infection, but only in situations of special risk, such as the thoracic unit described and where a sufficiently high cross-infection rate has been reported.

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EVOLUTION OF NATURAL RESISTANCE TO THE NEWER PENICILLINS

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The *raison d'être* of methicillin, and its continuing efficacy, are dependent on its virtual immunity to the hydrolytic action of penicillinase. Because of this, practically all strains of *Staphylococcus aureus* are sensitive to it; drug-resistance has not been reported during treatment; and, despite intensive screening and widespread use, very few naturally resistant strains have been reported (Jevons, 1961; Knox, 1961a; Barber and Waterworth, 1962). It has been known from the start, however, that *Staph. aureus* could be made resistant to methicillin in the laboratory (Rolinson *et al.*, 1960), though not always easily (Stewart, 1961; Barber and Waterworth, 1962). Such resistance, like that of the naturally resistant strains, is not associated with inactivation of the drug by penicillinase, and there is no evidence to date that any staphylococcus can form a specific methicillinase (Barber, 1961; Stewart, 1961). The situation is therefore different from that which obtained with penicillin G in its earlier days; nevertheless, fears have been expressed that the efficacy of methicillin will be ephemeral, and, indeed, the fate of all other antibiotics which joined battle with the staphylococcus would seem to justify such fears.

It is obvious that the usefulness of methicillin and its analogues in the future will depend upon the extent to which resistance emerges under clinical conditions. For this reason, when we began to use methicillin in 1959 we embarked at the same time upon a programme of assaying every staphylococcus isolated and reisolated from patients and staff against several derivatives of 6-aminopenicillanic acid to enable us to identify and trace resistant strains if ever they occurred. The present report gives the results obtained to June 15, 1962.

Methods

In general, our methods were similar to those described in previous papers (Stewart, 1960, 1961), with special attention in the present study to the following.

Microbiology

All strains of *Staph. aureus* isolated from lesions, nasal carriers, etc., were screened in agar flood-inoculated plates against 10 mm. blotting-paper disks of penicillin, methicillin (10 μg.), and, successively, of various newer derivatives of 6-aminopenicillanic acid, in appropriate concentration. Organisms showing colonies or haloes in the inhibitory zone, or other indications of resistance, were titrated in liquid media to their end-points.

Test for Inactivation of Penicillins.—Three methods were used. (1) Broth containing the highest concentration of antibiotic permitting full overnight growth of any given staphylococcus was centrifuged at 3,000 r.p.m. (20 minutes). The supernatant was assayed against *Sarcina lutea* in parallel with control solutions of the particular penicillin left in sterile broth at 37° C. for the same period, and also of similar broth inoculated with non-resistant penicillinase-forming staphylococci. The percentage inactivation was calculated from semi-log dose-response lines, constructed anew for each series of tests. (2) Whole-cell sediments from the broth residues of method 1 were added for two hours at 37° C. to standard solutions of the penicillins, which were then assayed similarly against *S. lutea*, with similar controls. (3) A two-stage membrane technique was developed by modifying our earlier method (Stewart *et al.*, 1961; Holt and Stewart, 1963). Staphylococci were inoculated on marked spots on broth-soaked cellulose-acetate membranes (Oxoid) placed on agar containing the particular penicillin in a concentration calculated to permit growth of the test organism on the overlying membrane and thereby act as an enzyme inducer, but to inhibit completely the indicator organism (Oxford staphylococcus or *S. lutea*). The membrane was inspected after overnight incubation on the drug-loaded agar to check that full growth had occurred. It was then removed and the agar flooded with 0.2 ml. suspension (standardized at about 10^8 organisms per ml.) of indicator organism. The plate was then reincubated for 18–24 hours. Destruction of the particular penicillin by diffusible enzyme was shown by growth of the indicator organism below the marked spots of active staphylococci and positive controls (excess of cereus 5B penicillinase).

Clinical

Our practice is to examine nasal swabs from patients and staff in the wards for acute cases at monthly intervals, or more often according to circumstances. The first methicillin-resistant *Staph. aureus* was detected in the course of this routine procedure. The phage-type and antibiotic sensitivity pattern of this organism were determined and thereafter a continuous check was maintained upon this patient and her contacts by repeated swabbings in this ward, and by exposing plates in various situations. When the resistant strain appeared in another ward the same intensified procedure was applied until eventually its incidence in the wards at large was determined. In parallel with this, records were kept of

the usage and administration of any form of penicillin in the patients and wards affected. Treatment was instituted whenever the resistant strain appeared, usually in the form of chlorhexidine, neomycin/bacitracin, fucidin, or other drugs topically to nasal carriers, and systemic antibiotics to the very few patients with lesions. Follow-up specimens were taken repeatedly in all instances to check persistence or clearance of the resistant strain or reinfection by another strain.

