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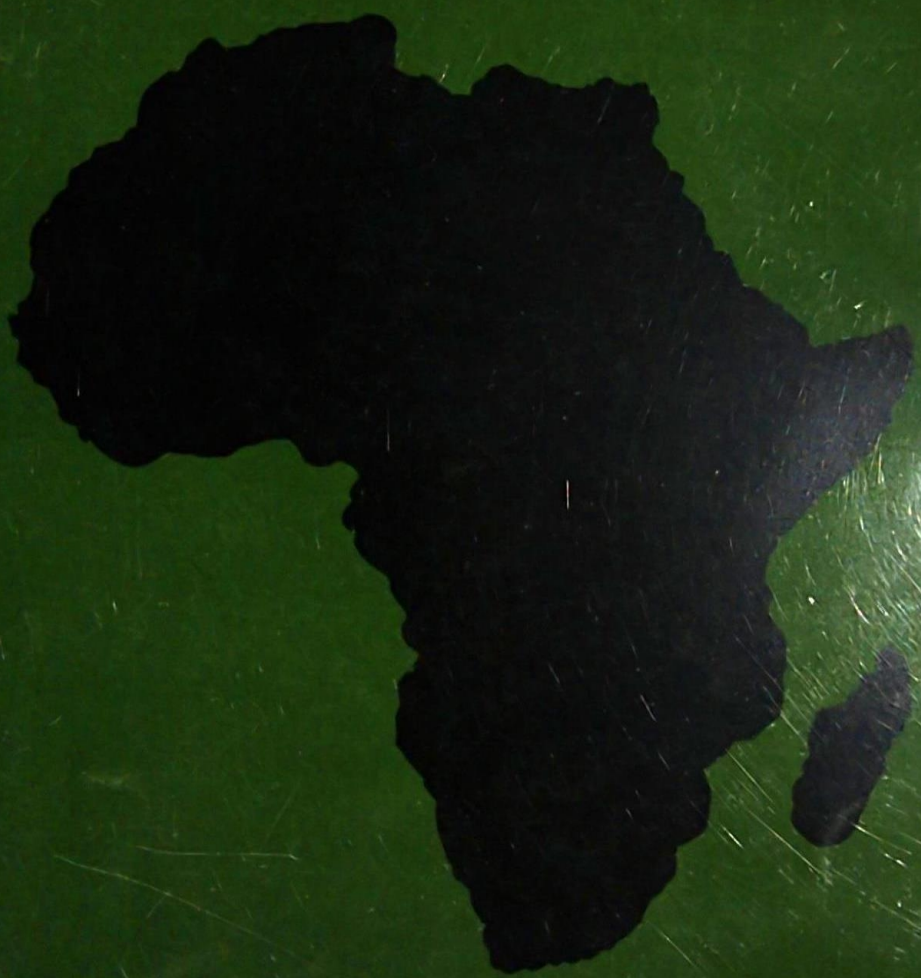

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Pattern of gram negative rods bacteraemia in diabetic patients in Ibadan, Nigeria.

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Summary
In a study of 100 patients in Ibadan between July and December 1995 to evaluate bacteraemia due to Gram-negative bacilli, 64% were culture positive, 44 (68.8%) of these yielded Gram-negative rods. The isolates were *Klebsiella* species (43.2%), *Escherichia coli* (27.3%), *Pseudomonas aeruginosa* (13.6%), *Proteus* species (11.4%) and *Bacteroides melaninogenious* (4.15%) by standard bacteriological methods. Antimicrobial sensitivity results suggested ofloxacin or ceftriaxone with metronidazole as empirical antibiotic therapy.

Keywords: *Bacteraemia, diabetics.*

Résumé
Dans une etude de 100 malades a Ibadan entre Juillet et Decembre 1995 pour eraluer le taux de bacteries cause par le Bacille de Negatif de Gram (Gram - Negative Bacilli), 64% de culture etaient positive, 44 (68.8%) de celle- ci ont ete des barres de Gram Negatif. Les especes isoles par les methodes de bacteriologie standards etaient: Klebsiella (43,2%), Escherichia coli (27,3%), Pseudomonas aeruginosa (13,6%), les especes proteus (11,4%) et les Bacteroides melaninogenicus (4,15%). Les resultats de sensitivite antimicrobiennes ont suggere ofoxacin ou cetriaxone avec metromidazole come antibiotique de traitement empirique.

