ISOLATION OF *FUSOBACTERIUM NECROPHORUM* FROM CANCRUM ORIS (NOMA)

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Abstract. A study of the predominant microflora in active sites of noma (cancrum oris) lesions was carried out in eight noma patients 3–15 years of age in Sokoto State in northwestern Nigeria. Paper point sampling and conventional anaerobic microbiological techniques were used. *Fusobacterium necrophorum* was recovered from 87.5% of the noma lesions. Oral microorganisms included *Prevotella intermedia*, alpha-hemolytic streptococci, and *Actinomyces* spp. which were isolated from 75.0%, 50.0%, and 37.5% of the patients, respectively. *Peptostreptococcus micros*, *Veillonella parvula*, *Staphylococcus aureus*, and *Pseudomonas* spp. were each recovered from one lesion. The *F. necrophorum* and *P. intermedia* isolates were tested for antibiotic sensitivity to clindamycin, tetracycline, metronidazole, and penicillin using the E-test, and all strains were observed to be sensitive to all of the antibiotics tested with the exception of one strain of *P. intermedia*, which showed resistance to penicillin. The first reported isolation from human noma lesions of *F. necrophorum*, a pathogen primarily associated with animal diseases, may have important etiologic and animal transmission implications.

Noma (cancrum oris) is a destructive gangrenous stomatitis occurring mainly in children. It may lead to devastating facial deformity, circumferential scarring, stenosis of the mouth, and in many cases death. This disease occurs almost exclusively among poor malnourished children in developing countries.1–3

Although cancrum oris has been described by ancient medical writers such as Hippocrates and Galen and studied as a disease entity for more than 150 years, the etiologic agents of this disease have never been convincingly documented.4 As a result of the microscopic observations of smears from infected tissues, as well as the logical progression of acute necrotizing gingivitis (ANG) to noma, the disease has been associated with the presence of large numbers of fusiform bacilli and spirochetal organisms.5–7 Hicken and Eldredge8 reported the presence of *Borrelia vincenti*, non-hemolytic streptococci, *Staphylococcus aureus*, diphtheroid bacilli, and gram-positive bacilli from the sloughing gangrenous area of the noma lesion. At the junction of the inflammatory and gangrenous areas they isolated *Bacillus fusiformis*, anaerobic microaerophilic non-hemolytic streptococci and anaerobic staphylococci. They suggested that the symbiotic association of *Bacillus fusiformis* with a nonhemolytic Streptococcus and *Staphylococcus aureus* was necessary to produce noma. Eckstein2 observed microscopically the presence of only *B. fusiformis* and *Borrelia vincenti* in healthy material just beyond the necrotic portion of acute noma lesions, and Emslie9 observed these organisms to be predominant in smears of acute cases of cancrum oris but also reported the presence of other organisms. MacDonald,9 using an infection model in guinea pigs, suggested that *Bacteroides melaninogenicus* might be an important associated microorganism in mixed infections of mucous membranes. *Bacteroides (melaninogenicus)*, *Bacillus fusiformis*, and *Borrelia vincenti* were observed in all acute noma lesions studied in a series of patients.10

Although based primarily on microscopic observations with limited cultural identification, the studies above suggest that the fusiform bacilli (*Bacillus or Fusiformis fusiformis*) and black-pigmented bacteria (*Bacteroides melaninogenicus*) observed in noma lesions are most likely members of the family *Bacteroidaceae*. Major taxonomic changes at both the generic and species level of the family *Bacteroidaceae* within the last decade have allowed better identification of specific members,11,12 and modern anaerobic microbiological techniques, which can be used in field studies, suggested means to better determine the microorganisms involved in noma. Studies were undertaken to isolate the predominant microflora and in particular members of the family *Bacteroidaceae* from noma lesions of children at the Outpatients Clinic of the Specialist Hospital in Sokoto in northwestern Nigeria.

SUBJECTS, MATERIALS, AND METHODS

This research was performed at the Specialist Hospital in Sokoto State in northwestern Nigeria. Growth and identification of the microbial isolates were performed in the microbiology laboratories of the Nigerian Medical Research Institute (Lagos, Nigeria) and the Department of Oral and Craniofacial Biological Sciences at the University of Maryland Dental School (Baltimore, MD).

