feature of tropical splenomegaly syndrome there may be a genetic basis for this type of immune response (McDevitt and Benacerraf, 1969). There was a high prevalence (58%) of splenomegaly among neighbour controls, which tended to cluster within families. The high frequency of splenomegaly among Rwandan immigrants is consistent with the observations of others (Hamilton *et al.*, 1965; Shaper 1969). Nevertheless, the frequency (100%) was significantly higher in the kindred than in the neighbour controls.

The possible relationship of tropical splenomegaly syndrome and endomyocardial fibrosis in the kindred is interesting in the light of the postulated autoimmune pathogenesis of endomyocardial fibrosis (van der Geld *et al.*, 1966). Shaper *et al.* (1968) described an immunological syndrome largely affecting Rwandan immigrants consisting of raised IgM levels and raised malarial antibody titres in association with circulatory antibodies to IgG, heart, thyroid, and gastric parietal cells. They suggested that these are associated with an altered immunological response to malaria in subjects who may have an inherently unusual immunological constitution. The question of autoimmune disorders and malaria has been examined by others (Greenwood *et al.*, 1971), and the problem is by no means settled.

The hypothesis that tropical splenomegaly syndrome is an "immune complex disease" receives support from several lines of evidence (Christian, 1969). The finding of low levels of serum complement among patients with the syndrome in the present study plus the finding of anticomplementary activity in sera of patients with tropical splenomegaly syndrome (Ziegler and Fife, unpublished) suggest in-vivo fixation of complement by antigen-antibody interaction. Cryoproteins observed in the sera of 84.6% of patients with tropical splenomegaly syndrome in New Guinea (Wells, 1970) most likely represent circulating immune complexes (Barnett *et al.*, 1970).

Since patients with tropical splenomegaly syndrome have high titres of malarial antibody and live in regions of high malarial transmission, repeated and persistent antigenaemia is likely to occur. In the presence of antibody excess immune complexes will form. Because of the predominant IgM nature of the antimalarial antibody in patients with tropical splenomegaly syndrome (Mody, 1970) the circulating complexes will be large and produce continuous particulate stress to reticulo-endothelial tissues. Such complexes might in themselves also be antigenic, including further antibody formation and macromolecular aggregation, thus perpetuating the disorder (Bonomo and Dammacco, 1971). Predisposition to the development of tropical splenomegaly syndrome may reside in the genetically-determined production of predominantly IgM antibody, as suggested in the present study.

Interruption of this postulated chain of events by the prevention of antigenaemia by prolonged malarial prophylaxis might explain the observed clinical amelioration and the return of IgM and complement levels towards normal after such treatment (Sagoe, 1970). Interruption of the immune response by immunosuppressive agents could also theoretically bring about clinical improvement, but it could not be recommended as specific therapy in view of the well-documented improvement on antimalarials.

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R Factors in Hospital Infection

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Summary

In order to assess the clinical importance of R factors 524 "coliform" infections were studied in a general hospital.

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Of these, 95 were caused by *Pseudomonas aeruginosa*. Among the remaining 429, 43% were caused by drug-resistant enterobacteria; resistance was frequently multiple and determined by transmissible R factors. Choice of therapy was thus limited but in every case at least one antibiotic, effective in vitro, was still available.

Introduction

Hospital infections with Gram-negative bacilli have become more important in the past 25 years—that is, since the introduction of antibiotics (Finland, Jones, and Barnes, 1959). In the

last 10-15 years antibiotic resistance determined by transmissible R factors has spread widely among the aerobic Gram-negative bacilli which inhabit the human intestine (Datta, 1969). R factors are of practical importance if the drug resistance determined by them interferes with effective treatment of bacterial infection. In order to assess this importance in a general hospital we made a survey of Gram-negative infections and of antibiotic resistance in the causative organisms. The infections under study were not necessarily acquired in hospital, but all were diagnosed in inpatients.

