ACINETOBACTER AND SIMILAR ORGANISMS IN EAR INFECTIONS

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SYSTEMIC human infections caused by organisms of the acinetobacter group, or "oxidase-negative moraxellas" as they are sometimes called, have been reported with increasing frequency since De Bord (1939) first drew attention to their medical significance as organisms capable of confusing the diagnosis of gonorrhoea by the smear method. Although subsequently described under such generic names as *Mima*, *Herellea*, *Neisseria*, *Bacterium*, *Achromobacter* and *Moraxella*, there now seems general agreement that they belong to the genus *Acinetobacter* (Cowan and Steel, 1974). Organisms of this group have also been isolated from infected ears (Brooke, 1951; Scott and Mahoney, 1953; Lutz *et al.*, 1956; De Torregrosa and Ortiz, 1961; Reynolds and Cluff, 1963; Brodie and Henderson, 1964; Shmilowiz, Avissar and Ephrati, 1968; Wright, 1970), but few attempts seem to have been made to determine their pathogenicity in this site.

The present paper reports the isolation of *Acinetobacter* strains from ear swabs and mastoid bone chips obtained from patients with acute or chronic otitis media, or otitis externa, and clinical details are given in an attempt to discover the pathogenicity of these organisms in this situation. An estimate is also made of the prevalence of *Acinetobacter* in normal ears.

MATERIALS AND METHODS

Most of the strains were isolated from clinical material obtained from patients attending the Royal National Throat, Nose and Ear Hospital, London. To ascertain the prevalence in normal ears, swabs were taken from the external auditory canals of volunteer students and staff, with no history of ear disease, at King's College Hospital Medical School, London.

Two type strains of *Acinetobacter anitratus* (NCTC nos. 7844 and 8102) and one of *Acinetobacter lwoffii* (no. NCTC5866) were obtained from the National Collection of Type Cultures. Two further type strains of *A. anitratus* and three of *A. lwoffii* were kindly provided by Dr A. Henderson of the Royal Maternity Hospital, Glasgow. All type strains were examined in parallel with the clinical isolates.

Identification of the clinical isolates was made in the first instance by their characteristic colonial appearance, after overnight aerobic incubation on MacConkey's agar at 37°C; their non-lactose-fermenting colonies were composed of non-motile gram-negative cocci or cocco-bacilli and bacilli. Confirmatory identification was made by standard diagnostic methods (Cowan and Steel, 1974), except that capsules were stained by the Prussian blue method of Berenbaum (1955), nitrate reduction was determined by the method of Cook (1950), and soap tolerance by means of a soap-agar plate (Billing, 1955). Sugar-fermentation reactions were tested with cultures in peptone water containing 1% of the test sugar.

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TABLE I
Number of strains of each main biotype showing the indicated properties

Total number of examples of each main biotype examined			Number of strains showing					
			capsular staining	urease activity	citrate utilisation	lecithinase activity	gelatin liquefaction	soap tolerance
<i>Acinetobacter anitratus</i>	clinical isolates	32	2	6	30	6	16	10
	type strains	4	0	4	3	1	1	0
<i>Acinetobacter lwoffii</i>	clinical isolates	22	4	6	16	3 (1 NT)	13	7 (1 NT)
	type strains	4	1	4	1	0	1	0
<i>Moraxella</i>		3	0	1	2	0	0	0
<i>Achromobacter</i>		1	0	0	1	0	1	0

NT = not tested.

The antigenic relationships of these strains were investigated by means of agglutination reactions with nine different antisera raised against strains of *A. lwoffii* isolated at the City Hospital, Aberdeen, (Brodie and Henderson, 1964) and kindly supplied by Drs J. Brodie and A. Henderson.

Antibiotic sensitivities were determined by means of the comparative (disk) method on blood-agar plates, with *Escherichia coli* as a control organism.

The pathogenicity for mice was determined by inoculating 0.2 ml of an overnight nutrient (digest) broth culture of each strain into the peritoneal cavity of each of two mice. The mice were then observed daily and any survivors were killed after 7 days. All the mice were examined *post mortem*, when cultures were taken from the peritoneum.