Introduction

Diabetes mellitus is a constellation of abnormalities caused by a relative or absolute lack of insulin [1] It is a state of chronic hyperglycaemia [2]. A random venous plasma glucose of 11.1 mmol/L or more on two occasions or a fasting value of 6.7 mmolL or more on two occasions is diagnostic [2,3,4,5].

Bacteraemia is the transient presence of bacteria in the blood without causing symptoms [6]. In healthy humans, blood is usually sterile due to the presence of natural defense system which include phagocytes that engulf invading bacteria. Thus the presence and subsequent establishment of bacteria in the blood is indicative of infection. The presence of Gram negative rods (GNB) in the blood is referred to as Gram negative rod bacteraemia [7].

Diabetics are more susceptible to infection than non-diabetics [8] because of impairment of the immune response resulting from combined factors. These include increased glucose content of blood and tissue, impairment of chemotaxis, serum opsonization and phagocytosis and lowered capacity of tissue reaction to antigenic stimuli [9].

Bacteraemia usually starts from an infected focus. Numerous localized infections are often accompanied by transient phase of bacteraemia [10] Some common focus. Numerous localized infections are often accompanied by transient phase of bacteraemia [10] Some common infections in diabetics include urinary tract infection, gram negative pneumonia, malignant otitis externa, acute pyelonephritis, diabetic foot, tuberculosis and acute cholecystitis. Diabetic patients have increased incidence of GNB infection [11] and the indiscriminate use of antimicrobial agents [12] has been an important factor underlying the increased incidence [13] of GNB which are more resistant to antibiotics than Gram positive ones [14]. Escherichia coli, Aerobacter aerogenes, Proteus vulgaria, Pseudomonas aeruginosa, Klebsiella pneumoniae and *Haemophilus* species are frequent isolates of GNB bacteraemia [15]. Studies of patients with bacteraemia particularly GNB, have shown high mortality rate. This has been explained by the fact that GNB bacteraemia was associated with development of septic shock [16] and sepsis syndrome [17]. With the increased incidence of GNB infection in diabetic patients, the problem will be more enormous in them. Studies have also shown that the in-vitro sensitivity tests on blood culture isolates are good predictors of the effectiveness of treatment regimen [18]. We therefore set out to study the pattern of GNB bacteraemia in patients with diabetes mellitus in the University College Hospital, Ibadan, Nigeria, at a time when there are reports of changing pattern of the causative agents and antimicrobial sensitivity of some infections in this environment [12,19].

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Patients, materials and methods

During the study period, July to December 1995, 100 known Non-insulin dependent diabettes mellitus patients attending the Medical Outpatient Clinic, University College Hospital, Ibadan were recruited into the study. These were consecutive patients who gave verbal consent. Those who had signs and symptoms of infection were excluded. 64 patients without diabetes mellitus, signs and symptoms of infection were used as control. About 10 ml of venous blood was collected aseptically by venipuncture from each patient and 5 ml of each blood sample was immediately inoculated into:-

(i) diphasic blood culture medium (for isolation of aerobes) and
(ii) thio-glycollate broth medium (for isolation of anaerobes).

The inoculated diphasic medium and thio-glycollate broth medium were incubated in atmosphere-plus 5-10% carbon dioxide at 37 °C in a humidified incubator. Daily, the dipasic medium was subcultured on the solid phase by tilting the bottle to allow the blood-broth to flow over the surface of the agar slope, care being taken not to wet the cap. All the culture bottles were examined daily for visible bacterial growth on the agar slope of the diphasic medium and turbidity in the thio-glycollate broth. Colonies on the agar slope appearing small translucent and confluent were taken as positive growth. Gas bubbles, cloudiness or change in colour of the broth of both media after overnight incubation were suggestive of positive growth.