In the case of wound infections we compared the incidence and severity of those yielding only "coliform" organisms with those caused by *Staphylococcus aureus*.

The Survey

The survey covered four five-week periods between November 1969 and November 1970 and was based on specimens sent for culture to the diagnostic laboratory. Cultures which yielded enterobacteriaceae and/or *Pseudomonas* species in "significant" numbers were collected. Blood cultures were collected throughout the 12 months. For the sake of brevity the organisms collected are referred to as "coliforms." Our criteria for "significant" numbers are defined below, according to the type of specimen.

The clinical history of each patient from whom the specimens came was obtained from the clinician. In particular, inquiry was made about signs and symptoms of infection and whether antibacterial therapy was considered necessary. Infections were classified according to site.

Blood Culture.—Blood for culture was inoculated into four bottles—two Casteneda (Tryptone Soya Broth (Oxoid) containing 0.03% Liqueid over slopes of Oxoid blood agar base), one Robertson's cooked meat, and one Brewer's thioglycollate broth. Culture was counted as positive (for the purpose of this survey) when three bottles yielded the same organism.

Urinary Tract Infection.—A semiquantitative method of culture was used. Significant numbers of bacteria, indicating infection, were taken as 10^5 /ml or more.

Wound Infection.—Wound swabs were sent to the laboratory for culture only when there was some clinical evidence of infection. Significant growth was taken as "2+"—that is,

growth at least on the areas of primary and secondary inoculation. During the last two periods of the coliform survey wound infections caused by *Staph. aureus* were also studied.

Chest Infections.—Specimens were included in the survey if they were (1) purulent sputum from patients not on antibiotics and (2) fluid from tracheostomy suction. Sputum was digested with pancreatin, diluted 1:10,000, and 0.1 ml spread on appropriate media (Dixon and Miller, 1965). Any growth was taken as significant. Coliforms were collected only if they were the majority organism. Tracheal washings were plated directly and significant growth was taken as "2+", as above.

Miscellaneous.—Small numbers of peritoneal dialysis fluids, swabs from burns, and umbilical swabs were cultured. Criteria for "significant" growth depended on the specimen.

Bacteriological Methods

Identification.—Bacteria were purified and identified by methods in routine use in the department (Darrell, 1967).

Sensitivity Tests.—Coliforms were tested by a disc-sensitivity method on nutrient agar (Oxoid), containing 4% lysed horse blood, against the following antibiotics: ampicillin (A), streptomycin (S), tetracycline (T), chloramphenicol (C), kanamycin (K), sulphonamide (Su), nalidixic acid (Na), polymyxin B (P), gentamicin (G), and trimethoprim (Tr). *Pseudomonas* species were also tested against carbenicillin (carb) and the minimal inhibitory concentration (M.I.C.) was determined by using a series of concentrations of drug in lysed-blood agar.

Classification of Resistant Bacteria.—In assessing resistance patterns different criteria were used for the different bacterial genera. Resistance to certain antibiotics was taken as characteristic of certain organisms, and not therefore likely to have been influenced by the spread of R factors. Characteristic resistances were taken to be as follows: the *Proteus* group, tetracycline and polymyxin; *Klebsiella* and *Enterobacter* spp., ampicillin; *Ps. aeruginosa*, all antibacterial agents except polymyxin, gentamicin, and carbenicillin.

Transfer of Resistance.—A representative sample of resistant coliforms were tested for transfer of resistance to *Escherichia coli* K12 during growth in mixed culture. Methods were as described by Datta (1968) except that in testing strains of *Ps. aeruginosa* for transfer of carbenicillin resistance the recipient used was a rifampicin-resistant mutant of *E. coli* K12; mixtures were plated on MacConkey's agar incorporating rifampicin 50 µg/ml and carbenicillin 100 µg/ml.

Results

Septicaemia.—There were 18 cases of coliform septicaemia. The organisms and their resistance patterns are listed in Table I.