The clinical details of the patients from whom the organisms were isolated were studied, with particular reference to the duration of the illness, severity of symptoms, and the nature of any antibacterial therapy.

RESULTS

Origins of clinical strains

Fifty-seven strains were isolated from patients with ear infections and one strain was isolated from swabs taken from 50 normal ears.

Properties of strains

All clinical strains were gram-negative cocci, cocco-bacilli or bacilli and gave positive reactions for catalase and negative reactions for H₂S and indole production. Most strains were non-motile, nitratase negative, oxidase negative, and gave negative methyl-red (MR) and Voges-Proskauer (VP) reactions; but one strain was motile, one was nitratase positive, three were oxidase positive and three gave positive reactions in one or both of the VP and MR tests.

TABLE II

Agglutination titres obtained with 23 clinical isolates of acinetobacter-like organisms and eight type strains of Acinetobacter in tests with antisera raised against nine strains of A. lwoffii

Strain number	Biotype	Titre of antiserum no.								Agglutination pattern	
		199465	18520	D8460	19677	D9682	D16870	18516	17750		19738
207 P	A	0	0	0	0	0	0	0	0	0	...
301	L	4	512	4	0	4	0	0	0	4	IV
766 P	A	4	512	4	4	32	0	64	4	4	...
827	A	32	1024	2048	0	4	0	128	0	4	I
909	L	512	64	4	512	32	256	1024	256	2048	...
2161	A	4	4	0	0	0	4	0	0	4	...
2411	L	0	0	0	0	0	0	0	0	0	...
2598	A	0	256	2048	0	4	0	4	0	0	II
2716	L	4	64	512	0	4	0	4	0	0	II
2991 P	A	0	0	0	512	0	0	0	0	0	...
3183	L	4	256	2048	0	4	4	64	0	4	I
3726	L	0	0	0	0	0	0	0	0	0	...
3752 P	A	4	256	2048	0	16	0	32	0	4	I
3834	A	0	0	0	0	0	0	0	0	0	...
3882	Ach	0	0	0	4	4	0	0	0	0	...
3916 P	A	32	128	4	0	16	4	0	4	32	III
3991	L	0	0	0	0	0	0	0	0	0	...
4261	A	4	128	256	0	4	0	4	0	0	II
4262 P	A	0	128	512	0	4	0	4	0	0	II
4727	L	4	64	2048	0	4	0	64	0	0	I
4742	L	0	0	0	4	32	0	512	32	4	...
5107	A	1024	0	1024	0	32	4	64	0	4	...
5563	A	4	1024	4	4	4	0	4	4	32	III
NCTC 5866	L	2048	0	512	2048	2048	4	2048	2048	2048	...
NCTC 7844 P	A	32	64	0	4	4	4	128	4	256	...
NCTC 8102 P	A	4	0	0	4	0	0	256	64	512	...
<i>A. anitratus</i> 1P		128	0	0	256	0	64	64	4	0	...
<i>A. anitratus</i> 2		4	64	0	0	4	0	4	0	0	IV
<i>A. lwoffii</i> 1		32	512	0	0	256	0	2048	2048	0	...
<i>A. lwoffii</i> 2		4	1024	0	4	0	4	64	0	4	...
<i>A. lwoffii</i> 3		4	0	0	0	0	0	0	0	0	...

A = *A. anitratus*, L = *A. lwoffii*, Ach = *Achromobacter*, P = pathogenic for mice.

Thirty-two strains produced acid in fermentation tests with glucose, galactose, *l*-arabinose, xylose and sometimes mannose, and 26 did not. The former strains could be thus identified as *A. anitratus*; 22 of the 26 non-saccharolytic strains were identified as *A. lwoffii*, the three oxidase-positive strains as *Moraxella* spp. and the one motile strain as *Achromobacter*.

There was considerable variation among the strains in capsular staining, urease and lecithinase activity, citrate utilisation, gelatin liquefaction and soap tolerance (table I).

Serological findings

These are detailed in table II. Four broad patterns of agglutination could be distinguished, but there was little demonstrable relationship between the type strains and the clinical strains, nor between any particular serological group and pathogenicity for mice.

