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Characterization of Non–Fermenting Gram–Negative Bacilli at the Lagos University Teaching Hospital – A Preliminary Report.

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SUMMARY

Clinically significant isolates of Gram–negative bacilli obtained in the Lagos University Teaching Hospital Laboratory between January 1995 and June 1996 were characterised, using Analytical profile index system (API 20E and API 20NE), and also by simple conventional laboratory methods.

Twenty–two percent (163) of all clinical significant isolates were found to be non–fermenting Gram–negative bacilli. The micro–organisms identified were *Pseudomonas aeruginosa* (55%), *Acinetobacter* species (29.5%), *Burkholderia (Pseudononas) cepacia* (9%), Pseudomonas fluorescens (2%), *Pseudononas stutzeri* (1%), *Stenotrophomonas (Xanthomonas) maltophila* (0.6%), *Chryseomonas luteola* (0.6%), *Ochrobacterium anthropi* (0.6%), *Agrobacterium radiobacter* (0.6%) and Alcaligenes xylosoxidans (0.6%).

Seventy percent of the isolates were obtained from specimens from hospitalised patients while 30% were obtained from specimens from outpatient clinics.

Most of the non–fermenting Gram–negative bacterial isolates were obtained from wound swabs (39%) and urinary specimens (36%). Nine percent was obtained from pulmonary specimens, 4.5% from eye swabs, 4.5% from ear swabs and 5.5% from blood. Very few (1.5%) isolates were cultured from the cerebrospinal fluid.

Key Words: Characterization, Gram–negative bacilli, non–fermentative.

INTRODUCTION

The glucose non–fermenting Gram–negative bacilli are a heterogenous group of bacteria which include the following genera: *Pseudomonas, Acinetobacter, Burkholderia, Stenotrophomonas (Xanthomonas), Alcaligenes, Moraxella, Ochrobacterium, Chryseobacterium (Flavobacterium), Achromobacter, Eikenella* and *Agrobacterium*[1]. This group of bacteria represents about 12–16% of all isolates encountered in clinical Microbiology Laboratories[2].

Apart from *Pseudomonas aeruginosa* which has been well studied, most of these organisms were thought to be environmental organisms. Some of these organisms have not been fully characterised, although they have been observed to cause diseases[3,4]. However, infection with this group of organisms is becoming increasingly important as they have been associated with life–threatening infections [5,6,7]. They cause nosocomial infections[8] which may be septicaemia, meningitis, pneumonia, urinary tract infections and surgical site infections. Recognised risk factors associated with infection or colonisation with these organisms include[9,10] antibiotic treatment, surgical instrumentation and stay in intensive care units. Outbreaks have also been known to occur with some of these organisms[10,11,12]. Unfortunately, there is scanty information on the prevalence of these organisms in developing countries.

In view of poor diagnostic facilities and lack of up–to–date information in developing countries, these organisms are difficult to identify in routine laboratories. Most of the time, the Gram–negative bacterial isolates are simply call "atypical coliform". However, given appropriate media and methods, most non–fermentative bacilli can readily be identified[1,13].

Awareness and routine identification of this group of organisms is becoming increasingly important because of their association with multiple drug resistance[14,15].

MATERIALS AND METHODS

All clinically significant isolates obtained from various specimens (blood, cerebrospinal fluid, urine, sputum and surgical site specimens) were subcultured from LUTH Laboratory between January 1995 and June 1996. They were identified properly by standard laboratory methods [1,2,16].

All the Gram–negative bacilli obtained were subcultured on Bacto Triple sugar iron (TSI) agar (DIFCO Laboratories, Detroit Michigan, USA). The bacterial isolates which showed growth but did not acidify the butt and slope of the TSI agar were considered to be non–fermenters and were further identified by API 20E and API 20NE (Analytical profile index system, bioMerieux Sa 69280 Marcy l'Etoile – France).

They were also identified by simple conventional biochemical methods (Table 1, Figure 1–a modification of the standard methods).

RESULTS

Table 2 shows the various non-fermenting Gram-negative bacilli isolated over eighteen months.

One hundred and sixty three (22%) non-fermenters out of a total of 749 clinically significant isolates were obtained.

Fifty-five percent of the non-fermenting bacilli were *P. aeruginosa* and 18.5% were *Acinetobacter baumannii*. All *Acinetobacter* spp constituted 29.5% of the total non-fermenters. Nine percent of the isolates were *Burkholderia cepacia*. The percentages of the remaining non-fermenters ranged from 0.6% for *Stenotrophomonas maltophila* to 2% for *Pseudomonas fluorescens*.

As shown in Table 3, 39% of the non-fermenters was obtained from surgical site infections, 36% from urinary specimens and 9% from sputum. The few remaining non-fermenting Gram-negative bacterial isolates came from eye swabs, ear swabs and CSF. All the isolates obtained from the eye swabs and most from the ear swabs were P. aeruginosa.