Urinary Tract Infection.—Urinary tract infections were classified as surgical, where there had been instrumentation, or medical. The findings are shown in Table II. Most medical infections were caused by *E. coli* and most surgical ones by other genera. In both groups 40-50% of *E. coli* strains were resistant to one or more antibiotics. Enterobacteriaceae other than *E. coli* showed much the same frequency of resistance to anti-

TABLE I—Coliforms Isolated from Blood Cultures

Organism	No. of Strains			Resistance Patterns
	Total	Characteristic Sensitivity*	Additional Resistance	
<i>E. coli</i>	7†	5	2	A S T C Su S T Su
<i>Klebsiella</i> sp. ..	5†	2	3	A Su A S T C K Su A S
<i>Proteus mirabilis</i> ..	1	1	0	—
<i>Ps. aeruginosa</i> ..	6	2	4	4 Carb

*Characteristic as defined in text.

†One blood culture from a premature baby yielded mixed *E. coli* and *Klebsiella*.

TABLE II—Coliforms Isolated from Urine

Organism	Surgical. No. of Strains			Medical. No. of Strains		
	Total*	Characteristic Sensitivity†	Additional Resistance†	Total*	Characteristic Sensitivity†	Additional Resistance†
<i>E. coli</i>	86 (45%)	45 (52%)	41 (48%)	108 (67%)	65 (60%)	43 (40%)
<i>Klebsiella</i> and <i>Enterobacter</i> spp. ..	34 (18%)	22 (65%)	12 (35%)	20 (12%)	11 (55%)	9 (45%)
<i>Proteus</i> sp.	43 (22%)	26 (60%)	17 (40%)	22 (14%)	14 (64%)	8 (36%)
<i>Ps. aeruginosa</i>	24 (12%)	22 (92%)	2 (8%)	4 (3%)	4 (100%)	0
Other	5 (3%)	—	5 (100%)	7 (4%)	2 (29%)	5 (71%)
Total	192	115	77	161	96	65

*Proportion of total coliforms represented by each genus is given in parentheses.

†Proportion of strains of each genus sensitive or resistant is given in parentheses.

TABLE IV—Coliforms Isolated from Infected Wounds

Class of Infection	<i>E. coli</i>	<i>Klebsiella</i> and <i>Enterobacter</i> spp.	<i>Proteus</i> spp.	<i>Ps. aeruginosa</i>	Others	Total	Resistant*
I	6	1	3	4	0	14	5
II	10	4	8	13	3	38	13
III	5	2	2	10	0	19	4
IV	1	0	0	1	1	3	2
Total	22	7	13	28	4	74	24

*Organisms with resistances additional to their characteristic pattern.

biotics, additional to their characteristic pattern. Table III shows the numbers of isolates (from both surgical and medical cases) resistant to four antibacterial drugs commonly used to treat urinary tract infection.

TABLE III—Resistance of Urinary Pathogens to Commonly Used Drugs

No. of Strains Resistant to:			
A (Su sens.)	Su (A sens.)	A Su	A Su Tr Na
70	19	33	30*

*28 of these were *Ps. aeruginosa*.

Wound Infection.—Wound infections were classified according to the report of the Public Health Laboratory Service (1960) as: I frank pus, II serous discharge, III erythema, and IV stitch abscess. There were 74 wound infections from which coliforms were isolated. There was no correlation between severity of infection and resistance of the organism (Table IV). Most infections were mild and systemic treatment was given only in 16 cases (22%). During the period when *Staph. aureus* wound infections were studied there were equal numbers of staphylococcal infections and coliform infections. The rate of infection per 100 operations was 5.6 for *Staph. aureus* and 5.3 for coliforms. Severity (according to the classification above) was similar, but staphylococcal infections were more often treated systemically than coliform infections (17 (43%) treated out of 39 cases).

Chest Infections.—The numbers of coliform infections and the proportion of resistant strains are shown in Table V.

