CHARACTERISTICS OF ANTIBIOTIC-RESISTANT *ESCHERICHIA COLI* IN THE RECTUM OF HEALTHY SCHOOL-CHILDREN

GUNILLA LIDIN-JANSON*†, E. FALSEN†, U. JODAL*†, B. KAUJER† AND
K. LINCOLN†

†Departments of Clinical Bacteriology and Immunity, Institute of Medical Microbiology, and

*Departments of Infectious Diseases and Paediatrics, University of Göteborg, Göteborg, Sweden

IN the host-parasite relationship in urinary tract infection (UTI), several characteristics of the invading micro-organism may be of importance (Hanson, 1973). The bowel is an important reservoir for strains of *Escherichia coli* that infect the urinary tract (Turck, Petersdorf and Fournier, 1962). During the treatment of patients with UTI the selective pressure of antibacterial agents may profoundly influence the gut flora (Winberg *et al.*, 1973). The aim of the present study was to define some characteristics of *E. coli* strains in the faecal flora of a community of school-children and especially to compare the drug-resistant *E. coli* strains with those of normal sensitivity in order to evaluate the selection of drug-resistant strains during antibacterial treatment in domiciliary practice.

MATERIALS AND METHODS

Plan of the investigation. In connection with a screening programme for asymptomatic bacteriuria in school-children (Lindberg *et al.*, 1975) 774 rectal swabs were taken from 655 children aged 7 to 16 years. Children from four schools (A, B, C and D) in different parts of the city (450 000 inhabitants) were studied—A and B in March 1971, C and D in November 1972, and B and D again in May 1974. In the course of the study, 20 and 96 pupils of schools B and D respectively were examined twice. In each school, classes of 18–31 pupils from each of the first to the sixth or seventh grades were examined. Questionnaires were distributed together with bottles for morning urine specimens. Histories of courses of antibiotics during the previous year, usually in respect of penicillin V, were reported in 136 cases (17.6%) and explicitly denied in 612 (79.1%). Nitrite tests and dip-slide cultures demonstrated infection in three cases (Lindberg *et al.*, 1975) and these children were excluded from the present study.

In each school, the pupils had lunch at a canteen. The food was prepared in separate kitchens for each school but supplies came from the same stores.

Bacteriological examinations. Faecal specimens were taken by swabbing the rectum with a cotton-tipped applicator stick which was inserted about 4 cm from the anal skin surface. Each swab was put into a tube containing transport medium (Möller, 1966), kept at 4°C and plated out later the same day. All plates were incubated aerobically at 37°C overnight.

Each swab was first used for heavy inoculation of one plate of peptone-free blood-agar. Antibiotic disks (AB Biodisk, Solna, Sweden) were placed on the agar surface. In 1971, new concentrations of certain antibiotics for these standard disks, came into use all over Sweden. The antibiotics and quantities in µg per disk were as follows, the amounts in brackets being those used up to 1971: sulphasomidine (Su) 250 (2400), tetracycline (T) 30 (50), streptomycin (S) 30 (50), chloramphenicol (C) 30 (30), ampicillin (A) 10 (12), nitrofurantoin (Ni) 30 (30),

Received 2 Aug. 1976; accepted 23 Dec. 1976.

nalidixic acid (Nal) 30 (30). In 1972 and 1974 trimethoprim (Trim), 1.2 µg per disk, was also used. The zones of inhibition around the antibiotic disks were measured in mm. Colonies of Gram-negative bacilli growing nearer to the disks than usual for sensitive *E. coli* were picked off for subculture.

The swabs were also cultured on modified Conradi-Drigalski agar plates (Kauffmann, 1966) to produce single colonies. One colony from each plate was subcultured. Selection irrespective of colony morphology was achieved by always picking the last colony growing from the streaks. Gram-negative bacilli were typed according to Edwards and Ewing (1972). Strains were held as deep agar-stab cultures under sterile paraffin oil.

