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Research Article

A meta-analysis of NOMA microbiome and the microbiological understanding of this infectious orofacial disease

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ABSTRACT
Background: Cancrum Oris, also called NOMA, also called Cancrum Oris, is a destructive disease that affects the oro-facial region, especially in malnourished children from the Mediterranean and Central African regions. If left untreated, it poses a 90% chance of causing death and survivors are often left disfigured and socially stigmatized. The exact cause is unknown, but it is thought to be caused by poor oral health and a poor host immune response, made worse by malnutrition and previous infections. This study purpose is to gather and examine the current knowledge on the microbes related to NOMA.
Methods: The search methodology followed the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines. Cochrane style MeSH terms, including NOMA, Cancrum Oris, Microarray, 16S rDNA and sequencing were used. A modified quality assessment tool called Strengthening the REporting of Genetic Association studies (STREGA) was used to assess paper quality. Publication must include reports on measure of genetic association to be eligible for meta-analysis.
Results: The search returned 143 entries, of which four publications were selected for full text review after removing publications not relevant to this study. The 4 studies reviewed were all case-control studies with 123 cases of acute NOMA, and 378 healthy controls. The studies used 16srRNA sequencing and microarrays for phenotype detection. *Prevotella*, *veillonella*, *peptostreptococcus*, and *streptococcae Spp.* were identified as potentially implicated in NOMA.
Conclusions: There exist some research gaps in summarizing NOMA candidate microbes therefore, the authors suggest a multi-omic approach to providing data to help with the understanding of NOMA.

INTRODUCTION

NOMA, also known as Cancrum Oris is a progressively destructive necrotizing disease that typically affects the oro-facial region in majorly young, malnourished children especially in the Mediterranean and Central African regions like Nigeria and Niger (Figures 2 and 3). A quasi-link between NOMA and HIV-endemic areas has been suggested to be an important co-factor in regions like Latin America, Asia, South Africa, and parts of Central and West Africa. When left untreated, it poses a 90% mortality rate that is associated with sepsis and pneumonia, which suggests an impaired anti-pathogen immune response, and survivors are left disfigured and socially stigmatized. On prevalence, the last WHO global incidence survey in 1998, recorded 770,000 affected cases with 140,000 new cases annually. While its aetiology remains ill defined, a complex interplay between dentogingival polybacteria originating from poor oral hygiene, and poor host immune response that is worsened by malnutrition and prior infections i.e., malaria, measles, tuberculosis and possibly oral fungal infection like Oral Candidiasis, have been suggested. Precisely, potential candidate microbes like fusobacterium necrophorum and prevotella intermedia have been implicated, but their exact role in NOMA pathogenesis remains unclear. Today's NOMA's risk factors amongst malnourished children are not all known, but malnutrition has an acknowledged NOMA association. Reports of approximately 20 million children of <5 years worldwide being severely malnourished and NOMA's high mortality rate, it makes the awareness of this disease all the more significant. Another suggested NOMA pathological precursor is acute necrotizing gingivitis (ANG) but only a small number of cases are reported to make this transition. The role of malnutrition and microbes in NOMA is further focalized by changes attributed to immune responses especially with reports of the complex interplay between the malnutrition and reduced immune response to which very little is still known. This dearth in knowledge is due in part, to poor data generation from contemporary or DNA based taxonomy techniques. However, it is unclear how many of such contemporary studies has been undertaken or if a catalogue or consensus on candidate microbes associated to NOMA exists.

The aim of this study is to review and catalogue the current evidence on contemporary data of microbes implicated in NOMA. The objective is to further appraise research gaps on proposed candidate microbes and recommend future studies.

METHODOLOGY

Search Strategy

This search methodology was modelled after the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines (Figure 1). Cochrane style MeSH terms and keywords comprising; NOMA, Cancrum Oris, Microarray, 16S rDNA sequencing and Metagenomics, were used for the initial search (Table 1) using the search tools; Pubmed [1950-2022], Ovid Medline [1946 - 2022] and Web of science [1900-2022]. English only publications that interrogated microbe taxonomy with contemporary techniques, were selected. Additional publications were sort from the reference lists of any selected articles from the initial search.

