Mima polymorpha infection

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SYNOPSIS Two cases of human infection by *Mima polymorpha* are described. From these cases, and from the literature, it is concluded that this species is heterogeneous with respect to its biochemical properties. From comparative studies it is concluded that the genus *Mima* is unrelated to the genus *Moraxella* or to *Bacterium anitratum*.

BACTERIOLOGICAL FINDINGS IN TWO CASES OF HUMAN INFECTION

As early as 1936, epidemics and sporadic cases of meningitis had been observed in which Gram-negative diplococci were found but which differed from the *Neisseriae* in growing on nutrient agar (Sen, 1936). In 1939, de Bord found that 33% of male patients, thought to be suffering from gonorrhoea because Gram-negative diplococci were present in the discharge, had in fact not been harbouring *N. gonorrhoeae* but organisms of a group unrelated to the *Neisseriae*. Accordingly, in 1942, he compiled a new tribe which he named the Mimeae, and which he divided into three genera named *Mima*, *Herellea*, and *Colloides*. These genera were apparently well marked from each other by indole production, citrate utilization, and the reduction of potassium nitrate to nitrite. The group as a whole was thought to be a non-oxidase producer, but de Bord found an oxidase-producing variant of *M. polymorpha*. In 1945, Deacon found that not only were some strains of *Mima polymorpha* atypical in producing oxidase but also in utilizing citrate or reducing nitrates. De Bord also noted in 1943 that, while typical species of *Mima* did not ferment carbohydrates, some strains from the vagina and conjunctiva might do so and, in this connexion, he investigated the action of the bacteria on glucose, maltose, sucrose, mannitol, dulcitol, salicin, and dextrin only.

Evidence of the pathogenic potentialities of the tribe grew. De Bord isolated *Mima polymorpha* var. *oxidans*, *Herellea vaginocolia*, and *Colloides anoxydana* from clinically normal conjunctivas and vaginas, but, in addition, he and Deacon (1945) isolated these from 'gonorrhoea', septic war wounds, and from brain tissue following injury. In 1948 de

Bord described the isolation of *Mima polymorpha* from the cerebrospinal fluid of a man with meningitis. Townsend, Hersey, and Wilson (1954) isolated *Mima polymorpha* from a patient with meningitis who died of the Waterhouse-Friederichsen syndrome. A contact of this man also developed meningitis, and he, too, yielded *Mima polymorpha*. The graver pathogenic possibilities of the organism were discussed by Faust and Hood (1949) who described the case of a woman who died of septicaemia after an illness lasting only 42 hours and from whom *Mima polymorpha* var. *oxidans* was recovered. Pike, Schulze, and McCullough (1951) described a boy of 6 years who had an operation for a congenital heart defect and six months later developed a low-grade pyrexia which persisted for two years, after which *Mima polymorpha* var. *oxidans* was recovered from blood and bone marrow cultures.

De Torregrosa and Ortiz (1961) have reviewed much of the literature concerning *Mima polymorpha*, and emphasized the importance of thorough biochemical investigation of Gram-negative cocci to avoid confusion with various species of *Neisseria*. Three of their five cases in young children suffered from meningitis and two from septicaemia. Of their fatal cases, two had meningitis and one had otitis media and pneumonia due to *Mima polymorpha*. Two of these instances are of particular interest. A female premature infant of 4 lb. 9 oz. developed meningitis on the fourth day of life and died 18 days later of meningitis and bronchopneumonia despite treatment with chloramphenicol, streptomycin, and sulphadiazine. The second case was the twin sister of the foregoing, and weighed 4 lb. 6 oz. She first developed symptoms one day after her sister, at the age of 5 days. She died at the age of 14 days, despite treatment with oxytetracycline, chloramphenicol, and polymyxin B. *Mima polymorpha* was isolated from the blood and from the purulent aural dis-

charge. These cases are of interest as they appear to indicate case-to-case transmission as in the infections reported by Townsend *et al.*

That the members of the genus *Herellea* also are pathogenic is shown by Sorrell and White (1953) and of Schuldberg (1953). Sorrell and White published the case of a 39-year-old man who was admitted to hospital suffering from acute bacterial endocarditis. The strain isolated differed from a typical *Herellea* sp. in giving a positive methyl red reaction and producing a yellow pigment. Schuldberg described the case of an 18-month-old girl who died of leptomeningitis with haemorrhage into the adrenal cortices. This strain was atypical in causing beta-haemolysis on blood agar.