Escherichia coli serotyping. A simplified O grouping was performed on Perspex plates as previously described (Lincoln, Lidin-Janson and Winberg, 1970) but with further development of the technique as follows. In a first step, 10 monovalent sera to the O antigen groups 1, 2, 4, 6, 7, 8, 16, 18, 25 and 75 were used. If a distinct agglutination did not occur, two sets of polyvalent antisera were used. Polyvalent antisera to 56 O-groups were produced by immunising altogether 15 rabbits in a crosswise manner, giving one set of seven sera and one set of eight sera, each set containing agglutinins to all 56 O-groups. A strain reacting in both sets could thus be classified by reference to a chequer-board chart. The 56 O-groups were selected in 1968, mainly on the basis of 8 years' experience of UTI strains sent from this laboratory to the International Escherichia Center in Copenhagen for serotyping. The O-groups were numbers 5, 10-13, 15, 17, 19-23, 26, 28, 33, 34, 36, 42, 46, 48, 51, 55, 59, 60, 65, 68, 69, 71, 76-78, 80-83, 85, 86, 88, 91, 98, 99, 101, 110, 111, 112 ab, 112 ac, 115, 117-120, 125, 128, 133, 140 and 145. There were also monovalent antisera against the O-groups 3, 9 and 14.

Cultures that were non-specifically agglutinated by all 10 sera in the first step were classified as spontaneously agglutinating (autoagglutinable) and designated Sa. Often, but not always, such strains were growing with rough colonies on the Conradi-Drigalski plate. Strains that were not agglutinated by the available antisera and did not spontaneously agglutinate were designated O Na. Groupable and O Na strains will together be referred to as smooth.

The final dilution of the antiserum in the wells was as a rule 1 in 500 to 2000, usually 1 in 1000. At this dilution weak cross-reactions were sometimes seen, especially between groups O1 and O2 and between O4 and O18. The pattern of agglutination as observed in the microscope usually made titration unnecessary, large coarse agglutinates representing a strong reaction and small, even agglutinates a weak one. Weak spontaneous agglutination could sometimes be combined with a strong agglutination with one specific antiserum, most often the O14 antiserum.

Identification of the K1 antigen. The K1 antigen was assessed by a modification (Kaijser, 1977) of the serum agar technique (Bradshaw *et al.*, 1971). The K typing was limited to antigen K1 since this is by far the most frequent *E. coli* K antigen and has been shown to be a virulence factor (Robbins *et al.*, 1974; Kaijser, 1973; Kaijser *et al.*, 1977).

Test for haemolysin production. Each colony to be tested was inoculated with a platinum needle on to an agar plate with 5% washed horse erythrocytes. The haemolytic zones were read immediately after overnight incubation.

Antibiotic sensitivity testing. Immediately after isolation, subcultures were subjected to antibiotic sensitivity testing with a disk diffusion method (Ericsson and Sherris, 1971) and the antibiotic disks described above. The medium was peptone-free nutrient agar with 5% defibrinated horse blood and the inoculum was rather light to facilitate reading of sulphonomide and nitrofurantoin inhibition zones. The prediffusion-time was 3 h.

In 1975, minimum inhibitory concentrations (MICs) of the agents tested were assessed by the agar dilution method (Ericsson and Sherris, 1971). The medium used was PDM Antibiotic Sensitivity Medium (ASM) supplied by Biodisk and detailed by Wretling, Nord and Wadström (1974). Plates (9 cm diameter) contained 25 ml of ASM either without antibiotic or with serial twofold dilutions of the following substances: sulphasomidine, batch Sdg 1106 (Hässel-Ciba-Geigy, Basel/Mölnal), tetracycline HC1, batch 310-2973 (Pfizer, Täby), streptomycin sulphate, batch 53556-01 (Kabi, Stockholm), chloramphenicol, batch LPG 113

Statistical calculations. Fisher's permutation test was used (Oden and Wedel, 1975). Two-tailed significance tests were done and values for $2p$ of less than 0.05 were considered significant.

Thirty-six of the 771 cultures yielded very few colonies of Gram-negative bacilli or none at all. Thus, 735 colonies were picked off from the Conradi-Drigalski plates, of which 709 were *E. coli*, eight *Klebsiella*, seven *Citrobacter*, four *Enterobacter*, five intermediary coliforms, one *Proteus vulgaris* and one *Pseudomonas aeruginosa*. The 709 *E. coli* colonies, each isolated from one Conradi-Drigalski agar plate, will be referred to as the unselected *E. coli* strains. Each of the six study groups, consisting of pupils from schools A and B in 1971, C and D in 1972, and B and D in 1974, contributed 99-134 unselected *E. coli*. Of these, 102 were resistant to one or more of the antimicrobial agents tested and 607 were fully sensitive.

