the mixture placed on a clean sheet of dental wax (EMscope Laboratories, Ashford, Kent). A copper grid coated with carbon and formvar was floated on the drop for 1 min, excess fluid removed with filter paper and the bacteria washed twice with water in situ on the grid. Bacteria were negatively stained with methylamine tungstate, pH 6.5 (EMscope Laboratories) for 1 min. Grids were examined with a Jeol 100CX electronmicroscope and micrographs taken of the different kinds of fimbriae observed on bacteria from different preparations.

RESULTS

Haemagglutinins produced

Each of the 48 strains of *P. mirabilis* formed MR/P-HA in moderate-to-large amounts (HP values of c. 350–1400 with the most sensitive of the erythrocyte species agglutinated). The MR/P-HA pattern of erythrocyte species agglutinated was usually broad-spectrum; 33 strains produced the FGHMS pattern and 12 strains produced patterns that differed from FGHMS by only one species (table). An MR/P-HA that reacted with fowl erythrocytes only (F) was formed by three strains that were of different P/S types (0/4, 7/5 and 7/10), none of which was known to have high affinity for the urinary tract.

All 48 strains of *P. mirabilis* failed to agglutinate untanned ox erythrocytes; therefore, it was possible to use tanned ox erythrocytes for the detection of MR/K-HA (Old and Adegbola, 1982). This HA was produced by all strains, usually in the first or second subculture in any series; the HP values were c. 400–1600. Thus, all 48 strains formed MR/K and MR/P haemagglutinins.

MS-HA is detected best by its activity with fowl, guinea-pig or horse erythrocytes
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TABLE

Haemagglutinins produced by strains of Proteus mirabilis of different P/S types

<table>
<thead>
<tr>
<th>Numbers of strains</th>
<th>P/S type producing HA of type</th>
<th>Patterns of MR/P-HA detected* (and numbers of strains)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>tested</td>
<td>MS</td>
</tr>
<tr>
<td>3/1,8</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>3/1,13</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>3/1,8,13</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>Others†</td>
<td>25</td>
<td>0</td>
</tr>
</tbody>
</table>

* For erythrocyte species, see Materials and methods.
† 23 P/S types were represented, see Materials and methods.

in the absence of d-mannose. If it had been produced, it would have been detected in cultures of the seven strains that produced the MR/P-HA of pattern FHMS by its activity with guinea-pig erythrocytes and in the three strains that produced the narrow-spectrum MR/P-HA of pattern F by its activity with guinea-pig or horse erythrocytes but detection of MS-HA would have been difficult in cultures of the remaining 38 strains that produced MR/P-HA of patterns FGHMS, FGHM or FGHS. However, MS-HA was not detected in cultures of any strain of P. mirabilis tested in this series (table).

Fimbriae

Because it was not possible to establish cultural conditions whereby MR/K-HA or MR/P-HA was expressed independently (Old and Adegbola, 1982), electronmicroscopic examination of cells for the presence of fimbriae was made on cultures that showed MR/K and MR/P haemagglutination. Bacteria from these cultures usually possessed two kinds of fimbriae: (i) non-channelled fimbriae of external diameter 4 nm (figure, a) associated in strains of Proteus with the presence of MR/K-HA (Old and Adegbola, 1982); and (ii) thicker, channelled fimbriae of external diameter c. 7–8 nm (figure, b). In strongly haemagglutinating cultures (HP values for each HA ≥1000), there were usually 50–200 fimbriae of each type arranged peritrichously on c. 80–100% of the bacterial cells (figure, c). Thick channelled fimbriae were present on the bacterial cells whether the MR/P-HA was of the common, broad-spectrum type or the rarer, narrow-spectrum type.

DISCUSSION

Strains of Proteus are less commonly implicated than strains of Escherichia coli in non-obstructive urinary-tract infections. However, in patients with urinary-tract abnormalities or those subject to repeated urinary infections, P. mirabilis is an important urinary pathogen with a predilection for the upper urinary tract (Svanborg Edén, Larsson and Lomberg, 1980). Furthermore, different strains differ greatly in their “clinical virulence” for the urinary tract (Fowler and Stamey, 1978). Epidemiological studies have shown that those strains of P. mirabilis associated with
urinary-tract infection generally belong to particular P/S types (3/1, 8, 3/1, 13 and 3/1, 8, 13); strains of *P. mirabilis* of other P/S types and other species of *Proteus* are seldom incriminated in serious urinary-tract infection (Senior, 1979).

Results in the present study with *P. mirabilis* strains of P/S types highly associated with urinary-tract infection or not, were similar. Moreover, these results were also similar to those obtained with *P. mirabilis* strains of diverse origin studied previously (Old and Adegbola, 1982); all strains were multi-haemagglutinating and formed thick and thin fimbriae. A narrow-spectrum MR/P-HA that reacted with only fowl erythrocytes was found in some strains in the present study, but was detected in only three strains, all of which were of P/S types not highly associated with urinary-tract infection. This suggests that this haemagglutinin which has been described previously in other species of *Proteus* and in *Providencia* (Old and Adegbola, 1982) is not an important factor in virulence.

Fowler and Stamey (1978) found that strains of *P. mirabilis* isolated from renal
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stones and other strains isolated from the anal region of patients in whom they had never caused bacteriuria bound equally well to human vaginal epithelial cells in vitro. Furthermore, in another in-vitro study, most of 335 strains of P. mirabilis tested adhered well to human squamous, but not transitional, epithelial cells, and no significant differences in this property were found between strains isolated from the urine, blood or faeces (Svanborg Edén et al., 1980; 1981). No relationship between adhesiveness, origin or apparent association with urinary-tract infection was established. The results from the present study with P. mirabilis have failed to show any correlation between P/S type—one measure of “clinical virulence” for the urinary tract—and haemagglutination pattern—a measure of in-vitro adhesiveness. These results, therefore, extend and complement the earlier observations.

Fimbriae-mediated adhesiveness was proposed to be an important virulence factor for P. mirabilis in vivo in experimental pyelonephritis in rats (Silverblatt, 1974; Silverblatt and Ofek, 1978), but this was based on observations made on a single strain. The lack of any such evidence in the present and other in-vitro studies using many strains of diverse origin, including strains known to be associated with urinary-tract infection, suggests that the importance of adhesiveness in urinary infection due to P. mirabilis remains to be established.

REFERENCES


SENIOR, B. W. 1979. The special affinity of particular types of Proteus mirabilis for the urinary tract. Journal of Medical Microbiology, 12, 1–8.