Two recent instances of the recovery of *Mima* spp. from wound infections in Aberdeen are now described.

CASE 1 A 29-year-old man suffering from Hodgkin's disease had his gall bladder removed on account of cholelithiasis; the wound became infected; coagulase-positive staphylococci and *Mima polymorpha* were isolated on culture.

CASE 2 A 57-year-old man was admitted to hospital after a history of osteomyelitis of the femur. From swabs taken from the sinus coagulase-positive staphylococci and *Mima polymorpha* were recovered on three occasions.

The organisms isolated from cases 1 and 2 were predominantly of coccid type; any tendency to become bacillary was minimal. Preparations from cultures were Gram negative, although a few Gram-positive forms were sometimes seen. They were non-motile at 22°C. and 37°C. Encapsulation was demonstrated by the Giemsa and Kirkpatrick methods. Aerobic growth was readily obtained on nutrient agar, horse blood agar, and MacConkey's bile-salt agar but not on Hoyle's tellurite medium. There was no growth anaerobically on horse blood agar or in Brewer's thioglycollate broth. The organisms failed to liquify gelatine or Loeffler's serum medium and were non-chromogenic on the latter. Catalase and ammonia were produced in peptone water. Indole, oxidase, and acetylmethyl-carbinol were not produced. Potassium nitrate was not converted to nitrite, nor urea to ammonia. The methyl red reaction was negative and citrate was not utilized. Acid only was produced from the pentoses arabinose and xylose, but not from rhamnose. From the hexoses, acid from mannose only was obtained but no splitting of glucose, fructose, or galactose was demonstrated. The organisms failed to ferment the disaccharides maltose, sucrose, lactose, and trehalose; the trisaccharide raffinose; the polysaccharide inulin; the

alcohols dulcitol, mannitol, sorbitol, and adonitol as well as the glycosides salicin and aesculin.

The strains were sensitive to neomycin, erythromycin, polymyxin B, framycetin, and colomycin but insensitive to chloromycetin, nitrofurantoin, novobiocin, oleandomycin, streptomycin, tetracycline, ristocetin, vancomycin, and penicillin, including D-alpha-aminophenylacetamido penicillanic acid and methicillin.

SEROLOGICAL INVESTIGATION A rabbit was injected intravenously on three successive days of each of three weeks with a suspension of the *Mima* sp. isolated from case 1. The organisms were administered in the live state, and, after the ninth injection, the serum was collected, preserved with merthiolate and stored in the deep freeze.

Doubling dilutions of this serum were made and to these were added live suspensions of the two strains of *Mima*. Agglutination was allowed to take place for four hours at 55°C. then overnight at room temperature. The homologous strain was agglutinated to a dilution of 1 in 2,048 of the antiserum and the second strain to 1 in 8,192.

COMPARISON OF *MIMA* STRAINS, *MORAXELLA*, AND *BACTERIUM ANITRATUM*

The investigations of de Bord (1943), Deacon (1945), Sorrell and White (1953), and Schuldberg (1953) would lead one to regard the tribe Mimeae as a group of organisms with essential biochemical characteristics which show considerable variation, e.g., they may or may not (1) produce oxidase, (2) utilize citrate, (3) give a methyl red reaction, (4) reduce nitrates to nitrites, (5) produce pigment, and (6) be haemolytic on blood agar.

Despite de Bord (1943) who stated that oxidase production by *Mima* spp. was variable, we find Henriksen (1952) suggesting that *Mima polymorpha* var. *oxidans* was in fact a *Moraxella* because of resemblances to *Moraxella* in its production of oxidase and lack of saccharolytic properties.

In 1948, Schaub and Hauber described a group of organisms which has become known as *Bacterium* (or *Achromobacter*) *anitratum*, and in 1949 Stuart, Formal, and McGann described a group of bacteria which they named the B5W group. In 1950, Ferguson and Roberts suggested that these groups were members of the same species, although it must be pointed out that the B5W group hydrolyses urea and does not give the methyl red reaction, whereas Schaub and Hauber state that their group gives the methyl red reaction and hydrolyses urea. Ewing (1949) suggested that this *B. anitratum* B5W group was related to the tribe Mimeae.