Among the *E. coli* colonies picked off from the blood agar plates as probably resistant (see *Methods*), another 204 resistant strains were identified and will be referred to as the selected resistant *E. coli*. Two or more colonies with identical properties and isolated from the same specimen were regarded as belonging to the same strain.

The MIC of each of the eight antimicrobial agents studied was determined for a random sample of 117 of the unselected *E. coli* strains (table I). The

TABLE I
Minimum inhibitory concentrations of eight antimicrobial drugs for 117 unselected strains of Escherichia coli

[illegible]

TABLE II

*Patterns of resistance shown by 306 faecal E. coli strains resistant to one or more of eight antimicrobial drugs**

Resistance pattern	Number of strains	Resistance pattern	Number of strains
SuTS	87	TSA	3
SuS	55	SuSC	3
T	52	SuTSNi	2
Su	35	SuSCA	2
TS	20	SuSA	2
SuTSA	13	SuT	1
SuTSCA	8	SuTSANi	1
SuTSC	6	SuTSCNi	1
A	6	SuTCA	1
Ni	5	TA	1
		SA	1
		S	1

* The abbreviations are explained in table I.

TABLE III

Demonstration of antibiotic-resistant E. coli in rectal swabs from 96 children who were examined both in 1972 and 1974

Number of children in whom (in 1972) anti-biotic-resistant <i>E. coli</i> were		Number of children in whom (in 1974) antibiotic-resistant <i>E. coli</i> were	
		found	not found
found	37	16	21
not found	59	18	41
Totals	96	34	62

highest MICs in the system used for *E. coli* of ordinary sensitivity were (μ g per ml): Su 256, T 4, S 4, C 8, A 16, Ni 16, Nal 8 and Trim 2.

The patterns of resistance of 306 *E. coli* strains are given in table II; the data for 276 strains were obtained by agar dilution tests and the rest by disk diffusion tests performed at the isolation stage. Twenty-six selected resistant strains had died during the study. Four strains had apparently lost R factors. In a few cases, the presence of segregated R factors was indicated; for instance O77 SuTSA- and SuSA-resistant bacteria occurred in the same specimen (Datta *et al.*, 1971). In such cases, only the most complicated pattern of resistance was counted.

Resistance to Su, T and S, often in various combinations occurred most frequently. Altogether, 217 strains (71%) were resistant to Su, 205 (67%) to S and 196 (64%) to T.

TABLE IV

A comparison between O antigen groups of antibiotic-sensitive, smooth E. coli strains and of strains resistant to at least one antibacterial agent

<i>E. coli</i> O group	Percentage number of the sensitive strains (n = 534)	Value of 2p for difference	Percentage number of the resistant strains		
			Total (n = 281)	Unselected† (n = 85)	Selected‡ (n = 196)
O1	8.8	0.006	3.5	1.2	4.6
O2	9.2	< 0.001	1.8	3.5	1.0
O6	5.2	0.02	1.8	2.3	1.5
O8	2.8	< 0.001	9.2	3.5*	11.7*
O18	5.4	NS	3.5	7.0	2.0
O25	5.1	NS	2.1	1.2	2.6
O77	0.4	< 0.001	6.4	9.3	5.1
Not agglutinable with the available antisera	23.8	0.002	34.4	29.1	36.7

* Value of 2p for difference here was 0.04; the differences between the other paired results in these columns were not significant (NS).

† Unselected = picked from antibiotic-free medium.

‡ Selected = picked because of antibiotic resistance.

Out of 190 strains resistant to the sulphonamide, 183 were not inhibited by 2048 µg per ml. Resistance to streptomycin in 185 strains was evenly distributed in the range 8–64 µg per ml, whereas resistance to tetracycline in 176 strains was distributed bimodally, 50 strains being inhibited by 16 µg per ml and 109 by 64–256 µg per ml. The high-level resistance group included 41 of 45 T monoresistant strains.

