Changes in antibiotic sensitivity patterns of Gram-negative bacilli in burns

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SYNOPSIS Sensitivity tests with 12 antibiotics on 1,018 strains of Gram-negative bacilli isolated in a burns unit between 1969 and 1971 showed some important differences from results in similar tests on a series of strains isolated between 1965 and 1967. These changes included the emergence of a large proportion of kanamycin-resistant strains of *Klebsiella aerogenes*, *Proteus mirabilis*, and *Escherichia coli* and of smaller proportions of trimethoprim- and gentamicin-resistant strains; also the complete replacement of *Proteus mirabilis* with dissociated resistance to ampicillin by strains showing linked resistance to ampicillin and carbenicillin. The probable relationship of these changes to the emergence of an R factor determining resistance to tetracycline, kanamycin, carbenicillin, ampicillin, and cephaloridine in Enterobacteria and *Pseudomonas aeruginosa* is discussed.

A survey of the types and antibiotic sensitivity patterns of Gram-negative bacilli isolated from burns in this Unit between 1965 and 1967 showed some unexpected features, including a high incidence of ampicillin-resistant *Proteus mirabilis* and *Escherichia coli*, a large proportion of which were sensitive to carbenicillin (Davis, Lilly, and Lowbury, 1969). During that period carbenicillin was introduced in the Unit for the treatment of infection with *Pseudomonas aeruginosa*, and in 1969 carbenicillin-resistant carbenicillinase-producing strains of this organism emerged (Lowbury, Kidson, Lilly, Ayliffe, and Jones, 1969); subsequent studies have shown that this resistance was transferable and probably acquired by the transfer of R factors from Enterobacteria colonizing the same burns (Sykes and Richmond, 1970; Fullbrook, Elson, and Slocombe, 1970; Roe, Jones, and Lowbury, 1971). The resistance of *Ps. aeruginosa* carrying the R factor was often unstable.

To find what changes have occurred following the emergence of transferable carbenicillin resistance in *Ps. aeruginosa*, we have tested the antibiotic sensitivity of a series of 1,018 strains of Gram-negative bacilli, excluding *Ps. aeruginosa*, isolated in the Unit between 1969 and 1971; the findings are reported here.

Materials and Methods

Swabs from burns were examined by culture methods described previously (Davis *et al*, 1969). Gram-negative bacilli—one isolate of each colony type per patient per week—were identified by tests described in that paper, using the criteria of Cowan and Steel (1965). All strains of identified Gram-negative bacilli were examined for sensitivity to a range of antibiotics by a ditch plate method identical with that described by Davis *et al* (1969). Tests of sensitivity to nitrofurantoin, which were not included in the previous series, were included in this one. 'Moderately sensitive' strains were classified as 'sensitive' in this series.

Results

Table I shows the proportion of nine species of Gram-negative bacilli which were resistant to 12 antibiotics or chemotherapeutic agents in the period 1969-71. Several interesting changes are found to have taken place.

PROTEUS MIRABILIS

Ampicillin-resistant isolates had fallen from 95 to 53%, but in the new series the proportions of strains resistant to ampicillin and to carbenicillin were the same; all strains resistant to ampicillin were also

Gram-negative Bacilli	Antibiotic and Concentration in Ditch (µg/ml)												Total No. of Strains Tested
	A	C	Ce	G	S	K	T	Cl	N	Na	Su	Tr	
	125	100	50	50	50	50	50	50	125	50	100	10	
<i>Proteus mirabilis</i>	52.6	52.6	53	0.37	10.8	70.9	93.7	6.7	98.1	3.0	38.4	14.9	268
<i>Escherichia coli</i>	40.7	41.7	24.5	0.9	18.5	16.2	48.6	14.8	34.3	1.9	31.5	7.4	218
<i>Klebsiella aerogenes</i>	75.8	83.3	77.7	4.7	29.8	55.8	72.1	17.2	77.4	3.7	45.7	12.1	215
<i>Enterobacter species</i>	32.4	11.8	79.4	0	5.9	10.8	29.4	2.9	71.6	4.9	15.7	4.9	102
<i>Bacterium anitratum</i>	45.8	21.5	99.4	40.2	48.6	23.4	58	64.5	90.7	27.1	35.5	44	107
<i>Citrobacter species</i>	16.7	38.9	61.1	5.6	30.6	16.7	27.8	8.3	47.1	5.6	27.8	13.9	36
<i>Proteus morganii</i>	52.4	23.6	85.7	14.3	14.3	33.3	71.4	14.3	66.7	0	23.6	23.6	21
<i>Proteus vulgaris</i>	12.5	25	87.5	0	0	25	75	0	62.5	0	50	37.5	8
<i>Providencia</i>	72.1	18.6	100	41.2	60.5	53.5	65.1	32.6	95.3	4.7	46.5	34.9	43

