

OCCURRENCE OF NONFERMENTATIVE GRAM-NEGATIVE BACILLI IN A MALAYSIAN HOSPITAL

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SYNOPSIS

Nonfermentative Gram-negative bacilli were commonly isolated from clinical material in a Malaysian hospital over a two year period of study. *Pseudomonas aeruginosa*, *Acinetobacter* sp and other pseudomonas were the most common organisms seen. Although these bacteria occurred regularly in clinical specimens their pathogenetic role could be established in only a small proportion of cases. Most of these organism were either contaminants or commensals of the normal bacterial flora. It is important to interpret the isolation of a nonfermentative Gram-negative bacillus with caution, and in the light of clinical findings. Failure to do so may result in the unnecessary use of antibiotics.

INTRODUCTION

The nonfermentative Gram-negative bacilli are an ill defined taxonomic group of bacteria that comprises at least 30 species in seven major genera. Apart from *Pseudomonas aeruginosa* these bacteria are generally thought to be harmless environmental saprophytes. In recent years however these organisms have been increasingly isolated from clinical specimens from hospitalised patients. They have been shown to cause hospital acquired infections especially among the immunocompromised (1). This paper describes the occurrence of this group of bacteria over a two year period in the Kuala Lumpur General Hospital.

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METHODS

The bacteriology laboratory of the Faculty of Medicine, the National University of Malaysia provides a diagnostic service for the university units in the Kuala Lumpur General Hospital. All clinical specimens received were processed in the usual manner by direct microscopy and culture onto suitable media. All plates were read after 24 hours incubation and again after 48 hours. Blood cultures were routinely subcultured after 2 days and 5 days incubation. Where necessary a third subculture was performed usually after 14 days.

All Gram-negative bacilli which grew in pure or moderate to heavy growth were identified. Scanty growth of gram-negative bacilli in mixed cultures were ignored. For urine specimens (with the exception of bladder taps) only isolates with a colony count of greater than 100,000 colonies per ml and in pure growth were further processed. However all isolates obtained from CSF, other body fluids and blood cultures were identified regardless of density of growth.

The nonfermentative Gram-negative bacilli were identified using the following battery of tests; colonial morphology, pigment production, odour, oxidase test, catalase test, O/F (glucose) test, triple sugar iron, IMVIC (indole, methy-red, Voges-Proskauer test and citrate) and motility. The majority of *Pseudomonas aeruginosa* and *Acinetobacter* sp could be identified in this manner. When identification was not possible after these preliminary tests, the Oxi/ferm (Roche) system was used. This system has already been described previously. (2,3).

An attempt was also made to establish the clinical significance of the isolates. This was done by a visit to the ward and discussion with the attending physician during the department's routine daily microbiology round or when this

was not possible by retrospective reference of the patients' records. Features taken to support clinical significance were symptoms and signs of infection, haematological and radiological findings and repeated isolates of the same organism from the patient.

RESULTS

From the period 1st January 1981 to 31st December 1982 a total of 64,209 clinical specimens were processed by the university bacteriology laboratory. From these a total of 2456 isolates of nonfermentative Gram-negative bacilli were obtained. This figure included repeated isolates from the same patient. The details of the isolates are summarised in Table 1.

Pseudomonas aeruginosa comprised the most common nonfermentative Gram-negative bacillus isolated accounting for nearly 70% of such isolates. This was followed by *Acinetobacter* sp (17%) and other pseudomonads (8%). *Moraxella* sp (26 isolates), *Flavobacterium* sp (21), *Alcaligenes* sp (16), *Bordetella bronchiseptica* (3), *Chromobacterium* sp (1) and *Achromobacter* sp (2) were among the other nonfermentative Gram-negative bacilli isolated but were encountered in far lesser numbers. The pseudomonads other than *Pseudomonas aeruginosa* comprised *Ps. stutzeri* (30 isolates), *Ps. cepacia* (26), *Ps. maltophilia* (21), *Ps. vesicularis* (15), *Ps. putida* (7) and *Ps. fluorescens* (5). Another 94 isolates could only be classified by the Oxi/ferm system as *Pseudomonas* sp. A total of 23 isolates or approximately 1% remained unidentified.

The majority of these isolates were obtained from pus specimens and other surface swabs followed by respiratory tract specimens, blood cultures, urines, CSF and other body fluids.

TABLE 1
NONFERMENTATIVE GRAM NEGATIVE BACILLI FROM CLINICAL SPECIMENS

ORGANISM	TYPE OF SPECIMEN						TOTAL
	1	2	3	4	5	6	
<i>Ps. aeruginosa</i>	638	528	338	6	100	130	1740
<i>Acinetobacter</i> sp	31	101	80	44	98	72	426
<i>Pseudomonas</i> sp	3	37	14	9	117	18	198
<i>Moraxella</i> sp	0	2	2	8	14	0	26
<i>Flavobacterium</i> sp	0	2	1	17	1	0	21
Others	1	3	3	1	14	0	22
Unidentified	2	4	5	0	12	0	23
TOTAL	675	677	443	85	356	220	2456

NOTE: 1: Pus, 2: Other swabs, 3: Respiratory specimens, 4: CSF and other body fluids, 5: Blood cultures, 6: Urine.

