ISOLATION OF SPECIES OF *YERSINIA* FROM PATIENTS WITH GASTROENTERITIS IN NIGERIA

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SUMMARY. From patients in Nigeria with acute gastroenteritis, strains of *Yersinia* were isolated from 14 (1.3%) of 1082 specimens of faeces examined specifically for yersiniae by direct plating and after cold enrichment. Clinical significance was ascribed to six isolates of *Y. enterocolitica* (serotypes O3, O5,27 and O9) but not to seven isolates of *Y. intermedia* or one isolate of *Y. frederikseni*.

INTRODUCTION

There continues to be a world-wide increase in the number of reports of infection with *Yersinia enterocolitica* in man. The most common clinical presentation is gastrointestinal disturbance, with isolates of serogroups O3, O5,27, O8 and O9 most frequently implicated.

Rhamnose-fermenting (Rha⁺) and other biochemically atypical strains, previously called *Y. enterocolitica*-like, are usually isolated from environmental sources including water, milk and foods or from animals (Lassen, 1972; Brenner *et al.*, 1976; Chester *et al.*, 1977; Kapperud, 1977; Schiemann and Toma, 1978). Recent studies of biochemical characteristics and DNA-relatedness have indicated, however, the existence of at least four species: *Y. enterocolitica sensu stricto* (Bercovier *et al.*, 1980a); *Y. frederikseni*—rhamnose-positive (Rha⁺) strains (Ursing *et al.*, 1980); *Y. intermedia*—rhamnose-, melibiose- and raffinose-positive (Rha⁺, Mel⁺, Raf⁺) strains (Brenner *et al.*, 1980); and *Y. kristensenii*—sucrose-negative (Suc⁻) strains (Bercovier *et al.*, 1980b).

Many of the reports of the isolation of yersiniae from man have come from laboratories in which these organisms were sought specifically. In other countries in which detailed studies have not been made, little is known of the distribution of yersiniae in man and the environment. For example, there are only two short reports of the isolation of *Y. enterocolitica* (one strain) or *Y. enterocolitica*-like organisms (two strains) from patients with gastroenteritis in Nigeria (Anjorin *et al.*, 1979; Agbonlahor, Odugbemi and Lasi, 1981). The aims of the present study, therefore, were to determine the prevalence and assess the clinical significance of *Yersinia* species isolated from patients with gastrointestinal disturbances in Nigeria.

MATERIALS AND METHODS

Antisera. Diagnostic antisera against O determinants O1, O2a, O2b, O3, O5, O5,27, O8 and O9 of *Y. enterocolitica* were kindly donated by Professor G. Wauters, Cliniques Universitaires St Luc, Brussels, and Professor S. Winblad, Institute of Clinical Bacteriology, Malmo, Sweden.

The survey. A total of 1082 specimens of faeces from patients with acute gastroenteritis was examined specifically for *Y. enterocolitica* at the Lagos University Teaching Hospital. Faeces were spread on plates of MacConkey Agar (Oxoid) and Salmonella-Shigella Agar (Oxoid) that were incubated at 23°C for 48 h. For cold enrichment, a loopful of faeces was inoculated into phosphate-buffered saline, pH 7.3, (Oxoid) and incubated at 4°C for 3 weeks with subcultures made at 2, 7, 14 and 21 days on to plates of MacConkey Agar and Salmonella-Shigella Agar that were incubated at 23°C for 48 h. Lactose non-fermenting colonies present on any selective agar

were tested for their reaction with Gram's stain, urease and phenylalanine deaminase activities, nitrate reduction and motility at 23°C and 37°C. The identity of cultures behaving biochemically like *Yersinia* was confirmed and they were assigned to species by their reactions in tests for fermentation of melibiose, raffinose, rhamnose and sucrose and for citrate utilisation (Bercovier *et al.*, 1980a). Isolates of *Y. enterocolitica* were serotyped.

Antimicrobial susceptibility tests were performed by standard techniques with the Multodisk No. 7402E (Oxoid) containing the following antimicrobial agents: ampicillin (2 µg); cephaloridine (5 µg); chloramphenicol (10 µg); gentamicin (10 µg); streptomycin (10 µg); sulphafurazole (100 µg); sulphatriad (300 µg); and tetracycline (10 µg). Susceptibilities of cultures were also tested with individual disks containing amoxycillin (20 µg) and augmentin (30 µg; i.e., amoxycillin 20 µg and clavulanic acid 10 µg) kindly donated by Beecham Research Laboratories, Great West Road, Brentford, Middlesex TW8 9BD. The inoculum was a 1000-fold dilution of an 18-h culture of the test strain grown in Mueller-Hinton Broth (Oxoid) at 23°C. The zones of inhibition obtained with each strain with different antibiotics were compared with those given by a culture of *Escherichia coli* strain NCTC10418.

β-Lactamase activity was assessed by a starch paper technique (Odugbemi, Hafiz and McEntegart, 1977).