Now that the importance of *B. anitratum* is recognized as a cause of meningitis in this country and now that we are recording the appearance of *Mima* spp., it seemed a suitable time to attempt to establish if there were any relationship between these groups.

Four strains of *B. (Achromobacter) anitratum* were

obtained from the National Collection of Type Cultures, London, and a comparison was made between their biochemical properties and those of our two strains of *Mima*. At the same time one strain each of *Moraxella lacunata*, *bovis*, and *liquifaciens* were obtained from the same source. The comparative results are set out in Table I.

TABLE I

COMPARISON OF PROPERTIES OF BOTH STRAINS OF *MIMA* AND OF *B. ANITRATUM* AND *MORAXELLA* SPP.

Characteristic	<i>Mima</i> spp. (Both Strains)	<i>M. lacunata</i> N.C.T.C. No. 7784	<i>M. bovis</i> N.C.T.C. No. 8561	<i>M. lique-</i> <i>faciens</i> N.C.T.C. No. 7911	<i>B. anitratum</i> N.C.T.C. No. 7363	<i>B. anitratum</i> N.C.T.C. No. 7364	<i>B. anitratum</i> N.C.T.C. No. 7642	<i>B. anitra-</i> <i>tum</i> N.C.T.C. No. 8102
Morphology								
Coccus	+	—	—	—	—	—	—	—
Bacillus	(+)	+	+	+	+	+	+	+
Blood agar								
Colony	Yellowish white	Grey, viscid	Grey, viscid	Grey, viscid	Grey, viscid	Grey, viscid	Grey, viscid	Grey, viscid
Haemolysis	—	—	+	—	—	—	—	—
Motility	—	—	—	—	—	—	—	—
Aerobic	+	+	+	+	+	+	+	+
Anaerobic	—	—	—	—	—	—	—	—
Gelatin liquefaction	—	No growth	+	+	—	—	—	—
Loeffler liquefaction	—	—	—	—	—	—	—	—
Oxidase	—	+	+	+	—	—	—	—
Catalase	+	+	+	+	+	+	+	+
Voges-Proskauer test	—	—	—	—	—	—	—	—
Methyl red test	—	—	—	—	—	—	—	—
Indole	—	—	—	—	—	—	—	—
Ammonia production	+	+	+	+	+	+	+	+
Citrate utilization	—	—	—	—	+	+	+	+
Nitrite production	—	—	—	+	—	—	—	—
Urea	—	—	—	—	—	+	+	—
Milk	—	No growth	Digested	—	—	—	—	—
Carbohydrates								
Pentoses								
Arabinose	— ¹	—	—	—	—	—	—	—
Xylose	— ¹	—	—	—	—	—	—	—
Rhamnose	—	—	—	—	—	—	—	—
Hexoses								
Glucose	—	—	—	—	—	—	—	—
Fructose	—	—	—	—	—	—	—	—
Galactose	—	—	—	—	—	—	—	—
Mannose	— ¹	—	—	—	—	—	—	—
Disaccharides								
Sucrose	—	—	—	—	—	—	—	—
Maltose	—	—	—	—	—	—	—	—
Lactose	—	—	—	—	—	—	—	—
Trehalose	—	—	—	—	—	—	—	—
Trisaccharide								
Raffinose	—	—	—	—	—	—	—	—
Polysaccharides								
Inulin	—	—	—	—	—	—	—	—
Dextrin	—	—	—	—	—	—	—	—
Alcohols								
Adonitol	—	—	—	—	—	—	—	—
Mannitol	—	—	—	—	—	—	—	—
Dulcitol	—	—	—	—	—	—	—	—
Sorbitol	—	—	—	—	—	—	—	—
Glycosides								
Salicin	—	—	—	—	—	—	—	—
Aesculin	—	—	—	—	—	—	—	—

+ = reaction obtained

—¹ = acid produced without gas

Suspensions of the four strains of *B. anitratum* were now agglutinated with the antiserum prepared against the first strain of *M. polymorpha*, as described above. After noting the results, the serum was now absorbed by aliquots of each of the four strains of *B. anitratum* till absorption was complete. The titre of the serum was now re-estimated by agglutinating suspensions of the homologous strain of *Mima*. The results are shown in Table II. It can be seen from these results that *B. anitratum* is unrelated to *M. polymorpha*.