This study was carried out with the approval of the Institutional Review Board of the University of Maryland School of Medicine, as well as that of the Ministries of Health and Education in Sokoto State in Nigeria. Informed consent was obtained from the children’s parents or guardians, and from the local village chiefs where necessary.

Subjects. Cancrum oris lesions from eight patients were cultured for anaerobic microorganisms. All patients presented with lesions that had been present for six weeks to two years (Figure 1). The individuals were 3–15 years of age and malnourished. Classification of the study subjects as malnourished was based on an abbreviated dietary history from the parents and anthropometric assessment based on weight-for-height (wasting) as an indicator of the present state of nutrition and on height-for-age (stunting) as an indicator of past nutrition.13 Several of the impoverished children exhibited varying degrees of edema of the extremities, skin lesions, hair changes, apathy, and hepatomegaly, usually seen in protein-energy malnutrition of the marasmic-kwashiorkor type.2,11 They all resided in northwestern Nigeria within a 2-hr drive of Sokoto City, the administrative capital of Sokoto State. The lesions were cultured and pa-
Patients were treated with antibiotics (metronidazole and penicillin G) at the Specialist Hospital in Sokoto.

**Sampling and culture procedures.** After isolation with cotton rolls to prevent salivary contamination, sterile endodontic paper points were intraorally inserted directly into active sites of noma lesions either between the tooth surface and gingival tissue or at the advancing margin of tissue damage and then placed into small, capped plastic tubes containing 1 ml of pre-reduced transfer fluid. Within 1 hr, the vials containing the samples were shaken to disperse the microorganisms from the surface of the points or swabs and immediately triple-streaked onto pre-reduced *Brucella* blood agar supplemented with hemin (0.05%) and menadione (0.1%) (BBHK) (Anaerobe Systems, Inc., San Jose, CA) or onto pre-reduced selective fusobacteria agar (Anaerobe Systems, Inc.). The streaked plates were immediately placed into a BBL GasPak Pouch (BBL, Becton Dickinson Microbiology Systems, Cockeysville, MD) and incubated at 37°C for 4–5 days. After incubation, the bags were opened one at a time and isolates were presumptively identified by colonial morphology and gram staining. The predominant colonies (taken from the third streak) on the BBHK plates were subcultured onto BBHK agar and the isolates on the fusobacteria selective plate were subcultured onto BBHK agar and fusobacteria selective agar. After incubation anaerobically in an anaerobic chamber (10% H₂, 5% CO₂, and 85% N₂) (Coy Laboratory Products, Ann Arbor, MI) for 4–5 days at 37°C, organisms were presumptively identified by colonial morphology, gram stain and phase contrast microscopy, and the air tolerance test. The anaerobic isolates were then identified using the AN-IDENT identification system (BioMerieux Vitek, Inc., Hazelwood, MO). The facultative isolates were identified following the routine procedures suggested in the *Clinical Microbiology Procedures Handbook*. The *Streptococcus* isolates were biochemically identified using the API-20S (BioMerieux Vitek, Inc.).

*Fusobacterium necrophorum* and *Prevotella intermedia* isolates from cancrum oris lesions were tested for antibiotic susceptibility with four different antibiotics using E-Test strips (AB Biodisk, Culver City, CA). An inoculum was grown in brain heart infusion broth supplemented with hemin and menadione (BHIHK) for 24–48 hr, and the turbidity was adjusted to a #1 McFarland standard. A 200-μl volume of broth culture was applied to pre-reduced BHIHK agar. The application of E-test strips to the plates followed the manufacturer’s instructions. Minimum inhibitory concentration levels were determined to be sensitive or resistant based on the National Committee for Clinical Laboratory Standards recommendations.