Results

Incidence of Resistant Staphylococci

Methicillin and other derivatives of 6-aminopenicillanic acid were first used in the hospital in the latter half of 1959. Between then and March, 1961, no resistant strains were detected. In March, 1961, a penicillinase-forming group III *Staph. aureus* (type 75/77) resistant to methicillin (20 μg./ml.) was isolated from the nostrils of an infant in a medical ward. This infant had been admitted six weeks previously with a neonatal staphylococcal infection of the umbilicus and skin; the staphylococcus responsible for this infection, of a different type, had been eliminated by parenteral therapy with penicillin G from January 27 to February 6; nasal swabs taken after this course of treatment grew no *Staph. aureus*, but the infant was kept in hospital for nutritional reasons. The methicillin-resistant strain of *Staph. aureus* was isolated from her nasal swab on March 13 in the absence of any lesion. It was not eliminated by repeated topical applications of chlorhexidine, neomycin, and bacitracin, and she was discharged from hospital on April 12. When she returned as an out-patient in August, 1961, a nasal swab yielded the same organism, which eventually disappeared after further topical applications of chlorhexidine, neomycin, and bacitracin.

On the first appearance of the resistant strain in this patient in March, 1961, nasal swabs were taken from 25 patients and staff in the ward. The same resistant strain was isolated from one other infant, whose nasal swab had been free from *Staph. aureus* a few days before. In this case the organism disappeared after topical application of chlorhexidine for a few days. In April, 1961, the resistant strain was isolated from three other patients in the same ward, once from a nasal swab in the course of a routine check on all patients and staff, once from the ear of an infant with otitis externa, and once from an infant with a patch of mild eczema. The original carrier had now left the ward.

At this stage vigorous measures were taken to restrict the spread of the resistant organism. The ward was closed to admissions, all patients and staff received topical applications of chlorhexidine, etc., to the nares and skin flexures, and the infected infants were isolated. In May the resistant strain was recovered once only, from a pharyngeal swab, and disappeared from that infant after oral treatment with erythromycin. In June, however, it reappeared in minor lesions in four infants, in one throat swab, from the same ward, and also in a swab taken from the stoma of an infant with a tracheotomy in the adjacent ward.

Thereafter (Table I) the resistant strain of identical phage-type began to appear in patients in various other wards, usually in nasal swabs, occasionally from minor lesions. In some of these passive carriers the strain disappeared spontaneously, in others after topical therapy. One patient only proved to be a persistent nasal carrier for about 28 days, apart from the original carrier. By May, 1962, 14 months after its first appearance, the resistant staphylococcus had been isolated on 71 occasions from 37 children in 8 out of 48 wards and from one nurse. Up to this point it had caused no serious lesions, but the situation then changed: the organism was isolated from the surgical wound of a child, freshly operated upon for spina bifida, who was receiving methicillin and streptomycin; a few days later septicaemia developed with positive blood cultures, and, despite a change of treatment to erythromycin (50–100 mg. eight-hourly), the child died. At necropsy the resistant strain was recovered from the spleen, right ventricle, and lung; as in all previous instances it was of phage-type 75/77.

TABLE I.—*Number of Children Each Month Harbouring Methicillin-resistant Staph. aureus*

Ward	No. of Children with Resistant Strain of Staphylococcus (1961–2)														
	Mar	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May
E1 Acute med. (babies)	2	3	1	5	3	1	2	1	1	1		1	3	2	1
E2 Acute med. (babies)				1											
B2 Acute med.					1	1	1					1		1	1
B4 Acute surg. (babies)									2				1	1	1*
D2 Orthopaedic									1						
B7 Gastroenteritis												1	1	1	
B3 Acute med.													1	1	4
F4 " "															1
Other wards (40)															
Ward staff													1		
Total cases	2	3	1	6	4	2	3	1	4	1	—	3	7	6	8
No. of patients in hospital (weekly averages)	472	481			465				606		657	683	675	667	655

* Fatal case.