The 306 resistant *E. coli* strains were derived from 261 (33.9%) of the 771 specimens. The range of variation in occurrence was 30.3–37.4% for the three years of the study and 24.4–38.8% for the six study groups. In the two schools examined twice the prevalence of resistant *E. coli* did not change significantly. However, in school B resistance to T decreased from 31.1% in 1971 to 14.3% in 1974 (2p = 0.003). Out of the 96 children from school D who were examined both in 1972 and in 1974, 16 were shown to harbour antibiotic-resistant *E. coli* on both occasions (table III). Strains with identical properties were found on both occasions in three children. This figure coincides with the difference from the expected 13, calculated from $34 \times 37/96$ (table III).

The O and K antigens of the antibiotic-resistant and the sensitive *E. coli* strains were compared. The distribution of the most common O antigen groups and of non-agglutinable strains is shown in table IV. In the whole group of smooth, antibiotic-resistant *E. coli* strains the O-groups 1, 2 and 6 were less frequent than among the sensitive strains and the groups O8 and O77 were more frequent, as were strains not agglutinable with the available test sera. O-group 8 was more preponderant among the selected resistant strains than among the unselected.

Resistant *E. coli* serotypes O8 and O77 were found in all study groups. O8 bacteria appeared with 11 different patterns of resistance, from mono-resistances

TABLE V

Occurrence of K1 antigen in antibiotic-sensitive and -resistant unselected E. coli strains*

O group	Proportion of K1 positive strains among	
	antibiotic-sensitive strains	strains resistant to at least one antibacterial agent
O1	38/47	0/1
O2	34/49	2/3
O3	0/8	0/2
O4	2/10	0/3
O6	0/28	0/2
O7	10/19	0/3
O8	0/15	0/3
O9	1/3	0/1
O10	0/5	...
O11	0/3	1/1
O12	4/4	...
O13	3/24	0/4
O15	0/4	1/2
O16	11/11	...
O18	14/29	6/6
O21	0/13	...
O22	1/3	0/1
O23	0/27	0/1
O42	1/2	...
O46	1/2	...
O51	2/4	...
O75	1/12	...
O77	0/2	0/8
O81	1/9	...
O83	13/15	1/1
O110	1/1	0/1
O112 ab	0/10	...
25 other O groups less than 10 of each	0/48	0/18
Subtotal, O groupable	138/407 = 33.9%	11/61 = 18.0%
Not agglutinable	1/127 = 0.8%	0/24 = 0.0%
Spontaneously agglutinating	39/73 = 53.4%	8/17 = 47.1%
All	178/607 = 29.3%	19/102 = 18.6%

* Unselected = picked from antibiotic-free medium.

Su, T and Ni to multi-resistances SuTCA and SuTSA. O77 bacteria had eight different patterns, from mono-resistances Su and T to multi-resistances SuTCSA and SuTSA.

Table V shows the occurrence of the antigen K1 among the sensitive and the resistant unselected strains. The K1 antigen frequently occurred together with the O antigens 1, 2, 7, 16 and 83 which were well represented among the sensitive strains. K1 was also common in the O18 strains, accounting for 6 of 11 resistant, smooth K1-positive strains. All the resistant O18 K1 strains

TABLE VI
Haemolysis in solid medium by antibiotic-sensitive and -resistant E. coli strains

O typability	Proportion of haemolytic strains among			
	antibiotic-sensitive strains		strains resistant to at least one antibacterial agent	
	Number*	Percentage	Number*	Percentage
Groupable into one of 69 O-groups	76/407	18.6	32/178	18.0
Not agglutinable with the available antisera (O Na)	18/127	14.2	3/93	3.2
Spontaneously agglutinating	11/73	15.1	2/23	8.7
Total	105/607	17.3	37/294	12.6

* Number of strains showing haemolysis out of number tested in the stated category.

occurred in school D, four in 1972 and two in 1974, and all were mono-resistant to Su. Out of eight resistant Sa K1 strains, six were mono-resistant to Su and occurred in school D, four in 1972 and two in 1974. Altogether, the K1 antigen was found in 29% of the sensitive and in 19% of the resistant strains ($2p = 0.03$).