**RESULTS**

The predominant microorganisms isolated from the eight lesions are listed in Table 1. *Fusobacterium necrophorum* and *P. intermedia* were isolated from seven and six of the eight lesions, respectively. When *F. necrophorum* was isolated from the lesions, *P. intermedia* was also isolated from the same lesion in 71.4% of the cases. Alpha-hemolytic streptococci were isolated from six of the eight lesions and *Actinomyces* spp. were isolated from three of the eight lesions. *Staphylococcus aureus, Veillonella parvula, Peptostreptococcus micros,* and *Pseudomonas* spp. were each iso-
TABLE 1
Recovery of predominant microorganisms from cancrum oris (noma) lesions

<table>
<thead>
<tr>
<th>Predominant microorganisms</th>
<th>Hemolysis observed</th>
<th>Isolates/no. of patients sampled</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fusobacterium necrophorum</td>
<td>Beta 7/8</td>
<td></td>
</tr>
<tr>
<td>Prevotella intermedia</td>
<td>Beta 6/8</td>
<td></td>
</tr>
<tr>
<td>Alpha-Streptococcus spp.</td>
<td>Alpha 4/8</td>
<td></td>
</tr>
<tr>
<td>Actinomyces spp.</td>
<td>None 3/8</td>
<td></td>
</tr>
<tr>
<td>Peptostreptococcus micros</td>
<td>None 1/8</td>
<td></td>
</tr>
<tr>
<td>Veillonella parvula</td>
<td>None 1/8</td>
<td></td>
</tr>
<tr>
<td>Pseudomonas spp.</td>
<td>None 1/8</td>
<td></td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>Beta 1/8</td>
<td></td>
</tr>
</tbody>
</table>

The predominant microorganisms were subcultured in a microbiology laboratory of the Nigerian Medical Research Institute in Lagos, Nigeria. The subcultures after incubation for four days were then brought under anaerobic conditions within 24 hr to the University of Maryland Dental School for identification of the microorganisms. The isolation of many black-pigmented bacteria indicated that good anaerobic conditions prevailed and this method for culturing anaerobes in the field appears ideal.

DISCUSSION

The present investigation represents an initial attempt to culture, using anaerobic methodology, the microorganisms present in cancrum oris lesions in patients from Sokoto, Nigeria, and the study limitations must be appreciated. Specimens from eight cancrum oris patients were cultured and although specimens were taken at active lesion sites, the study subjects presented with various degrees of oral tissue destruction representing noma of various durations and severity. The specimens were initially cultured on location using prereduced media and BBL GasPak pouches for anaerobiosis and incubated at the Specialist Hospital in Sokoto.

The first observation of the BBHK agar plates after triple streaking and incubation of the noma samples revealed four unusual observations when compared with cultures of samples of ANG and healthy sites from age-matched children, which had been performed in Nigeria a few months before (Falkler WA and others, unpublished data). The first observation was the degree of hemolysis on the BBHK plates, which was not observed with the ANG cultures. The second was the high number of black-pigmented colonies on the plates, which although were present in the ANG cultures, were not as predominant as observed on the noma samples and were totally absent on the cultures from healthy sites. The third was that the majority of the culture plates, regardless of the active noma site cultured, looked almost identical.
with regard to the microorganisms present. The fourth was the presence on both the BBHK agar and the fusobacteriaselective medium of F. necrophorum, which has never before been observed in our laboratory after culturing oral samples for Fusobacterium species for more than 20 years.\textsuperscript{18-25} Another interesting observation and a major difference from the ANG samples, which we previously cultured in Nigeria, was the absence of F. nucleatum colonies and its replacement with F. necrophorum.

Actinomyces. Veillonella, and alpha-streptococci are normal oral flora and the majority of the other microorganisms (staphylococci, pseudomonads) isolated from one or a few cases had been previously reported associated with noma lesions\textsuperscript{6} and may represent secondary invaders. The two main organisms isolated were P. intermedia and F. necrophorum. The association and virulence of F. necrophorum with necrobacillosis in wallabies\textsuperscript{26} and the similarity of this phorum. The association and virulence of F. necrophorum from human noma lesions.