TABLE II

RELATIONSHIP OF *B. ANITRATUM* TO *M. POLYMORPHA*

	<i>Mima polymorpha</i>		<i>B. anitratum</i>		
	Case 1	7363	7364	7462	8102
Unabsorbed serum	2,048 ¹	128	256	256	0
Absorbed serum	0	1,024	1,024	1,024	1,024

¹Titre is expressed as the reciprocal of the final dilution giving complete agglutination of the bacterial suspensions.

The paucity of biochemical properties of *Mima* and the variability of its positive attributes raises difficulty in classification. For example, as Gram-negative cocci are rare in nature, being confined to the genus *Neisseria*, the new tribe Mimeae and the genus *Lampropedia* (which is not recognized in Bergey's manual), it might be thought that organisms of the tribe Mimeae are related to *Neisseria* as several of the species in the latter genus are able to grow at 22°C. and on nutrient agar in a similar manner to *Mima*; and while it is typical of species belonging to the genus *Neisseria* that they produce oxidase, several strains of *Mima* have been noted to do so also.

The features which appear to differentiate *Mima* from *B. anitratum* are not sufficiently definite. Some strains of *B. anitratum* hydrolyse urea but this is

variable. The strains of *B. anitratum* examined in this investigation can utilize citrate as the sole source of carbon for growth whereas this is not a feature of *Mima*; but as stated above, Deacon (1945) showed that some strains of *Mima* show this feature. Also while the four strains of *B. anitratum* studied did not ferment glycosides, alcohols, or carbohydrates (with the possible exception of glucose in one instance) and our strains of *Mima* did ferment certain carbohydrates, it is said that typical organisms of the *Mima* group also do not ferment carbohydrates.

Similarly, the lack of biochemical activity among the organisms of the genus *Moraxella* inevitably causes them in this respect to resemble the relatively inert *Mima*, but this is no evidence of relationship.

There is no doubt that the strains of *B. anitratum* and *Moraxella* studied were all bacilli, by which they are distinguished from the strains of *Mima* since the latter were all cocci with only slight tendency to bacillary morphology. Although it is usually unsafe to rely on morphological appearances in identifying an organism, they may be helpful in instances such as this.

REFERENCES

- Deacon, W. E. (1945). *J. Bact.*, **49**, 511.
 De Bord, G. G. (1939). *Ibid.*, **38**, 119.
 — (1943). *J. Lab. clin. Med.*, **28**, 710.
 — (1948). *J. Bact.*, **55**, 764.
 De Torregrosa, M. Vda., and Ortiz, A. (1961). *J. Pediat.*, **59**, 35.
 Ewing, W. H. (1949). *J. Bact.*, **57**, 659.
 Faust, J., and Hood, M. (1949). *Amer. J. clin. Path.*, **19**, 1143.
 Ferguson, W. W., and Roberts, L. F. (1950). *J. Bact.*, **59**, 171.
 Henriksen, S. D. (1952). *J. gen. Microbiol.*, **6**, 318.
 Pike, R. M., Schulze, M. L., and McCullough, M. (1951). *Amer. J. clin. Path.*, **21**, 1094.
 Schaub, I. G., and Hauber, F. D. (1948). *J. Bact.*, **56**, 379.
 Schuldborg, I. I. (1953). *Amer. J. clin. Path.*, **23**, 1024.
 Sen, B. B. (1936). *J. Bact.*, **32**, 293.
 Sorrell, W. B., and White, L. V. (1953). *Amer. J. clin. Path.*, **23**, 134.
 Stuart, C. A., Formal, S., and McGann, V. (1949). *J. infect. Dis.*, **84**, 235.
 Townsend, F. M., Hersey, D. F., and Wilson, F. W. (1954). *U.S. Armed Forces med. J.*, **5**, 673.