Properties of the Resistant Strain

Seventy-one isolates of the resistant strain were examined from 37 individuals, of whom at least 30 were carrying it passively in one or more sites. All strains belonged to group III and were confirmed by Drs. M. T. Parker and M. P. Jevons, of the Staphylococcal Reference Laboratory at Colindale, as being of virtually identical phage-pattern, of the type 75/77.

In its morphology and colonial and biochemical properties the resistant strain was a typical *Staph. aureus*. In primary culture and early subcultures the colonies were uniform in appearance, in resistance, and in rate of growth. In some later subcultures smaller more slowly growing colonies appeared; these also were highly resistant to the drugs. In the presence of methicillin (5–20 μg./ml. in agar) the organism grew largely in the form of small colonies of abnormal swollen cells.

Cross-resistance

All 71 isolates were resistant to methicillin (20 μg./ml. or more) and also to the entire range of therapeutic penicillins at present available, including the isoxazole derivatives of 6-aminopenicillanic acid (Table II). Unlike its sensitive counterpart, the resistant strain showed an inoculum-size effect with methicillin and other 6-aminopenicillanic acid derivatives, in that small inocula were inhibited by 20 μg./ml., whereas larger inocula required 50–100 μg./ml. for inhibition; this effect, unlike that obtained with penicillin G, was insufficient to enable the newer penicillins to inhibit small inocula in therapeutic concentrations.

The sensitivity pattern of all 71 isolates of this strain to other antibacterial agents was fairly uniform—namely, sensitive to 1 μg. of erythromycin, novobiocin, and vancomycin per ml., but resistant to 10 μg. or more of streptomycin and tetracyclines per ml.

TABLE II.—*Effect of Different Penicillins and Related Compounds Upon the Atypical Carshalton Strain of Resistant Staph. aureus, Compared with a Typical Penicillinase-forming Strain*

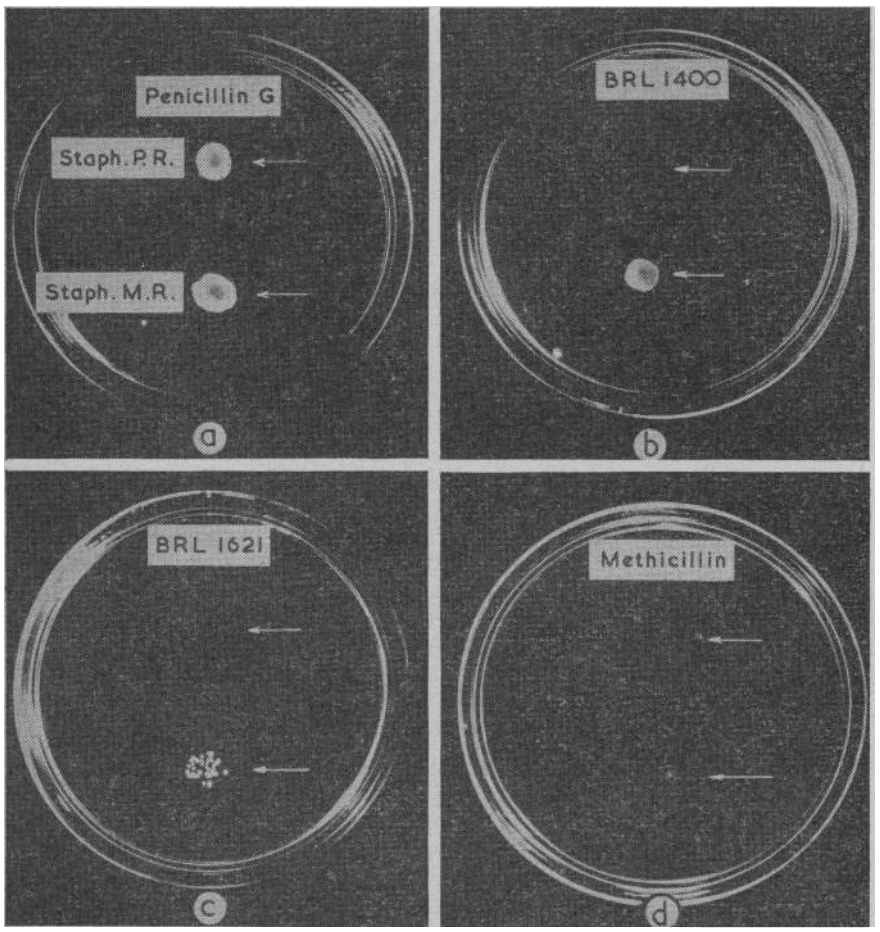
Compound	Common Name	Bacteriostatic Concentration against *Staph. aureus*	
		Typical Penicillinase-forming Strain	Carshalton Strain
Benzylpenicillin ..	Penicillin G	>100	>100
p-Aminobenzylpenicillin	" T	>100	>100
α-Aminobenzylpenicillin	Ampicillin	>100	>100
Phenoxyethylpenicillin	Phenethicillin	50	>100
Phenoxypropylpenicillin	Propicillin	50	>100
5-Methylisoxazolyl penicillin ..	Oxacillin	0·5–2·0	10–50
3-Chloro-5-methylisoxazolyl penicillin ..	Cloxacillin	0·1–1·0	5–20
Isoxazole 1577 ..	—	0·1–1·0	10–50
Penicillin C95	—	5	100
Dimethoxybenzylpenicillin	Methicillin	2–4	20–50
Amino-adipic acid penicillin	Cephalosporin N	>100	>100
Cephalosporin C ..	—	50	>100
" G ..	—	1–5	5–10