Table 4 shows the sources of various specimens from which the non-fermenters were isolated. Seventy percent of the isolates were from patients on hospital admission while 30% were from outpatients. Specimens were obtained from numerous clinics and wards with the surgical wards (33%) and surgical clinics (11%) giving the highest percentages of specimens from which non-fermenters were obtained.

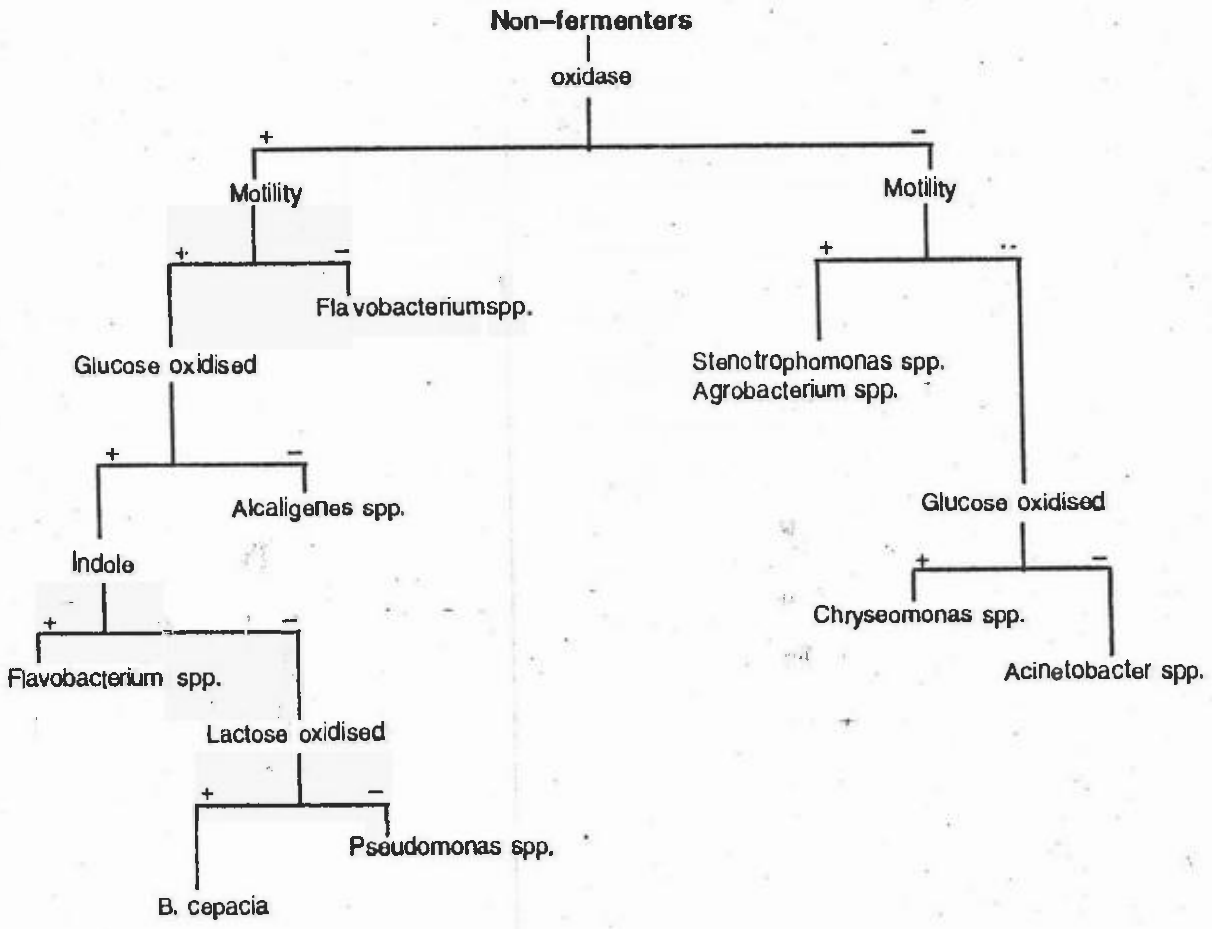

Figure 1: Biochemical Characteristics of Non-fermenting Gram-negative Bacilli

Table I

Simply conventional methods of identification of Gram–negative non–fermenting bacilli

Cultural characteristics of Gram–negative non–fermenting bacilli on MacConkey agar.

1. Greenish/dark green/yellowish green Flat spreading colonies Sweet smelling.
   - P. aeruginosa
2. Non–lactose fermenting/non–pigmented colonies
   - P. aeruginosa
   - P. fluorescens
   - F. meningosepticum
   - O. anthropi
3. Tiny pink colonies
   - B. cepacia
4. Mucoid/light pink/non lactose fermenting colonies
   - Acinetobacter species
   - Chryseomones luteola
   - Agrobacterium radiobacter

Table II

The occurrence of non–fermenting Gram–negative bacilli in LUTH Microbiology Laboratory between January 1995 and June 1996.