Antibiotic sensitivities

All of the biochemically typical clinical strains were resistant to penicillin. One type strain of *A. lwoffii* was penicillin sensitive, and all three oxidase-positive clinical isolates were also sensitive to penicillin. The one nitratase-positive strain and the one motile strain were penicillin resistant. All strains

TABLE III
Results of antibiotic-disk-sensitivity tests

Antibiotic (content per disk)	Total number of strains of each category examined and the number showing sensitivity (S) or resistance (R)											
	<i>A. anitratus</i>				<i>A. lwoffii</i>				<i>Moraxella</i> 3 strains		<i>Achromo- bacter</i> 1 strain	
	32 clinical isolates		4 type strains		22 clinical isolates		4 type strains		S	R	S	R
	S	R	S	R	S	R	S	R				
Penicillin (1.5 units)	0	32	0	4	0	22	1	3	3	0	0	1
Streptomycin (10 µg)	30	2	3	1	17	5	4	0	3	0	1	0
Tetracycline (10 µg)	27	5	4	0	20	2	4	0	3	0	0	1
Chloramphenicol (10 µg)	1	31	2	2	9	13	4	0	3	0	1	0
Erythromycin (10 µg)	27	5	4	0	21	1	4	0	3	0	1	0
Novobiocin (5 µg)	0	32	0	4	1	21	1	3	0	3	0	1
Neomycin (30 µg)	32	0	4	0	22	0	4	0	3	0	0	1
Polymyxin B (100 units)	32	0	4	0	22	0	4	0	3	0	1	0
Ampicillin (25 µg)	27	5	1	3	19	3	4	0	3	0	0	1

were sensitive to polymyxin B. Details of the other sensitivity patterns are given in table III. There was no essential difference between the sensitivity patterns of the saccharolytic and the non-saccharolytic biotype.

Animal pathogenicity

Of the 65 strains tested for pathogenicity, 19 killed one or two mice within 7 days. Details of these pathogenic strains are given in table IV. Post-mortem examination of the mice killed by these strains showed hyperaemia of the peritoneum with some exudate formation but no involvement of other organs. The strain isolated from a normal ear was not tested for pathogenicity.

Clinical findings

Fifty-seven strains were isolated over a period of 26 months from 55 patients whose ages ranged from 7 months to 75 years. In 12 instances the isolations were made in pure culture, but usually the organisms were found in mixed growths, together with such potential pathogens as *Streptococcus pyogenes*, *Staphylococcus aureus* and *Haemophilus influenzae*, or with commensals of the skin or respiratory tract, or with faecal organisms. Table V shows, in relation to the three clinical diagnoses of acute otitis media, otitis externa and chronic otitis media, isolations of *Acinetobacter* that consisted either of pure cultures or cultures mixed only with normal skin flora; the responses obtained to anti-bacterial therapy are also shown.

Two patients with acute otitis media from whom *Acinetobacter* was isolated together with, respectively, penicillin-sensitive *Staph. aureus* and tetracycline-resistant *Strept. pyogenes*, are of special interest. The first patient failed to respond to systemic penicillin, but when tetracycline was substituted the ear condition cleared within 2 weeks. The second patient failed to respond to systemic tetracycline, but the substitution of systemic penicillin together with local neomycin and polymyxin cleared the ear in 10 days.

TABLE IV
Results of pathogenicity tests

Organism	Total number of strains examined	Number that killed 1-2 mice in 1-7 days	Number of pathogenic strains recovered from peritoneum	Number of pathogenic strains showing capsular staining
<i>A. anitratus</i> (clinical isolates)	32	13	8	0
<i>A. anitratus</i> (type strains)	4	3	3	0
<i>A. lwoffii</i> (clinical isolates)	21	3	3	1
<i>A. lwoffii</i> (type strains)	4	0	0	0
<i>Moraxella</i>	3	0	0	0
<i>Achromobacter</i>	1	0	0	0

In the patients with otitis externa and chronic otitis media from whom mixed cultures were obtained that usually included *Staph. aureus*, with or without *Esch. coli*, *Proteus* spp., *Pseudomonas* spp., *Strept. pyogenes* and *Strept. faecalis*, the clinical courses varied a great deal. Some ears were clear in a week or so whereas others continued to give trouble many months later; most received some form of antibiotic treatment.