The type of disease associated with animals is typified by necrosis of the tissues involved, abscess formation, and usually a characteristic putrid odor. Bacteremia is present. It is a commensal in the gut of herbivores and infection arises from fecal contamination of damaged mucous membranes or skin.\textsuperscript{34}

There are three animal biovars of F. necrophorum. Type A, which will agglutinate chicken, human, and pigeon erythrocytes and is highly virulent, causing bovine hepatic abscesses\textsuperscript{35}, Type AB, which is associated with bovine and ovine foot abscesses, and Type B, which is least virulent and can be isolated from the rumen of animals and is also found within lesions caused by the A or AB biotypes.\textsuperscript{35} There are no convenient methods for distinguishing between isolates within the three biotype categories. Biotype A has been called F. necrophorum subsp. necrophorum and biotype B F. necrophorum subsp. funduliforme.\textsuperscript{36} Initial work trying to determine if human strains will fit into current animal biovar designations suggests that strains isolated from humans may not follow the animal biovar designation.\textsuperscript{37}

Although not frequently isolated, infections in humans involving F. necrophorum include necrotic tonsillitis with post-anginal septicemia (Lemierre’s syndrome).\textsuperscript{38-41} Oral and dental infections, brain abscesses, empyema, hepatic and intra-abdominal abscesses, and endocarditis,\textsuperscript{42-44} and mastoiditis, meningitis, and venous sinus thrombosis.\textsuperscript{45}

Whether P. intermedia was the black-pigmented member of the organisms considered as Bacteroides melaninigenicus in earlier work associated with noma lesions is not clear.\textsuperscript{9,10} Prevotella intermedia is a gram-negative anaerobic cocci-
sult in a minimal infectious dose) via animal-fecal contamination caused by shared living quarters with animals, food contamination, finger feeding, or water source as animals share drinking vessels with the impoverished children.77

Once establishing itself at a damaged mucosal site, possibly with the aid of an adhesin or hemagglutinin,73 F. necrophorum could trigger the infectious process as the sole pathogen or as a result of a polymicrobial infection. Fusobacterium necrophorum has been shown to display a classical endotoxin,74,75 a dermonecrotic toxin,76 a cytoplasmic toxin,77 and a hemolysin,78,79 all of which will result in destruction of tissues and the production of a low oxidation-reduction potential. These conditions as well as essential growth factors from damaged tissues (i.e., hemin from hemolyzed red blood cells) allow for multiplication of F. necrophorum, other anaerobes, and facultative anaerobes. Of special interest was our observation of the increased number of P. intermedia associated with the noma lesion. It has been demonstrated that F. necrophorum produces a growth-stimulating factor for P. intermedia.80

The invasive-destruction period could result from a breach in the host immune response compounded by the F. necrophorum toxins previously mentioned and the other virulence factors of the secondary invader microorganisms. The rapidity of lesion development and the discoloration of the tissue that supercedes the sloughing of the tissue suggest initial destruction via a necrotizing toxin or tissue destroying enzyme. Besides the classical endotoxin,74,75 a cytoplasmic toxin,77 and dermonecrotic toxin,76 F. necrophorum also produces substances destructive to tissues such as volatile sulfur compounds and proteolytic enzymes such as phosphatase B.81–83 The rapid progression of the lesion may be due to F. necrophorum producing a leukotoxin82,83,84–88 that is active against a variety of white blood cells, especially polymorphonuclear neutrophils, which would be the first cells at the site of infection. Prevotella intermedia could add to tissue destruction with its ability to degrade lipid materials59 and the production of proteolytic enzymes such as dipeptidyl peptidases and cysteine proteases, with the latter also involved in the breakdown of IgG.89–91 The degradation of immunoglobulin molecules would limit opsonic activity and complement-mediated lysis of these gram-negative microorganisms.

The role of spirochetes in this disease process is not understood at present and was not investigated in this study. These organisms are present in the noma lesion1,4,7,8,92 and have been observed by dark-field microscopy of material obtained from lesions in this study (Falkler WA and others, unpublished data). Spirochetes may participate in the invasion process since spirochetes were previously observed with fusiform bacilli in samples taken directly from healthy tissue at the advancing edge of active noma lesions.7 The potential role of spirochetes in the disease process merits investigation.

There are many questions to be answered regarding the balance between the immune response and advancing noma lesions. The observation of some patients with damage, scarring, and fibrosis following noma suggests that there may be a limitation of lesion development. Whether this is due to a positive shift in immunity as a result of an increase in nutritional state or some immune recovery following elimina-


62. Matteo J, Saarela M, von Troil-Linden B, von Troil-Linden B,