Bacterial Isolates	Number of strains seen (% of total)
Pseudomonas aeruginosa	90 (55)
Pseudomonas fluorescens	3 (2)
Pseudomonas stutzeri	2 (1)
Burkholderia (Pseudomonas) cepacia	13 (9)
Burkholderis picketti	1 (0.6)
Acinetobacter baumannii	29 (18.5
Acinetobacter haemolyticus	2 (1)
Acinetobacter calcoaceticus	12 (7)
Acinetobacter junii/johnsonii	2 (1)
Flavobacterium meningosepticum	2 (1)
Stenotrophomonas (Xanthomonas) maltophila	1 (0.6)
Chryseomonas luteola	1 (0.6)
Ochrobactrum anthropi	1 (0.6)
Agrobacterium radiobacter	2 (1)
Alcaligenes xylosoxidans	2 (1)
Totals	163 (100)

Total number of isolates obtained from clinical specimens between January 1995 and June 1996 = 729

Percentage of non–fermenting Gram–negative bacilli = 22.3

Table IV

Sources of various specimens from which non–fermenting Gram–negative bacilli were cultured

Outpatients

Clinics	Number of non–fermenters isolated
Surgical out–patients	17 (11%)
Medical out–patients	10 (5 %)
Paediatric out–patients	3 (1%)
Antenatal clinic	2 (1%)
Gynaecological out–patients	1 (0.6%)
Children's emergency	3 (2%)
Staff clinic	3 (2%)
Ear, nose and throat clinic	5 (4%)
Adult casualty	3 (2%)
Dental clinic	1 (0.6%)
Guinness Eye Centre	1 (0.6%)
Total	49 (30%)

In–patients

Hospital Wards	Number of non–fermenter isolated
Surgical wards	55 (33%)
Medical wards	18 (12%)
Paediatric wards	25 (16%)
Obstetric wards	8 (5%)
Gynaecological wards	8 (4%)
Total	114 (70%)

Tabel III

Distribution of common non–fermenting Gram–negative bacilli in various clinical specimens

Bacterial isolates	Eye Swabs	Ear Swabs	Surgical Site Samples	Blood	Sputum	CSF	Urinary Specimens	Intravenous fluid	Totals
P. aeruginosa	7	9	41	1	5		28		90
A. baumannii		2	13	2	3		11		29
Acinetobacter spp			2		4		10		14
Pseudomonas spp			4	1			8		5
B. cepacia				4				1	13
F. meningosepticum						2			2
Percentages	4.5	4.5	39	5.4	9	1.5	36	0.6	100

DISCUSSION

The proportion of non-fermenting Gram-negative bacilli isolated in LUTH during the period of this study was 22%. This rate is higher than that reported in developed countries[2] (12 -16%). This finding may be related to socio-economic factors, as this group of micro-organisms usually infect the less immuno-competent host.

The predominance of *P. aeroginosa* (55%) and *Acinetobacter species* (29.5%) is consistent with the finding of previous authors13.

Most of the *P. aeruginosa* strains were isolated from surgical wound infections and urinary specimens. *P. aeruginosa* is a well-recognised agent of nosocomial urinary tract and wound infections, as well as community-acquired wound infections even in immunocompetent patients[2,6]. All the non-fermenters isolated from eye and ear swabs were *P. aeruginosa*. This organism is a well established agent of otitis externa and eye infections which may follow minor or major trauma to the eye[17].

In view of the fact that non-fermenting Gram-negative bacilli are more often associated with nosocomial infections, it is not surprising that many of these organisms are multi-resistant to commonly used antimicrobial agents like ampicillin, co-trimoxazole and tetracycline[14].

It is therefore important for the clinician to note that in a recent review[18] *P. aeruginosa* was found to be usually susceptible to ceftazidime, ciprofloxacin and amikacin. However, resistance to beta-lactams like imipenem and ceftazidime may emerge during treatment.

Out of all the *Acinetobacter* species, *A. baumannii* was the most frequently isolated one in this study. This is consistent with findings from other parts of the world[9,15]. *Acinetobacter*spp were isolated most frequently in urinary specimens, wound swabs and pulmonary specimens. This is not suprising since they have been found to be associated with nosocomial wound infection[6], urinary tract infection and xhest infection. None was isolated from the CSF in this study although *Acinetobacter* spp have been reported to cause meningitis [3,19].

Acinetobacter spp are usually sensitive to amoxycillin-clavulanic acid, doxycycline, and co-trimoxazole. However, it is essential to perform antimicrobial susceptibility tests for each clinically significant strains in the *Acinetobacter* calcoaceticus-baumannii complex as multiple resistant strains have been reported.

Seventy percent of the non-fermenters were isolated from hospitalised patients. This pattern was expected since the organisms are known to primarily attack the debilitated host.

Non-fermenting Gram-negative bacilli should be sought as agents of infections in our routine microbiology Laboratories, as it is possible to identify them using simple conventional methods. Awareness of this group of micro-organisms and their sensitivity pattern is quite important because they cause nosocomial infections, and strains may be multiply – antibiotic resistant.

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