One strain of *A. lwoffii* was isolated from a normal ear in a mixed culture with *Staph. epidermidis* and diphtheroid organisms.

DISCUSSION

Most of the organisms isolated in this study clearly belong to the genus now usually called *Acinetobacter*. Eight, however, were atypical—three gave a positive oxidase reaction, one reduced nitrate, two were VP positive, one was MR and VP positive and one was motile.

Two main biotypes have been described for this group—those that produce acid from glucose and a few other carbohydrates and those that do not. The former have been given the specific name *Acinetobacter anitratus* and the non-saccharolytic strains are called *Acinetobacter lwoffii* (Cowan and Steel, 1974); the present strains can be grouped in this way. Such properties as the ability to liquefy gelatin, to utilise citrate, and to produce urease, have been reported as being more variable and were found in this study to be shared between members of both main biotypes.

Another property found in both biotypes was soap tolerance, originally described by Billing (1955) for strains of "*Achromobacter anitratum*". Of the 17 strains with this property in the present series, six fell into the *lwoffii* group, and there was no relation between soap tolerance and the ability to liquefy gelatin. Lecithinase production was likewise variable.

The results of the serological studies are less definite. Four main patterns

TABLE V
Isolations in relation to clinical diagnosis and response to antibacterial treatment

Number of patients with the indicated diagnosis	Strains isolated in pure culture or with normal skin flora only	Antibacterial therapy		Duration of therapy	Response
		Local	Systemic		
Acute otitis media, 13	<i>A. lwoffii</i> †	...	...	...	...
	<i>A. lwoffii</i> †	...	...	...	...
	<i>A. anitratus</i>	None	Penicillin	1 wk	None
		Polymyxin and neomycin	Tetracycline	1 wk	Good
	<i>A. lwoffii</i> *	Chloramphenicol	Tetracycline	1 wk	Good
	<i>A. anitratus</i> with skin flora	None	None	...	Good
Chronic otitis media, 34	<i>A. anitratus</i> †	...	...	...	...
	<i>A. lwoffii</i>	None	None	...	Good
	<i>A. anitratus</i>	Framycetin	None	1 wk	Good
	<i>A. lwoffii</i> ‡	None	None	...	Good
	<i>A. anitratus</i>	Polymyxin and neomycin	None	1 wk	Good
	<i>Moraxella</i>	Polymyxin and neomycin	None	2 months intermittent	Poor until operative treatment performed
	<i>Moraxella</i>	Polymyxin and neomycin	None	1 wk	Good
	<i>Achromobacter</i> * with skin flora	Neomycin	Penicillin	1 wk	Good
	<i>A. lwoffii</i> with skin flora	Neomycin	Penicillin	1 wk	Poor until operative treatment performed
	<i>A. lwoffii</i> with skin flora	None	None	...	Moderate until operative treatment performed
Otitis externa, 8	<i>A. lwoffii</i> ‡ with skin flora	None	None	...	Good
	<i>A. lwoffii</i> †	...	...	...	...

* = Strains resistant to the antibiotics given, † = no clinical details obtained, ‡ = isolated from bone chips obtained at mastoidectomy, ... = no observations possible.

of agglutination were found, but many strains gave individual patterns and some did not react at all with the antisera; this suggests that there is a diversity of antigenic types. A degree of antigenic similarity was found between members of the two main biotypes, but no definite relationship could be demonstrated between the type strains and the clinical isolates. It is evident from the literature that a considerable number of serological types exist that do not correspond with the biochemical groupings, and that some antigens are shared with organisms of otherwise unrelated groups such as *Klebsiella* (Aiken, Ward and King, 1956; Daly, Postic and Kass, 1962; Lothe and Griffin, 1965), and *Chlamydia* (Matthiesen and Volkert, 1957). Further studies on the antigenic relationships of *Acinetobacter* are required.