TABLE V—Coliforms Isolated from Respiratory Tract

Organism	No. of Strains		
	Total	Characteristic Sensitivity	Additional Resistance
<i>E. coli</i>	2	2	0
<i>Klebsiella</i> sp. ..	13	9	4
<i>Proteus</i> spp. ..	1	0	1
<i>Ps. aeruginosa</i> ..	23	18	5
Total	39	29	10

Meningitis.—There was no case of coliform meningitis diagnosed in the hospital in the whole 12-month period.

PATTERNS OF RESISTANCE

The proportion of isolates of each genus resistant to each drug is shown in Table VI. Multiple resistance was commoner than

TABLE VI—Proportion of Isolates Resistant to Each Drug

Organism	Total No.	No. of Strains Resistant to:											
		Character- istic Pattern	A	S	T	C	K	Su	Tr	G	Na	P	Carb
<i>E. coli</i>	240	140 (58)*	57 (24)	60 (25)	53 (22)	14 (6)	21 (9)	54 (23)	2 (1)	0	9 (4)	0	NT
<i>Klebsiella</i> sp. and <i>Enterobacter</i> ..	88	54 (61)	88 (100)	22 (25)	12 (14)	7 (8)	10 (20)	18 (20)	4 (5)	0	6 (7)	0	NT
<i>Proteus</i> spp. ..	83	50 (60)	15 (19)	6 (7)	71 (85)	0	2 (2)	13 (16)	13 (16)	0	2 (2)	83 (100)	NT
<i>P. aeruginosa</i> ..	95	78 (82)	—	—	—	—	—	—	—	0	—	0	17 (18)
Other	18	3 (17)	13 (73)	6 (33)	3 (17)	3 (17)	4 (22)	—	5 (28)	0	0	1 (6)	NT

*Percentages are given in parentheses.
NT = Not tested.

resistance to single drugs. For *E. coli* the commonest patterns are shown in Table VII. For the *klebsiella/enterobacter* group the commonest patterns were resistance to A S T Su, A S, and A S Su. These patterns were found with 14 isolates; of the remaining 20 resistant strains, 12 were resistant to three or more drugs and three to five or more drugs.

TABLE VII—Multiplicity of Resistance of All Strains of *E. coli* Isolated: Commonest Patterns of Resistance

Pattern No. of strains	A	A S T Su	S	A T	A S K	S T Su	S Su	Su	Total
13	11	6	5	5	5	5	5	5	55

The remaining 45 resistant strains showed a variety of patterns: 28 of them were resistant to three or more drugs and 11 were resistant to five or more drugs.

The patterns and multiplicities of resistance were similar in strains from all sites of infection. All strains of *Ps. aeruginosa* were inhibited by 1,000 µg of carbenicillin per ml.

TRANSFER OF RESISTANCE

A representative sample of strains was tested for transfer of resistance to *E. coli* K12. The proportion which transferred resistance to one or more drugs is shown in Table VIII.

TABLE VIII—Incidence of Transferable Resistance

Organism	<i>E. coli</i>	<i>Klebsiella</i> sp.	<i>Proteus</i> spp.	<i>Ps. aeruginosa</i>	Other
Total tested	52	18	15	14	10
Transfer of resistance to <i>E. coli</i> K12	34 (64%)	7 (39%)	0	0	4 (40%)

Discussion

During four five-week periods 524 cases of infection with "coliforms," as defined earlier, were studied in inpatients in a general hospital. The hospital admitted about 16,000 patients a year, giving an overall rate for coliform infection of 8.5%. If enterobacteriaceae are considered without pseudomonas 43% of the infecting organisms were drug-resistant—that is, resistant to more antibacterial agents than is characteristic of the particular organism (see above). Multiple resistance was usual and was often determined by transmissible R factors. Patterns of resistance were very variable. There was no evidence for the spread among hospital bacteria of any characteristic R factor.