Destruction of the Penicillins

All penicillins except methicillin were inactivated by growth of the resistant strain in 18 to 24 hours. Whole-cell suspensions from induced cultures inactivated penicillin G and some other penicillins in two hours but did not affect methicillin at all under these conditions, and produced only marginal inactivation of the isoxazole penicillins (Table III). When tested by the more sensitive membrane-plate method, with continuous induction, the inactivating enzyme had a strong effect upon the isoxazole penicillins, though it still failed to destroy methicillin (see Fig.).

TABLE III.—*Differences in Susceptibility of Penicillins to Staphylococcal Penicillinase*

Penicillin	Effect of Staphylococcal Penicillinase	
	Typical Strains	Atypical (Methicillin-resistant Strains)
Benzyl-(G) ..	Rapid, complete hydrolysis	
p-Aminobenzyl-(T)		
Ampicillin		
Cephalosporin N		
Phenethicillin	Complete hydrolysis, less rapidly	Rapid complete hydrolysis
Phenoxypropyl		
Propicillin		
C-95		
Cephalosporin C	Incomplete hydrolysis, slowly	Incomplete or complete hydrolysis, slowly
Isoxazole derivatives		
Methicillin ..	Minimal hydrolysis	



Inactivation of various penicillins by the totally resistant strain of *Staphylococcus aureus* (two-stage membrane technique). The white patches are zones of indicator growth below, Staph. P.R.: Control strain of penicillinase-forming staphylococcus, resistant to penicillin G but sensitive to methicillin, etc. Staph. M.R.: Carshalton strain, resistant to methicillin, etc. (a) Agar contains 2 μg. penicillin G per ml.: indicator growth below both strains. (b) Agar contains 1 μg. oxacillin (B.R.L. 1400) per ml.: no growth below P.R., full growth below M.R. (c) Agar contains 1 μg. cloxacillin (B.R.L. 1621) per ml.: no growth below P.R., partial growth below M.R. (d) Agar contains 2 μg. methicillin per ml.: no growth below P.R., trace only below M.R.

Incidence in Relation to Penicillin Therapy

The majority of patients harbouring the resistant strain received penicillin during their stay in hospital, mainly before the resistant strain was isolated (Table IV). Only 5/37 received methicillin, while two received an isoxazolyl penicillin (cloxacillin). Twenty-two received penicillin G or V. Eight had no penicillin therapy.

Discussion

The sequence of events reported here illustrate chronologically and quantitatively how natural resistance to methicillin and the newer penicillins may appear and spread. The resistant strain appeared suddenly, without any obvious relationship to methicillin therapy. Its spread was gradual and very slow, involving only a fraction of the population at risk—less than 2% of patients at the end of over 12 months' spread; though this was a period during which a fairly vigilant prophylactic campaign was operating. For 13 months the organism was passive in behaviour, and then it suddenly broke through a wound infection and caused a fatal septicaemia; this happened in a

TABLE IV.—*Penicillin Therapy Received by Patients Harbouring Methicillin-resistant Staph. aureus*

Resistant Strain Acquired	No Penicillin	No. of Patients Receiving Penicillin		
		Pen. G or V	Methicillin	Isoxazole Pens.
Before therapy ..		—	—	—
During ,, ..		7	1 (fatal)	—
After ,, ..		15	4	2
Total ..	8	22	5	2

patient who was receiving methicillin at the time of colonization of her wound.