Most authors have reported *Acinetobacter* to be resistant to penicillin, and all but three of the clinical isolates studied here were found to be so. These

three penicillin-sensitive strains were also oxidase positive, and this suggests that they should be considered as belonging to the genus *Moraxella* (Wilson and Miles, 1974). Surprisingly, one type strain of *A. lwoffii* was penicillin sensitive. Most of the strains were sensitive to erythromycin and ampicillin, but they varied in their reactions to tetracycline and streptomycin; a high proportion was chloramphenicol resistant, a property to which Lothe and Griffin (1965) drew particular attention.

Most of the clinical isolates were non-pathogenic for mice; the majority of those that were pathogenic to mice belonged to the *anitrat* group, and a similar relationship was also noted for the type strains. No clear-cut pattern of pathogenicity is described in the literature.

It is difficult to assess the pathogenicity of the present strains for the human ear. That pure cultures were relatively uncommon suggests pathogenicity of a low order, and indeed many strains were isolated together with an organism such as *Staph. aureus* or *Proteus* that might be expected to play a more prominent part in any inflammatory process; other workers have also noted this association (Brooke, 1951; Scott and Mahoney, 1953).

Further evidence of lack of pathogenicity is provided by the two patients in this series who underwent mastoidectomy. In both instances *Acinetobacter* was isolated from the bone chips obtained at operation, suggesting fairly deep penetration of the organisms, yet neither patient required special post-operative treatment or antibiotic therapy. It may, however, be of some significance that these strains were both of the *lwoffii* group.

In contrast, evidence pointing to some measure of pathogenicity was obtained from the patients with acute otitis media. Pure cultures of *Acinetobacter* were obtained from four of these, and at least one required appropriate antibiotic treatment before the ear was satisfactorily healed; the infecting strain belonged to the *anitrat* group and was pathogenic for mice. In a second patient with acute otitis media a penicillin-sensitive *Staph. aureus* was the only other organism isolated. The ear did not respond to treatment until tetracycline, to which the acinetobacter was sensitive, was substituted for the penicillin given initially. This strain was also a member of the *anitrat* group but was not pathogenic for mice.

On balance the evidence points to a degree of pathogenicity for the ear that varies with individual strains, with a suggestion that saccharolytic strains are more likely to be pathogenic. In most instances, however, their presence would seem to indicate little more than their opportunistic potential.

There is evidence that *Acinetobacter* organisms may form part of the normal skin flora (Taplin, Rebell and Zaia, 1963; Greer, Mikhail and Livingood, 1965; Donald, 1966) and the isolation of one of the present strains from a normal external ear supports this concept. Garrison (1963) has suggested that the frequent occurrence of this group of organisms in soil may account for their isolation from the respiratory tract; it is perhaps surprising that they are apparently so seldom isolated from such sites as the ear.

A few of the present strains were not entirely typical. One of these reduced nitrate, but this property no longer excludes it from the genus *Acinetobacter*

(Jyssum and Joner, 1965); nor does exclusion seem justified for the three strains that gave positive MR or VP reactions.

The three non-saccharolytic oxidase-positive strains fit best into the genus *Moraxella*. Thornley (1967) found a close relationship between the oxidase-positive, penicillin-sensitive strains and typical oxidase-negative, penicillin-resistant *Acinetobacter* strains, but evidence from other studies is considered sufficient to justify their generic separation (Baumann, Doudoroff and Stanier, 1968; Johnson, Anderson and Ordal, 1970).

SUMMARY

Fifty-seven strains of acinetobacter-like organisms were isolated over a period of 26 months from the ears of 55 patients with acute or chronic otitis media, or otitis externa, and one strain was isolated in a survey of 50 normal ears. After comparison with eight reference strains, 32 of the isolates were identified as *Acinetobacter anitratus*, 22 as *Acinetobacter lwoffii*, three as *Moraxella* spp. and one as *Achromobacter* sp.

Analysis of the clinical findings suggests that although most of these organisms played little part in the disease process, a few strains were probably pathogenic in this situation.

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