Cultures with signs of bacterial growth were subcultured onto two blood and MacConkey agar. One of the blood agar and MacConkey agar plates were incubated aero-

bically and the other blood agar plate incubated anaerobically using Gaspak system at 37 °C for 24 hours. Cultures showing no sign of growth were similarly sub-cultured on day 3, 5, and 7. Identification of the bacterial isolates was done according to standard bacteriological methods [2]. In-vitro antimicrobial sensitivity testing of the isolates was done by using agar diffusion method [20]. *Escherichia coli* NCTC 10418 was used as control.

Results

Of the 100 diabetic patients, 60 were females, while 40 were males. Of the 40 blood samples from the males, 26(65%) were culture positive, with 18 (69.2%) of these growing GNB. About 38(63.3%) of the female blood samples were culture positive, 26(68.4%) of which yielded GNB isolated from these diabetic patients are shown in Table 1. Table 2 shows the comparison of prevalence of GNB

Table 1: GNB isolated from blood of diabetic patients

GNB	Male No (%)		Female No (%)		Total No (%)	
Baceteroides melaninoge nicus	1	5.6	1	3.8	2	94.5
Escherichia coli	5	27.8	7	26.9	12	27.3
Klebsiella species	8	44.4	11	42.3	19	42.2
Proteus species	2	11.1	3	11.4	5	11.4
Pseudomanas aeruginosa	2	11.1	4	15.4	6	13.6
Total	18	100	26	100	44	100

in diabetic and control patients indicating significant association between diabetes mellitus and GNB bacteraemia. The prevalence of GNB bacteraemia in diabetic patients by age and sex is shown in Table 3. The frequency of GNB bacteraemia increased with the age of patients. The in-vitro antimicrobial susceptibility profile of the GNB is shown in Table 4.

Discussion

Positive blood culture is the only conclusive evidence of the existence of GNB bacteraemia. Bacteraemia is the most easily interpreted bacteriological finding in sepsis syndrome patients. Blood can be sampled easily and aseptically in every patient, any organism cultured from the blood has clearly invaded the host though the problem of contamination of specimen must always be remembered [18]. In this study, 64 out of the100 blood samples cultured were positive, out of which 44 yielded GNB representing 68.8% of the total positive cultures. This is similar to other reports [14,21]. GNB formed 69.2% of the total positive cultures in males and 68.4% of those in females. There is no significant difference between the sexes.

Table 2: Prevalence of GNB bacteriamia in diabetics

	GNB Bacteraemia	No GNB Bacteraemia	Total
Diabetes mellitus	44	56	100
Control (No diabetes mellitus)	2	62	64
Total	46	118	164

X2 = 32.49, P < 0.001

Table 3: Age and sex distribution of diabetic patients with gram negative rod bacteraemia.

Age range (years)	Male no. (%)		Female no. (%)		Total no. (%)
45 - 50	2	(11.1)1	(3.8) 3		(6.8)
51 - 55	1	(5.6) 2	(7.7) 3		(6.8)
56 -60	2	(11.1)2	(7.7) 4		(9.1)
61 - 65	3	(16.7)8	(30.8)11		(25.0)
66 - 70	6	(33.3)7	(26.9)13		(29.6)
>70	4	(22.2)6	(23.1)10		(22.7)
Total	18	(100)26	(100)44		(100)

In this study, 44 (44%) of 100 diabetic patients had GNB bacteraemia while 2 of the 64 non-diabetic control patients had bacteraemia. There is significant association between diabetes mellitus and GNB bacteraemia ($P < 0.001$). Aubertin *et al.* in 1982 [8] had reported increased bacteraemia in diabetics than in the non-diabetics. GNB bacteraemia is more prevalent in older diabetic patients than the younger ones. This can be due to the weaning immunological status associated with old age in addition to the immunosuppressive effect of diabetes.

While other workers reported Escherichia coli as the leading agent of GNB bacteraemia [7], we found *Klebsiella* species to be the most frequent agent (43.2%) in this environment. This was followed by Escherichia coli (27.3%), Pseudomonas aeruginosa (13.6%), *Proteus* species (11.4%) and Bacteroides melaninogenicus (4.5%). Some workers noted that in some cases more than one Gram negative organism may be isolated from the blood culture of diabetes mellitus [22,23], but in our study, only one organism was isolated from each of the patients with positive cultures. All our patients were out-patient. GNB isolated from their blood are more likely to be community acquired. It is pertinent to note that the these agents of bacteraemia will eventually be the culprits in diabetics with septicaemia. This because Bacteraemia has been associated with the development of septic shock and sepsis syndrome [18].