Clinical evaluation. We followed essentially the recommended criteria of Bottone (1978). Thus, the recovery on primary plating of a *Yersinia* strain from the faeces of patients with bloody or non-bloody diarrhoea, with either or both of abdominal pain and fever, in the absence of parasites or other bacterial pathogens such as *Salmonella*, *Shigella* or *Vibrio* species and enteropathogenic serotypes of *E. coli*, was considered clinically significant; viruses and *Campylobacter* species were not sought. The sera of patients were not tested for antibodies to *Yersinia*.

RESULTS AND DISCUSSION

Characters of *Yersinia* isolates

Yersinia species were isolated from only 14 of 1082 specimens of faeces examined in this survey. All 14 isolates gave positive results in tests for urease production, nitrate reduction and motility at 23°C and negative results in tests for motility at 37°C and phenylalanine deaminase production. In extensive tests with a wide range of substrates (unpublished data) the observed biochemical reactions suggested that each isolate behaved like *Y. enterocolitica* or was *Y. enterocolitica*-like (Bercovier *et al.*, 1980a). Six of the 14 isolates did not ferment rhamnose,

TABLE
Isolation of Yersinia species from patients with gastroenteritis in Nigeria

Patient no.	Sex	Age (years)	<i>Yersinia</i> sp. isolated	Isolation method	Serotype	Other pathogenic species isolated
1	F	16	<i>Y. enterocolitica</i>	DP	O3	none
2	F	30	<i>Y. enterocolitica</i>	DP	O3	none
3	M	1.5	<i>Y. enterocolitica</i>	DP	O3	none
4	M	10	<i>Y. enterocolitica</i>	DP	O3	none
5	F	<1	<i>Y. enterocolitica</i>	DP	O5,27	none
6	M	58	<i>Y. enterocolitica</i>	CE	O9	none
7	F	<1	<i>Y. intermedia</i>	CE	NT	<i>Entamoeba histolytica</i>
8	F	26	<i>Y. intermedia</i>	CE	NT	<i>E. histolytica</i>
9	F	34	<i>Y. intermedia</i>	DP	NT	none
10	M	2.5	<i>Y. intermedia</i>	CE	NT	ETEC
11	M	3	<i>Y. intermedia</i>	CE	NT	<i>Giardia intestinalis</i>
12	M	4	<i>Y. intermedia</i>	CE	NT	ETEC
13	M	33	<i>Y. intermedia</i>	CE	NT	<i>Trichuris trichiura</i>
14	M	<1	<i>Y. frederikseni</i>	CE	NT	<i>G. intestinalis</i>

DP=direct plating; CE=cold enrichment; NT=not serotypable with available antisera (see *Methods*); ETEC=serotypes of enterotoxigenic *Escherichia coli*.

melibiose or raffinose and did not utilise citrate and, hence, behaved like strains of *Y. enterocolitica sensu stricto*; these isolates were of O serotypes common in human infection (table). The other eight isolates fermented rhamnose (Rha⁺); none was serotypable with the available *Y. enterocolitica* O antisera. Seven of the eight Rha⁺ isolates also fermented melibiose and raffinose and utilised citrate and were, therefore, probably strains of *Y. intermedia* (Brenner *et al.*, 1980). The remaining Rha⁺ isolate was Mel⁻, Raf⁻ and did not utilise citrate; it appeared to be a strain of *Y. frederikseni* (Ursing *et al.*, 1980).

Each of the 14 isolates produced β -lactamase and was resistant to ampicillin, amoxycillin and cephaloridine but sensitive to augmentin. Most of the 14 isolates were also sensitive to chloramphenicol, gentamicin, streptomycin, sulphafurazole, sulphatriad and tetracycline.

Clinical significance

Five of the six cultures of *Y. enterocolitica* were of serotypes O3 or O5,27 and were isolated in significant numbers on primary plating independently of other intestinal pathogens (table); thus, these isolates were the probable causative agents of the gastrointestinal infections in patients 1 to 5. From patient 6, an isolate of *Y. enterocolitica* of serotype O9 was obtained, but only after cold enrichment, and it is difficult to ascribe to that isolate a definitive role in the patient's illness. However, in view of the recurrent nature of the illness in that patient, prolonged carriage of the strain cannot be excluded, and it is generally agreed that cold enrichment is desirable for the isolation of *Y. enterocolitica* from carriers who may excrete few organisms (van Noyen *et al.*, 1981).

On the other hand, isolation of *Y. intermedia* and *Y. frederikseni* from the faeces of seven patients was made only after cold enrichment, and other intestinal pathogens were present which might have accounted for their illness (table). Thus, the association between these *Yersinia* isolates and the gastrointestinal illnesses of these seven patients would seem doubtful (van Noyen *et al.*, 1981). The strain of *Y. intermedia* from patient 9, however, was isolated after direct plating of the faeces. Although it might be tempting to ascribe a pathogenic role to it on the basis of its presence in significant numbers in the faeces of that patient, it must be noted that the presence of other potential pathogens such as viruses and *Campylobacter* species was not excluded.

Further work, including studies of healthy populations, will be required to increase our understanding of the distribution, epidemiology and pathogenicity of species of *Yersinia* in Nigeria and other countries.

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