Eligibility criteria

Titles and abstracts of selected publications were proofread to identify publications suitable for this review. Studies considered and articles retrieved were - cross sectional studies, case control studies and controlled clinical trials. The full texts of these retrieved articles were proofread to identify those suitable for study inclusion. Purely clinical or pathological papers, conference papers and abstract only articles were excluded.

Quality appraisal

To assess the quality of each study, a modified quality assessment tool (QAT) called Strengthening the Reporting of Genetic Association studies (STREGA), was used. In summary, the QAT-STREGA tool assessed the presence of case population characteristics, case and control screening, NOMA diagnosis, power calculation, measure of association i.e., odds ratio, Hardy-Weinberg equilibrium and any inclusion of National Centre for Biotechnology Information (NCBI) taxonomy ascension or ID number. The QAT-STREGA tool scrutinized publications using these 7 characteristics, and each allowed a score of 0 or 1, to add up to a maximum overall score of 7. A quality score of $\geq 4/7$ was deemed an acceptable study quality.

Data extraction

All results were extracted onto a data form for tabulation. The sample collection technique, experimental design, and the contemporariness of their respective taxonomic data generating techniques were also appraised. Detected and novel phenotypes were also evaluated and any phylotype present in $\geq 50\%$ of selected studies was adjudged suggestive of association to NOMA. SPSS 27 software package (IBM Company, Armonk, NY, USA) was used for statistical analysis. Descriptive statistical information of individuals with paired specimens in addition to sample sizes for case and control, case types, sequencing methods and phenotypic associations were captured.

Meta-analysis

To be eligible for meta-analysis, a publication must include reports on measure of genetic association i.e., risk odds ratio. Because of varying measure of association methods, and to remove this covariate, studies were further sub-divided into groups along measure tool.

RESULTS

The electronic search yielded 143 entries; comprising 123 publications from; PubMed - 120, Ovid Medline – 13 and Web of Science – 10. A total of 140 publications were removed for having no correlation to taxonomic data generated from contemporary techniques on NOMA samples, and this resulted in the selection of three publications for full text review. Following this review, an additional publication was selected from the reference texts, resulting in four publications being adjudged eligible for study inclusion (Figure 1). With one exception, all selected studies were case-control studies with confirmed ANG and acute NOMA cases i.e., diagnosis of ANG was made by the presence of pain, spontaneous bleeding and gingival ulceration, while acute NOMA was by visible exposure of intraoral bone or extraoral oedema and necrosis with less than 10 days onset (Figure 2 & 3). In addition, all three case-controlled studies were part of the Geneva Study Group on NOMA (GESNOMA), published between 2002 - 2018 with study location in Niger (See Table 1).

Table1: Selected Study Characteristics and Methodologyy

No	Study Type	Case Type	Location	Sample Case	Size Control	Sequencing Method	Phenotypic Association	Detected Phylotypes	Novel Phylotypes
1.	Controlled*	Acute	Niger	23	23	16S rRNA	Yes	339	81
2.	Controlled*	Acute	Niger	84	343	Microarray	Yes	82	15
3.	Cohort	Chronic	Nigeria	4	0	16S rDNA	Yes	67	25
4.	Controlled*	Acute	Niger	12	12	16S rRNA	Yes	NS	NS

Key: 1 Bolivar *et al*., 2012; 2 Huyghe *et al.,* 2013; 3 Paster *et al*., 2002; 4 Whiteson *et al*., 2014

*= No. of acute necrotizing gingivitis (ANG) being part of study (study 1 – 9, study 2 – 37 and study 4 - 12); NS = Not Stated

The four studies cumulatively included 123 cases of NOMA, 58 ANG and 378 healthy controls and, 488 phenotypes were detected of which 121 were never before described. All three GESNOMA studies utilized 16S rRNA sequencing while the fourth, non-GESNOMA study used high-density microarrays (Table 1). The sample collection protocol was undertaken with cotton rolls for lesion saliva isolation and paper points for subgingival lesion sampling. The remaining study characteristics are as captured in Table 1. The three GESNOMA publications had a QAT-STREGA score of ≥4 and additional details are shown in Table 2. *Prevotella spp*, *veillonella spp*, *peptostreptococcus* and *streptococcae spp.* were implicated in two or more studies (Table 3).