The frequency of resistance in urinary infections in hospital found by us seems to be similar to that reported by McAllister *et al.* (1971) for coliform infections. In general, our figures are somewhat lower than those reported by Salzman and Klemm (1966) for infections in a New York hospital. Much higher frequencies of resistance, especially in *Klebsiella*, have been reported in particular situations. For example, Gardner and Smith (1969) reported a prevalence of severe pulmonary and renal infections caused by multiple resistant *Klebsiella* spp in surgical wards of Massachusetts General Hospital. Similar infections were recorded in a Scottish neurosurgical unit (Price and Sleight, 1970). Both these outbreaks were associated with a heavy consumption of antibiotics, often used prophylactically. In the Scottish unit the outbreak ceased on the withdrawal of all antibiotics.

In this hospital, during the period of the survey, only 25% of patients received any antibacterial therapy: 54% of total antibiotic used was some form of penicillin, 11% was tetracycline.

Coliform infections severe enough to merit antibacterial therapy must be considered separately for each site of infection. All cases of septicaemia and urinary infection merit treatment.

There were only 18 cases of "coliform" septicaemia in the whole 12-month period. Twelve of these were infection with enterobacteriaceae and six with *Ps. aeruginosa*. Among the enterobacteriaceae, multiple resistance limited the choice of therapy in five cases (Table I). Of the six strains of *Ps. aeruginosa* isolated from blood culture four showed some degree of resistance to carbenicillin. None, however, had an M.I.C. for carbenicillin higher than 1,000 µg/ml of medium, and none transferred resistance.

We have examined the 353 organisms causing urinary tract infection in order to assess the clinical importance of resistance—219 were sensitive to ampicillin and 270 to sulphonamides. Sixty-three were resistant to both drugs and half of these were resistant to nalidixic acid and trimethoprim as well. Of the latter only two (one *E. coli* and one *Kl. aerogenes*) were organisms other than *Pseudomonas* sp. Therefore, except for *Pseudomonas* sp., >99% of urinary infections in this survey could be treated with one of the commonly used drugs—sulphonamides, ampicillin, trimethoprim, or nalidixic acid. Of the 28 urinary strains of *Ps. aeruginosa* (Table II) two were resistant to carbenicillin; all were sensitive to polymyxin and gentamicin.

Coliforms isolated from wound infections showed a similar frequency of drug resistance and of R factors. Most wound infections were mild and required no systemic treatment.

The significance of coliform bacteria in sputum is often very doubtful and therefore indications for antibiotic treatment are not clear-cut. Stringent criteria were used for this survey before

patients were counted as having coliform chest infections, and for this reason the numbers are small. *Ps. aeruginosa* and *Klebsiella* spp. were most often isolated. The *Klebsiella* strains were not notably resistant and in each case (as with wound infections) at least one antibiotic, active in vitro, was available.

Ps. aeruginosa, because it is characteristically resistant to most antibacterial agents, has always presented clinicians with therapeutic problems. All strains are sensitive to the polymixins. Gentamicin and carbenicillin are the drugs of choice. Among the 95 strains of *Ps. aeruginosa* in this survey, none was resistant to gentamicin but 17 were resistant to carbenicillin. Only one strain had an M.I.C. for carbenicillin as high as 1,000 µg/ml of medium and in no case was the carbenicillin resistance transferable. R factors so far identified in *Ps. aeruginosa* give an M.I.C. for carbenicillin of 4,000 µg/ml., or higher (Ayliffe, Lowbury, and Roe, 1972).

Among the enterobacteriaceae causing parenteral infections the incidence of drug resistance and of R factors is high. Although R factors determining gentamicin resistance have been reported (Witchitz and Chabbert, 1971) all strains isolated in this survey were gentamicin-sensitive. Trimethoprim resistance was rare (Table VI) and in no case transmissible, although R factors determining high-level trimethoprim resistance have been identified (Fleming, Datta, and Grüneberg, 1972). R-factor-determined resistance, in the infections reported here, often limited the choice of antibacterial therapy, but in no case was the resistance spectrum so wide as to render effective treatment impossible.

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