The first ward affected was a medical ward for infants, but the spread thereafter was haphazard, not local; the adjacent medical ward, also for infants, acquired only one infection. Curiously, two wards which had been sprayed with methicillin (Elek and Fleming, 1960), for 6 and 12 weeks respectively, in 1960 failed then and subsequently to show any resistant strains, despite intense searching.

The resistant strain described here differs in several ways from the first naturally resistant strain reported by Jevons (1961). This strain contains a mixed population of cells; the majority of cells yield normal colonies of *Staph. aureus* and are sensitive to methicillin (2.5 μg./ml.); a minority grow slowly and form small atypical colonies which are resistant to methicillin (10 μg./ml. or more). In contrast our Carshalton strains are composed largely, though not always entirely, of cells which grow at a normal rate into normal colonies, but though fully resistant in that state do not grow normally in the presence of methicillin. All strains so far examined belong to group III as defined by phage-typing, form a powerful penicillinase, and show cross-resistance to all the natural and semisynthetic penicillins and to certain cephalosporins. In the case of our Carshalton strain this penicillinase destroys not only penicillin G, in common with several other penicillins (Table III), but also, to some extent by the membrane technique, the isoxazole penicillins, which we found to be relatively resistant to penicillinase from all other staphylococci tested. Despite this, it causes only marginal destruction of methicillin. The inhibitory effect of methicillin is, however, influenced by inoculum size, and this is a departure from the usual behaviour of methicillin in relation to penicillinase-forming staphylococci.

With penicillin G there are well-known differences between staphylococci with natural resistance, which are invariably penicillinase-forming, and those with laboratory-induced resistance, which are not (Barber, 1947; Knox, 1961b). Methicillin-resistant strains, whether natural or artificial, do not show this difference, and indeed there is little obvious difference between any of the 71 isolates of the naturally resistant Carshalton strain and some of the group III strains which have been rendered resistant artificially in our laboratory. In view of their cross-resistance throughout the penicillin series and their ready acquisition of resistance to other antibiotics, such strains are potentially very dangerous indeed, especially in the light of their gradual spread and increasing pathogenicity, as described here. On the other hand, the incidence of such strains is low; their emergence in the present sequence of events does not seem to be a direct consequence of therapy with methicillin or other new derivatives of 6-aminopenicillanic acid; and they would not have been identified in such numbers unless an organized search had been maintained for nearly three years. The great majority of penicillinase-forming staphylococci remain fully sensitive to methicillin and to the isoxazole penicillins.

Nevertheless, some practical cautions must be observed. One is that fears expressed at an early stage by some authorities (Barber, 1960; *British Medical Journal*, 1961) have been at last justified in small measure; another is that one of us must eat some if not all of his own words (Stewart, 1961), albeit in good company (Chain, 1960); lastly, and most important, patients harbouring these rare strains must be isolated, vigorously treated, and preferably should be sent out of hospital as soon as possible.

Summary

The properties and invasiveness of 71 isolates of a group III staphylococcus with natural resistance to all available therapeutic penicillins are described.

The organism first appeared, passively, in a nasal carrier; it spread into 8 out of 48 wards in a children's hospital, affecting 37 patients, most of whom developed no active infections; one child died from septicaemia after acquiring the organism in a surgical wound.

Penicillinase formed by this strain of staphylococcus inactivates most penicillins readily, but the isoxazole penicillins are affected only during active growth of a large population of cells, and even under those conditions methicillin is not significantly inactivated. This type of resistance appears, therefore, to be unlike that responsible for natural resistance to penicillin G.

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Since 1950 general practitioners in South-west London have had unlimited access to chest radiography through the Mass Radiography Service. The facility is used by over 500 doctors in the area, and to date 186,692 examinations have been made at the request of general practitioners. As a result 1,600 cases of active pulmonary tuberculosis requiring treatment have been discovered. About half of these patients were sources of infection. Most of them have been rendered non-infectious. Many other intrathoracic abnormalities have been detected. Evidence of bronchial neoplasm was revealed in one man in every forty aged over 45 sent by his doctor. (S.W. London Mass *X*-Ray Service, Seagrave Road, London S.W.6.)