Meta-analysis

Only 2 studies reported a measure of association score, and both used different measurement methods, i.e., the phylogenies reconstruction by probability method and the indicator species analysis (Table 3). *Prevotella spp*, *veillonella spp*, *peptostreptococcus* and *streptococcae spp.* were implicated.

Table 2. Selected study Quality Assessment (QAT- STREGA)

No.	Power calculation	NOMA diagnosis	Screening of Controls	Case population characteristics	NCBI Ascension No.	Assessment of HWE	Data with risk ratios	Quality score
1.	No	Yes	Yes	Yes	Yes	No	Yes	5
2.	No	Yes	Yes	Yes	Yes	No	No	4
3.	No	Yes	No	Yes	Yes	No	No	3
4.	No	Yes	Yes	Yes	Yes	No	Yes	5

Key: 1 Bolivar *et al*., 2012; 2 Huyghe *et al.,* 2013; 3 Paster *et al*., 2002; 4 Whiteson *et al*., 2014
HWE = Hardy Weinberg equilibrium; NCBI = National Center for Biotechnology Information ascension numbers can be obtained from NCBI Taxonomy available at https://www.ncbi.nlm.nih.gov/taxonomy

Table 3. NOMA associated phylotypes by systematic review and meta-analysis.

No.	Publications	Detected phylotypes in 50% of NOMA samples	Detected Phylotypes by meta-analysis (measure of association)
1.	Bolivar et al. 2012	***Prevotella spp., Peptostreptococcus stomatis, Prevotella intermedia, Streptococcus pneumoniae, Prevotella intermedia strain C, Veillonella parvula***	***Peptostreptococcus spp., Prevotella spp.***
1.	Huyghe et al. 2013	***Peptostreptococcus spp., Prevotella spp.,*** *Nocardioidaceae spp.*	
2.	Paster et al. 2002	*Achrombacter xylosoxidans, Afipia genomospecies 8, Bacillus fusiformis, Brevundimonas diminuta, Leptothrix strain DhA-71, Ochrobactrum anthropi, Propionibacterium acnes, Rhizobium loti, Sphingomonas strain JSS-28, Staph. aureus, Staph. epidermidis, Stenotrophomonas maltophilia.*	
4.	Whiteson et al. 2014	*Coprobacillaceae sp., Prevotella intermedia^, Catonella^, Aggregatibacter sp.^, Peptostreptococcus spp, Porphyromonas endodontalis, Treponema (Spirochates), Seleomonas sp.^,* ***Prevotella sp. ^, Prevotella tannerae,*** *Porphyromonas,* ***Prevotella nigrescens, Veillonella dispar, Streptococcae sp.****, Bacteroidales sp., Neisseriaceae sp,*	*Coprobacillaceae,* ***Veillonella, Prevotella,*** *Treponema and* ***Peptostreptococcus***

Key: Taxonomies in bold were detected in 2 or more studies; ^ = Detected in unaffected site of NOMA case.