The group of sensitive strains was compared with the entire group of resistant strains for the capacity to produce haemolysis in solid medium (table VI). Twelve strains that had died are excluded from the table. There was a tendency towards a greater proportion of haemolytic strains in the sensitive group (17.3% compared with 12.6%, $2p = 0.08$). The O Na sensitive strains were more often haemolytic than the O Na resistant strains ($2p = 0.009$).

DISCUSSION

In the present study of *E. coli* collected from healthy school-children during a 3-year period, the sensitive strains and the strains resistant to at least one antimicrobial agent were two somewhat different populations. Resistant strains were less likely than sensitive ones to belong to the O groups 1, 2 and 6, and more likely to belong to the O groups 8 and 77 or to be non-typable with the sera used.

Two possible virulence factors were unevenly distributed among sensitive and resistant *E. coli*. The K1 antigen was less common among the resistant strains. This antigen is of pathogenic significance, occurring more often in strains from UTI, especially pyelonephritis, than in strains from normal faeces (Kaijser *et al.*, 1977) and is also strikingly common among *E. coli* strains causing neonatal meningitis (Robbins *et al.*, 1974). In the large group of resistant strains not agglutinable with the O-antisera used, haemolytic strains were infrequent. Haemolytic capacity of *E. coli* in solid medium seems to correlate with ability to infect the urinary tract (Cooke and Ewins, 1975).

Aspects of plasmid physiology offer possible explanations for a different distribution of properties among antibiotic-resistant and sensitive strains. Different cell envelope characteristics may confer different recipient abilities (Wiedemann and Schmidt, 1971). Plasmid incompatibility (Datta and Hedges, 1971) may be the reason for the low coincidence of haemolytic capacity and antibiotic resistance found among the O Na strains of the present study.

The resistance factors selected by the use of antibiotics in animals and man both contribute to the maintenance of a pool of antibiotic-resistant enterobacteria that would otherwise tend to diminish (Swann Committee, 1969). Spread of hospital-derived, antibiotic-resistant enterobacterial strains from former patients to healthy people has been shown to occur (Damato, Eitzman and Baer, 1974). Also, animal *E. coli* strains may reach the human population via food (Cooke *et al.*, 1970) and may become established in the bowel (Shooter *et al.*, 1970).

In Sweden, chlortetracycline, oxytetracycline, bacitracin, spiramycin, oleandomycin, and the nitrofurans nitrovin, nitrofenid and nitrofurazone, are permitted feed additives for calves, swine, sheep and poultry (Statens Jordbruksnämnd, 1972). Tetracyclines are not permitted for growth-promotion purposes, but were so before 1 April 1971. The decrease in the prevalence of tetracycline resistance in school B from 1971 to 1974 is of some interest in this context. Chlortetracycline is the agent most used for group therapy with medicated feeds. Sulphonamides and streptomycin as well as penicillins are used extensively for individual antibacterial treatment in veterinary practice (Dr L. Rutqvist, personal communication). Sulphonamides are used as feed additives in other parts of the world (Jukes, 1971). More than 60% of the 306 resistant *E. coli* in the present study, were resistant to sulphonamides, and the frequency of resistance to streptomycin and tetracycline was similar.

Bettelheim *et al.* (1974), with full O and H serotyping facilities, found that O-typable strains were less commonly derived from animals and were more commonly derived from man. This is not surprising since the *E. coli* antigen scheme was based on work on strains of mainly human origin (Kauffmann, 1966). Serotypes commonly associated with UTI in humans, and probably derived from the normal human flora, were more often found among the human strains than among the animal strains.

Of antibiotic-resistant *E. coli* isolates from calves, Hartley *et al.* (1975) found that the O-groups 8, 9 and 101 accounted for 24%. In human clinical isolates, the O-group distribution differed between the antibiotic-resistant and sensitive strains (Petrocheilou and Richmond, 1976), groups 8, 9, 17 and 101 being considerably more common among the resistant *E. coli*, whether or not known to be carrying plasmids. These observations are in agreement with the concept that a high proportion of the antibiotic resistant *E. coli* found in our healthy school-children were animal-derived strains.