There is a picture of multi-drug resistance to most of the commonly used antibiotics such as cotrimoxazole, amoxycilllin and augumentin and 50% of the strains of *Pseudomonas aeruginosa* demonstrated resistance to ofloxacin, a new quinolone, a situation which calls for caution with indiscriminate antibiotic therapy, a practice which is rampant in Nigeria [12,19]. However, a greater percentage of strains of *Klebsiella* species were sensitive to ofloxacin, ceftriaxone(Rocephine), amikacin and ceftazidime, while greater percentage of the strains of *Escherichia coli* were sensitive to ciprofloxacin (ciprotab), ofloxacin, amikacin and azithromycin. Amikacin is not readily available in the Nigerian market. There is evidence derived from clinical studies of the effect of antibiotics to support the idea that bacterial invasion is harmful. Since the demonstration that early antibiotic treatment led to greatly reduced mortality in *Pseudomonas* bacteraemia, antibiotics have been used empirically for patients in whom bacteraemia is suspected [24]. It has also been documented that delay in introduction of appropriate antibiotics was associated with significant increase in hospital stay and with development of acute organ failure [18]. Hence we will suggest a combination of the quinolone (ofloxacin or ciprofloxacin) or ceftriaxone and metronidazole as empirical treatment in diabetic patients with features of septicaemia pending the arrival of the reports of microbio-

Table 4: In-vitro antimicrobial disc susceptiblity of the GNB

Antimicrobial agents	Sensitivity	B.melan-inogenicus	E. coli	Klebsiella spp.	Proteus spp.	Pseudo .aeruginosa
Erythromycin	S (%)	2 (100)	0	-	-	- -
	R (%)	0	12 (100)	-	-	-
Cefuroxime	S (%)	-	-	6 (32)	4 (80)	2 (20)
	R (%)	-	-	13 (68)	1 (20)	4 (80)
Ceftazidime	S (%)	-	10 (83)	12 (63)	0	5 (83)
	R (%)	-	2 (17)	7 (37)	5 (100)	1 (17)
Augumentin	S (%)	2 (100)	7 (58)	5 (26)	4 (80)	1 (17)
	R (%)	0	5 (42)	14 (74)	1 (20)	5 (83)
Amoxycillin	S (%)	-	3 (25)	0	0	0
	R (%)	-	9 (75)	19 (100)	5 (100)	6 (100)
Ceftriaxone	S (%)	-	12 (100)	19 (100)	3 (60)	6 (100)
	R (%)	-	0	0	0	0
Azithromycin	S (%)	-	10 (83)	4 (21)	1 (20)	1 (17)
	R (%)	-	2 (17)	15 (79)	4 (80)	5 (83)
Ofloxacin	S (%)	-	12 (100)	18 (95)	5 (100)	3 (50)
	R (%)	-	0	1 (5)	0	3 (50)
Amikacin	S (%)	-	12 (100)	17 (89)	4 (80)	4 (67)
	R (%)	-	0	2 (11)	1 (20)	2 (33)
Ciprofloxacin	S (%)	-	9 (75)	17 (89)	4 (80)	2 (33)
	R (%)	-	3 (25)	2 (11)	1 (20)	4 (67)
Cotrimoxazole	S (%)	2 (100)	1 (8)	1 (5)	0	1 (17)
	R (%)	0	11 (92)	18 (95)	5 (100)	5 (83)
Genticin	S (%)	-	8 (67)	16 (84)	3 (60)	4 (67)
	R (%)	-	4 (33)	3 (16)	2 (40)	2 (33)

Key: S = Sensitive; R = Resistant.

logical investigations. In addition, a continuous education programme aimed at eradicating the practice of inappropriate usage of antibiotics should be directed at (i) the masses through the mass media, town-fora and religious gatherings, and (ii) the medical and paramedical personnel through seminars and scientific meetings. These measures will greatly reduce, if not totally eradicate, the menace of multi-drug resistance in Nigeria.

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