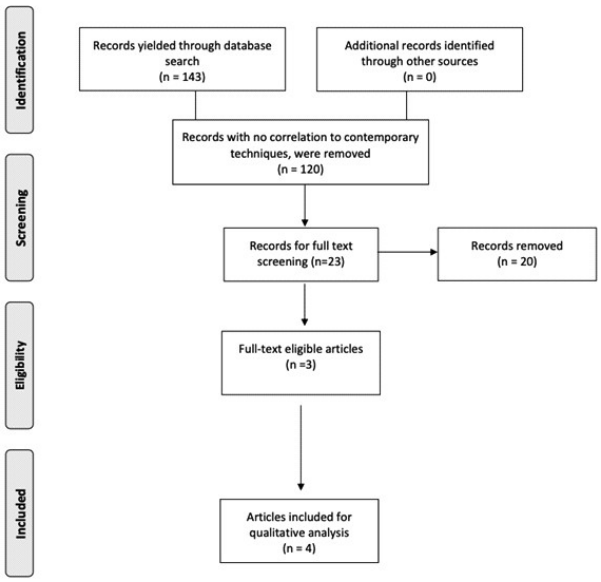

Figure 1: Prisma Flow Diagram

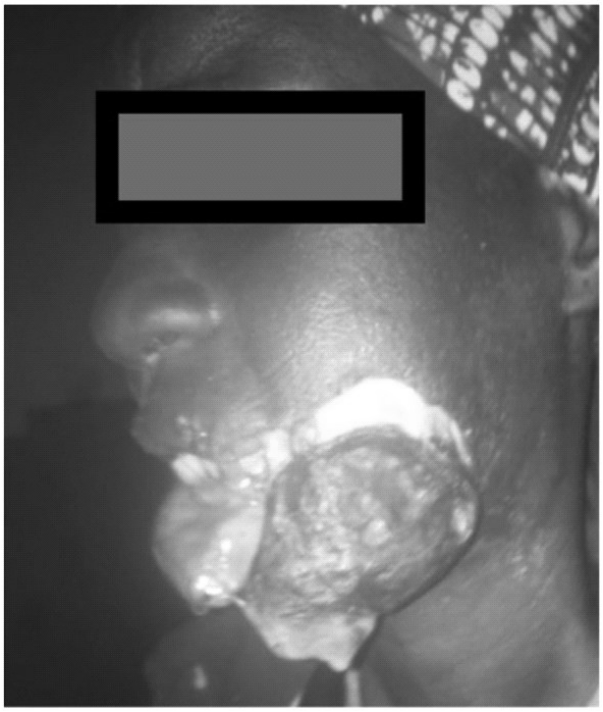

Figure 2: Gross extraoral oedema with myonecrosis at < 10 days onset

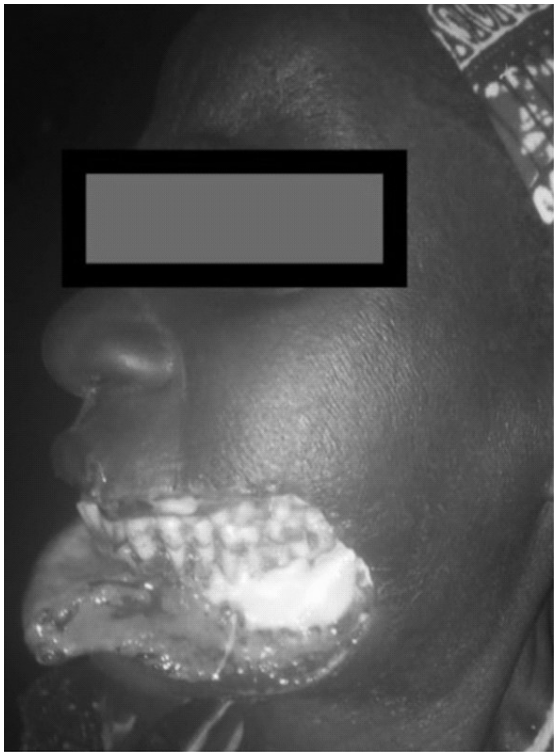

Figure 3: Exposure of intraoral bone

DISCUSSION

NOMA was originally classified along pathological phenotypes, i.e., ANG, oedema, gangrene, scarring and sequela. While NOMA is believed to begin as necrotising gingivitis and progress to necrotizing periodontitis and then to stomatitis, the term acute NOMA is applied only when necrotizing stomatitis aggressively progresses to myonecrosis and osteonecrosis that is typically recognizable as gross oedema with discolouration of overlying skin. The affected hard and soft tissue rapidly sloughs leaving the irregular defect, characteristic of the disease. There are reports that this destruction could be attributed to an immunopathological response to microbes rather than microbial action alone, i.e., possibly linked to deranged plasma immunoglobulins and components often associated with malnourished children. Increased research on NOMA is necessary to providing evidence-based policy for management and improving awareness considering that it is a consensus amongst clinicians that improved awareness would likely lead to the elevation of NOMA to a WHO neglected tropical diseases (NTD) status which would ultimately improve its awareness and lead to better prevention and control.