DISCUSSION

Bacteremias.

A total of 356 nonfermentative Gram-negative bacilli were isolated from blood culture. Of these only 44 isolates from 37 patients were considered significant. *Pseudomonas aeruginosa* and other pseudomonads were the most common isolates. Significant bacteremia due to *Pseudomonas aeruginosa* was seen in 35 patients. They were cases of severe burns (15 cases), neonatal sepsis (7), orthopaedic (5), leukaemias and lymphomas (4) and bacteremias secondary to urinary tract infections (4). *Acinetobacter* sp was considered significant in two neonates with presumed sepsis clinically. Of the other pseudomonads only one isolate of *Pseudomonas maltophilia* isolated from a neonate with respiratory distress syndrome was considered clinically significant. The other isolates were attributed to contamination of the blood culture during the taking of blood. Organisms of the *Moraxella-Acinetobacter* group has been shown to be common contaminants of blood cultures as they occur frequently as commensals on the skin of patients in hospital (4). In this survey *Pseudomonas aeruginosa* as well as other pseudomonads were also seen as common contaminants of blood cultures.

Urine

There were 220 isolates of nonfermentative Gram-negative bacilli from instances of significant bacteriuria. These were obtained from 167 patients. Of these 142 were patients with in-dwelling urinary catheters. *Pseudomonas aeruginosa* was the offending organism in 130 of these instances of significant bacteriuria. This underlines the fact that *Pseudomonas aeruginosa* is a common cause of catheter-associated urinary tract infection in our hospital and stresses the importance of good catheter care in the prevention of these infections.

CSF and other body fluids

All 17 isolates of flavobacteria were clinically significant isolates from neonates with meningitis. The high incidence is in part due to the fact that the university paediatric unit receives referrals of such cases from all over West Malaysia. Meningitis due to *Flavobacterium meningosepticum* is not uncommon in Malaysia and has been described previously (5). During this two year period two cases of *Pseudomonas aeruginosa* meningitis were also seen. Both occurred in infants, one of which was a shunt-associated infection. As in blood cultures the majority of isolates of nonfermentative Gram-negative bacilli from CSF were regarded as contaminants and these growths were not accompanied by positive cytological or biochemical of CSF. A case of neonatal meningitis in the paediatric unit due to this organism has previously been reported (6). *Pseudomonas cepacia* was isolated from the peritoneal fluid of a patient who was on chronic ambulatory peritoneal dialysis and who had evidence of clinical peritonitis.

Respiratory specimens

Of the 338 isolates of *Pseudomonas aeruginosa* from respiratory specimens, 225 were isolates from tracheal aspirates. These were obtained from 53 patients who were all on artificial ventilation in the intensive care units of the hospital. Of these 45 had radiological evidence of pneumonia and the clinicians were advised to institute anti-pseudomonal therapy. The other isolates of nonfermentative Gram-negative bacilli were obtained from throat swabs and sputa where it was very difficult to attribute any pathogenetic role to the isolates. These isolates were regarded as colonisers of the upper respiratory tract.

Pus and other swabs

Over 60% of the isolates of nonfermentative Gram-negative bacilli were obtained from pus and other surface swabs. These organisms have been described in association with various suppurative lesions (1,7). Although these organisms occur frequently in these specimens in our hospital, we have found it difficult to establish with certainty their pathogenetic roles. This is because these organisms were often isolated in mixed growth with Enterobacteriaceae and staphylococci. Repeated sampling of the infected site often revealed a different bacterial flora. Far too few clinicians send actual pus and placed too much reliance on pus swabs. Most of these swabs if not properly taken will only sample the superficial flora. This has been shown on several occasions when a repeat swab taken by the bacteriologist revealed an entirely different set of organisms. Of the isolates which were considered clinically significant, *Pseudomonas aeruginosa* was isolated from 67 cases of post-surgical wound infections, infected burns (27 cases), cutaneous abscesses (15 cases), umbilical sepsis (7 cases) and keratoconjunctivitis (6 cases). *Pseudomonas maltophilia* was considered a significant isolate in 3 cases of post-surgical wound infection. *Pseudomonas cepacia* was isolated in pure growth from a cutaneous abscess and *Chromobacterium violaceum* was isolated in pure growth from a wound following a snake bite. There were no cases of melioidosis seen during this two year period. In all other instances the isolates were considered to be either contaminants or surface colonisers.

Nonfermentative Gram-negative bacilli were commonly encountered in clinical specimens in the Kuala Lumpur General Hospital. *Pseudomonas aeruginosa* was the most common species seen. It was however difficult to establish a pathogenetic role for the majority of the isolates. They are common contaminants in blood cultures and CSF specimens. It is important to interpret the significance of these isolates in the light of clinical findings. Failure to do so may lead to the unnecessary use of antibiotics.

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