Table I Percentage of 1 018 strains of Gram-negative bacilli isolated from burns in 1969-71 resistant to antibiotics by ditch-plate test

A = ampicillin
K = kanamycin
Su = sulphadiazine

C = carbenicillin
T = tetracycline
Tr = trimethoprim

Ce = cephaloridine
Cl = chloramphenicol

G = gentamicin
N = nitrofurantoin

S = streptomycin
Na = nalidixic acid

resistant to carbenicillin. The increase in the proportion of strains resistant to kanamycin (from 0.9 to 70.9%) was dramatic; other changes were smaller, but there was, in the second survey, some resistance to gentamicin, nalidixic acid, and trimethoprim, which were active against all strains in the previous survey. Tetracycline resistance (incorrectly expressed in one table of the 1965-67 series; see erratum, 1970) was little changed in the second series.

ESCHERICHIA COLI

As with *Proteus mirabilis*, the second series showed a lower incidence of ampicillin resistance and a higher incidence of carbenicillin resistance; in contrast with findings in the earlier series, ampicillin and carbenicillin resistance were found in a similar proportion of strains. There was an increased number of kanamycin-resistant strains and an emergence of strains resistant to trimethoprim and gentamicin.

KLEBSIELLA AEROGENES

The principal changes in the second series were a dramatic emergence (from 0 to 55.8%) in the proportion of kanamycin-resistant strains, and an emergence of some trimethoprim- and gentamicin-resistant strains. Nalidixic acid and chloramphenicol-resistant strains were less common in the second series.

ENTEROBACTER SPP

A fall in ampicillin and tetracycline resistance and the emergence of resistance to kanamycin and trimethoprim are the principal changes.

BACTERIUM ANITRATUM

There was a fall in ampicillin and chloramphenicol resistance and a rise in resistance to carbenicillin,

gentamicin, streptomycin, kanamycin, nalidixic acid, and sulphadiazine.

CITROBACTER SPP

The main features are a fall in ampicillin, sulphadiazine, and tetracycline resistance and the emergence of resistance to gentamicin, kanamycin, nalidixic acid, and trimethoprim.

PROVIDENCIA

The emergence of carbenicillin resistance and an increase in strains resistant to gentamicin, kanamycin, sulphadiazine, and trimethoprim were the main features.

PROTEUS MORGANII

Resistance to gentamicin, kanamycin, and trimethoprim emerged; more strains were resistant to carbenicillin and tetracycline, and fewer were resistant to chloramphenicol and sulphadiazine.

Table II shows the resistance patterns of strains of the three most frequently isolated Gram-negative bacilli which were resistant to tetracycline, kanamycin, carbenicillin, ampicillin, and cephaloridine. These strains were selected for study because a large proportion of the strains of *Proteus* spp. and *Klebsiella* spp. recently isolated in the Burns Unit have shown the presence of an R factor determining this pattern of resistance (tetracycline, kanamycin, carbenicillin, ampicillin, cephaloridine); such strains were rarely found in other hospitals (Ayliffe, Lowbury, and Roe, 1972). The most striking feature in the new series is the large variety of resistance patterns, and the emergence of a large proportion of strains resistant to TKCACE; in the previous series 0/22 strains of *K. aerogenes* and only 1/59 *P. mirabilis* and 3/123 *E. coli* showed this