This review appraised research on the knowledge and taxanomic data on the prevalent microbe implicated in NOMA. A total of 4 published articles studying 123 cases, met the inclusion criteria, suggesting a dearth of contemporary research and data, on this disease. This dearth is due in part to the absence of research infrastructure and low prioritisation for contemporary microbiome profiling in affected regions like Africa. This is further buttressed by the observation that three of the four studies were GESNOMA project, a Switzerland based research group, while the fourth was a collaboration between a USA and a Nigerian Institution. Its worthy to note that the Paster study did use 16S rRNA, for chronic NOMA case types which suggests their report may reflect microbial conditions from activities, other than direct aetiology . Previous investigations have included microscopy and culture but this grossly underscored the variety of uncultivable organisms. In addition, because the disease develops rapidly in regions with very limited healthcare systems, data from acute phase diseases are limited and reports from advance stage of the disease may reflect flora conditions from environmental, habitual or lifestyle, rather than direct aetiology.

This review suggested *prevotella spp*, *veillonella spp*, *peptostreptococcus* and *streptococcae Spp.* as possible NOMA-trigger microbes with additional 121 novel phenotypes that requires further interrogation. While all techniques used were contemporary, all but micro-array contributed to these suggested NOMA trigger microbes. When compared to microbe microscopy and culturing, microarray is more advance however, prior knowledge of the targeted genomic features is necessary and there is a high possibility of cross hybridization between similar sequences.

These are in contrast to DNA sequencing that requires no prior knowledge of genome annotation, as its sequencing by synthesis approach, admittedly eliminates cross-hybridization. Furthermore, while previous studies have suggested fusobacterium necrophorum and prevotella intermedia as possible NOMA trigger microbes, this review appears to implicate only prevotella spp. and not fusobacterium spp. and implicitly three additional microbes in *veillonella spp, peptostreptococcus and streptococcae spp.* would be interesting organisms that should warrant further interrogation. While the reports were unclear on the taxonomy on the 121 novel phenotypes, metagenomics i.e., sequencing of undefined mixture of organism in an environment, might be a technique that may unravel these phenotypes. However, metagenomics alone may not provide all the answers, thus including additional omic tools like salivary metabolites of NOMA cases (metabolomics), may provide additional insights on the disease pathogenesis.

None of these studies reported a power calculation or a genotyping error, thus it was impossible to rule out underpowering of these studies which would have decreased genotyping error. To rule out underpowering, an effective sample size using an appropriate web browser program at a threshold of $\geq 80\%$ statistical power with suggested average disease prevalence, odd ratio, and case-control ratio of 1:2, would be essential. Besides, some of the association data reported were not statistically significant again probably due to an underpowered study. It is therefore imperative to appropriately power scale studies of these nature to assess risk or causal microbes amongst these vulnerable children group. Three out of the four studies were from the same GESNOMA study group with the same study location and samples. While this is convenient considering the affinity of the disease to focus on regions, it does not allow for the impact of geographical and environmental variation accrued from other affected regions.

In conclusion, translational research on Mediterranean and African-based population diseases is predicated on large scale multinational genetic studies and confirmation of actionable points that proffers better understanding of the risk factors, therapy and attaining relevant prevention and screening programs specific to the region. Clearly, there exists some research gaps in summarizing candidate microbes for NOMA, therefore, a multi-omic approach may provide data that may help with the understanding of NOMA pathogenesis.

Declaration

The authors declare no conflict of interest

Ethical approval: Adjudged none required.

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