It is well known that during the months immediately following treatment of UTI, recurrences are most likely to occur (Winberg *et al.*, 1974; Stansfeld, 1975). In that situation the composition of the normal *E. coli* flora might be of crucial importance. A selective pressure of antimicrobial substances, applied

in a hospital milieu, may act upon a flora adapted to infect a sensitive human host (Lincoln *et al.*, 1970). Antibiotic treatment of outpatients may also select for an *E. coli* flora resistant to one or more antibacterial agents. However, that flora will probably not be especially rich in factors likely to favour symptomatic reinfection of the urinary tract.

SUMMARY

During a 3-year period, 771 rectal swabs were taken from abacteriuric school-children. Out of 709 *E. coli* strains, each isolated from one faecal specimen, 102 were found to be resistant to one or more antibacterial agents, and 607 to be fully sensitive. Another 204 resistant strains were found by selection for antibiotic resistance. The antibiotic-sensitive and the resistant strains were found to be two somewhat different populations, distinguished by a different distribution of O antigen types. Also, the K1 antigen was more common among the sensitive than among the resistant strains. Resistant strains that were not O typable were very seldom haemolytic.

This work was supported by grants from the Medical Faculty of Göteborg, the Göteborg Medical Association, the First of May Flower Annual Campaign for Children's Health, and the Swedish Medical Research Council (Project no. 215). The skilful technical assistance of Helena Lomberg, Ingela Delgado, Siv Mangél, Lilian Nilsson, Kaety Plos and Anette Wetterstrand is very much appreciated.

REFERENCES

- BETTELHEIM, K. A., BUSHROD, F. M., CHANDLER, M. E., COOKE, E. M., O'FARREL, S. AND SHOOTER, R. A. 1974. *Escherichia coli* serotype distribution in man and animals. *J. Hyg., Camb.*, **73**, 467.
- BRADSHAW, M. W., PARKE, J. C., JR, SCHNEERSON, R. AND ROBBINS, J. B. 1971. Bacterial antigens cross-reactive with the capsular polysaccharide of *Haemophilus influenzae* type b. *Lancet*, **1**, 1095.
- COOKE, E. M. AND EWINS, S. P. 1975. Properties of strains of *Escherichia coli* isolated from a variety of sources. *J. med. Microbiol.*, **8**, 107.
- COOKE, E. M., SHOOTER, R. A., KUMAR, P. J., ROUSSEAU, S. A. AND FOULKES, A. L. 1970. Hospital food as a possible source of *Escherichia coli* in patients. *Lancet*, **1**, 436.
- DAMATO, J. J., EITZMAN, D. V. AND BAER, H. 1974. Persistence and dissemination in the community of R-factors of nosocomial origin. *J. Infect. Dis.*, **129**, 205.
- DATTA, N., FAIERS, M. C., REEVES, D. S., BRUMFITT, W., ØRSKOV, F. AND ØRSKOV, I. 1971. R. factors in *Escherichia coli* in faeces after oral chemotherapy in general practice. *Lancet*, **1**, 312.
- DATTA, N. AND HEDGES, R. W. 1971. Compatibility groups among f_i^- R factors. *Nature, Lond.*, **234**, 222.
- EDWARDS, P. R. AND EWING, W. H. 1972. Identification of Enterobacteriaceae, 3rd ed., Minneapolis.
- ERICSSON, H. M. AND SHERRIS, J. C. 1971. Antibiotic sensitivity testing. *Acta path. microbiol. scand.*, Sect. B, Suppl. 217.
- GARRETT, E. R. AND WRIGHT, O. K. 1967. Kinetics and mechanisms of action of drugs on microorganisms. VII. Quantitative adherence of sulfonamide action on microbial growth to a receptor-site model. *J. pharm. Sci.*, **56**, 1576.
- HANSON, L. Å. 1973. Host-parasite relationships in urinary tract infections. *J. infect. Dis.*, **127**, 726.