Gram-negative Bacilli	Number of Strains Identified	Total Number of Sensitivity Patterns	Resistance Patterns of Gram-negative Bacilli Resistant to TCKACe	Percentage of Strains Resistant to TCKACe
<i>Proteus mirabilis</i>	268	62	TCKACe(5) TCKACeN(5) TCKACeCl(1) STCKACeN(1) TCKACeNSu(30) STCKACeNSu(6) TCKACeNTr(4) STCKACeNSuTr(6) TCKACeNSuTr(23) TCKACeNCl(6) STCKACeN(1) STCKACeNTr(1) GSTCKACeNSu(21)	41
<i>Escherichia coli</i>	218	72	TCKACe(7) TCKACeSu(2) TCKANaCe(1) TCKACeN(1) STCKACeSu(4) TCKANaCeN(1) TCKACeSuTr(1) TCKACeNTr(3) TCKACeNSu(2) TCKClACeSu(2) STCKClACeSu(1) STCKACeNSu(1) TCKClACeNSu(1) TCKANaCeNSuTr(1) TCKACeNSuTr(1) GSTCKClACeNSu(1) STCKClANaCeNSuTr(1)	14
<i>Klebsiella aerogenes</i>	215	65	TCKACe(2) TCKACeN(35) TCKACeSu(7) TCKACeNSu(9) STCKACeN(1) STCKACeSu(23) STCKACeNSu(3) TCKClACeNSu(8) STCKACeNSu(12) STCKACeNSuTr(4) GSTCKACeSuTr(1) GSTCKClANaCeNSuTr(1) TCKClACeNSuTr(1) TCKClANaCeNSu(1) GTCKClACeNTr(2) TCKClACeNSuTr(1) STCKClANaCeNSuTr(1)	52

Table II Resistance patterns of strains of Grams-negative bacilli isolated from burns resistant to tetracycline, kanamycin, carbenicillin, ampicillin and cephaloridine 1969-71

G = gentamicin
C = carbenicillin
K = kanamycin
N = nitrofurantoin

S = streptomycin
A = ampicillin
Cl = chloramphenicol
Su = sulphadiazine

T = tetracycline
Na = nalidixic acid
Ce = cephaloridine
Tr = trimethoprim

resistance pattern (with or without resistance to other agents).

Discussion

The emergence of carbenicillin-resistant *Ps. aeruginosa* of several different phage types in 1969 followed the use of carbenicillin in the Burns Unit for three years (Lowbury *et al.*, 1969). These strains of *Ps. aeruginosa* were found to transfer carbenicillin resistance to *E. coli* K12, and produced β -lactamase with a substrate profile of the type produced by some strains of *E. coli* and other Enterobacteria, but not by typical *Ps. aeruginosa* (Sykes and Richmond, 1970; Fullbrook *et al.*, 1970). From this it was inferred that the R factor determining resistance to carbenicillin (which was linked with resistance to tetracycline, carbenicillin, ampicillin, and cephaloridine) was acquired by *Ps. aeruginosa* from one of the Enterobacteria.

This hypothesis is supported by further studies in our Unit, which showed that *Proteus mirabilis* and *Klebsiella aerogenes* isolated from burns carried an R factor that transferred the resistance pattern TCKACe in mixed cultures on mouse burns to *E. coli* K12, from which it could be further transferred to *Ps. aeruginosa* (Roe *et al.*, 1971). The emergence of frequent kanamycin resistance in *Proteus mirabilis*, *Klebsiella aerogenes*, and *E. coli* and of the resistance pattern TCKACe in the survey of Gram-negative bacilli described above are probably associated

with the spread of this R factor and the use of carbenicillin in the Unit.

The dissociated ampicillin resistance of carbenicillin-sensitive *Proteus mirabilis* and *E. coli*, which was common in the first survey, was no longer found in the second survey. Cultures of strains which showed dissociated resistance in the earlier period were found to have become sensitive to both antibiotics on storage. The instability of this resistance suggests that it was determined by an R factor. It seems that these unstable ampicillin-resistant strains, which became predominant in the Burns Unit when the antibiotic was in frequent use, may have been the primary source of the R factor determining carbenicillin resistance in *Ps. aeruginosa*.

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