- HARTLEY, C. L., HOWE, K., LINTON, A. H., LINTON, K. B. AND RICHMOND, M. H. 1975. Distribution of R plasmids among the O-antigen types of *Escherichia coli* isolated from human and animal sources. *Antimicrob. Agents Chemother.*, **8**, 122.
- JUKES, T. H. 1971. The present status and background of antibiotics in the feeding of domestic animals. *Ann. N.Y. Acad. Sci.*, **182**, 362.
- KAIJSER, B. 1973. Immunology of *Escherichia coli*: K antigen and its relation to urinary-tract infection. *J. infect. Dis.*, **127**, 670.
- KAIJSER, B. 1977. A simple method for K typing of *E. coli* bacteria. *FEMS Letters*, in the press.
- KAIJSER, B., HANSON, L. Å., JODAL, U., LIDIN-JANSON, G. AND ROBBINS, J. B. 1977. Frequency of *E. coli* K antigens in urinary tract infections in children. *Lancet*, **1**, 663.
- KAUFFMANN, F. 1966. The bacteriology of Enterobacteriaceae, *Copenhagen*.
- LINCOLN, K., LIDIN-JANSON, G. AND WINBERG, J. 1970. Resistant urinary infections resulting from changes in resistance pattern of faecal flora induced by sulphonamide and hospital environment. *Br. med. J.*, **3**, 305.
- LINDBERG, U., CLAËSSON, I., HANSON, L. Å. AND JODAL, U. 1975. Asymptomatic bacteriuria in schoolgirls. I. Clinical and laboratory findings. *Acta paediat. scand.*, **64**, 425.
- MÖLLER, A. J. R. 1966. Microbiological examination of root canals and periapical tissues of human teeth: methodological studies, *Göteborg*, p. 365.
- ODÉN, A. AND WEDEL, H. 1975. Arguments for Fisher's permutation test. *Ann. Statistics*, **3**, 518.
- PETROCHEILOU, V. AND RICHMOND, M. H. 1976. Distribution of R plasmids among the O-antigen types of *Escherichia coli* isolated from various clinical sources. *Antimicrob. Agents Chemother.*, **9**, 1.
- ROBBINS, J. B., MCCracken, G. H., GOTSCHLICH, E. C., ØRSKOV, F., ØRSKOV, I. AND HANSON, L. Å. 1974. *Escherichia coli* K1 capsular polysaccharide associated with neonatal meningitis. *New Engl. J. Med.*, **290**, 1216.
- SHOOTER, R. A., COOKE, E. M., ROUSSEAU, S. A. AND BREADEN, A. L. 1970. Animal sources of common serotypes of *Escherichia coli* in the food of hospital patients: possible significance in urinary-tract infections. *Lancet*, **2**, 226.
- STANSFELD, J. M. 1975. Duration of treatment for urinary tract infections in children. *Br. med. J.*, **3**, 65.
- STATENS JORDBRUKSNÄMND'S CIRKULÄR NO. 95. 1972, *Stockholm* (Circular from the National Agricultural Marketing Board).
- SWANN, M. M. 1969. Report of the Joint Committee on the use of antibiotics in animal husbandry and veterinary medicine, H.M.S.O., *London*.
- TURCK, M., PETERSDORF, R. G. AND FOURNIER, M. R. 1962. The epidemiology of non-enteric *Escherichia coli* infections: prevalence of serological groups. *J. clin. Invest.*, **41**, 1760.
- WIEDEMANN, B. AND SCHMIDT, G. 1971. Structure and recipient ability in *E. coli* mutants. *Ann. N.Y. Acad. Sci.*, **182**, 123.
- WINBERG, J., ANDERSEN, H. J., BERGSTRÖM, T., JACOBSSON, B., LARSSON, H. AND LINCOLN, K. 1974. Epidemiology of symptomatic urinary tract infection in childhood. *Acta paediat. scand.*, Suppl. 252.
- WINBERG, J., BERGSTRÖM, T., LINCOLN, K. AND LIDIN-JANSON, G. 1973. Treatment trials in urinary tract infection (UTI) with special reference to the effect of antimicrobials on the fecal and periurethral flora. *Clin. Nephrol.*, **1**, 142.
- WRETTLING, B., NORD, C.-E. AND WADSTRÖM, T. 1974. In vitro sensitivity of isolates of *Pseudomonas aeruginosa* to carbenicillin, gentamicin, tobramycin, and some other antibiotics. *Scand. J. infect. Dis.*, **6**, 49.