was analysed using Nucleotide Basic Local Alignment Search Tool (BLASTN) to search for highly similar sequence alignments within the nucleotide collection database. e contig was entered into MacVector 12.5 sequence analysis so ware and open reading frames (ORFs) 150 nucleotides or greater 50 amino acids) were identi ed. e ORFs were translated using MacVector 12.5 and the protein sequences were entered into Protein BLAST (BLASTP) and used to search for non-redundant protein sequence alignments.

While we have not attempted to characterise these viral proteins, our preliminary database search showed that the majority of these sequences have high homology to viral (phage) proteins (Table 1). ese viral proteins were identi ed by setting up the selection search to the minimum size of the ORF's to be more than 50 amino acids in length. Generally, the majority of the identi ed phage related proteins seemed to be associated with an insertion into the Neisseria genome. A signi cant number of the identi ed phage proteins have strong association with Neisseria meningitides, which might suggest that the identi ed virus might be a speci c prophage for the bacteria Neisseria meningitidis. However, based on the fact that almost all of the rest of the identi ed proteins were associated with insertion into di erent Neisseria genome, we can safely speculate that the identi ed virus is a speci c phage for the Neisseria genus.

Furthermore, the preliminary sequence analyses have revealed a number of interesting observations including ndings of putative bacterial promoter sequences, which were identi ed at -35 (TTGACA) and -10 (TATAAT). Intriguingly, these sequences were identi ed for the vast majority (79%, 19/24) of the identi ed phage related genes (Figure 1). However, it is rather di cult to accurately identify the exact promoter sequence for the identi ed phage proteins without experimental validation. Hence, further analyses and characterisation of the identi ed phages are being undertaken on a high scale by our research group.

Discussion and Conclusion

ere is an ever constant increase in the reported cases of multiple antibiotic-resistant pathogenic bacteria [12], which has prompted many researchers to revisit an older antibacterial therapy that utilises bacteriophages. Multi drug resistant bacterial pathogens pose a major threat to human health as well as to the long term e cacy of commonly used antibiotics [13]. e last few years have seen a signi cant increase in the number of new bacteriophage research programs, encompassing di erent delivery routes, the most popular being oral and parenteral [5,11]. Research that focuses on the oral route of 'anti-bacterial' therapy revolves around the scenario of the potential isolation of speci c bacteriophages from the human oral cavity, and investigating the possibility of utilising these phages as potential antibacterial agents. Bacteriophages isolated from the human oral cavity will more likely be useful in the development of antibacterial therapies for antibiotics resistant oral pathogens. Also, most of the published reports on human oral lytic phage isolation have encountered and/or reported the formation of lysis zones [2].

Bachrach et al. (2003) [14] reported the isolation of a lytic bacteriophage which they speculate contributes to the ecosystem of the human oral cavity and also possibly to overall human health since the phage is ubiquitously associated with its bacterium host. Oral pathogens are therefore noted to be found both in healthy and diseased individuals [3]. us, the presence of phages in healthy individuals may, although does not necessarily prove, the theory that they, phages somehow contribute to the overall health of the oral ora. While a number of studies who reported the isolation of bacteriophages from healthy human individuals indicated somewhat the likelihood of phages contributing on maintaining the oral ora [1,11,15], many others have argued otherwise [16,17].

is study provides proof of concept that bacteriophages may be isolated from healthy human individuals and that this fact (isolation from healthy individuals), prompted us to speculate that the isolated phage is likely to play a role in the maintenance of the oral ora. is warrants further investigations into the promising utility of bacteriophages therapy as an antibacterial modality. Furthermore, the identi ed bacteriophage could be utilised or further developed into making speci c antibiotic treatments that could potentially target its host, Neisseria meningitidis, or the Neisseria species in general. Currently, we are further validating and characterising this isolated phage as well as determining the biological role that these phages might play in health and disease- which we believe can signi cantly improve the development of speci c bacteriophage therapy.

Acknowledgment

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Query       1   MAATLNLPINNSLGFAYIVPFQ------NRK--------EN-------V-------T---    29
ZP_05985602 64  MAATLNLPINNSLGFAYIVPFQ------NRK--------EN-------V-------T---    92
ZP_00135136 72  MAATLNLPLQNGLGFAYIVPYQ------NRK--------EK-------K-------T---   100
ZP_06066245 70  MAATLNLPINNNLGFAYIVPFK------NNK--------EN-------K-------I---    98
ZP_05992197 69  MAATLNLALQNGLGFAYIVPYQ------NRK--------EK-------K-------          T---    97
ZP_08250835 68  MAATLNLPLQNGLGFAYIVPFR------NNK--------EK-------K-------T---    96

Query       30  --EAQFQLGYKGFIQLAQRSGQFKRINACPVYD-TD-V--------E------------    64
ZP_05985602 93  --EAQFQLGYKGFIQLAQRSGQFKRINACPVYD-TD-A--------E------------   127
ZP_00135136 101 --EAQFQLGYKGLIQLAQRSGQFKRLVAVPVYE-KQLI--------A------------   136
ZP_06066245 99  --EAQFQLGYKGYIQLAQRSGQFSRIAATPVYD-GQLI--------S------------   134
ZP_05992197 98  --EAQFQLGYKGLIQLAQRSGQFKRLVAVPVYE-KQ-LL-------A------------   133
ZP_08250835 97  --EAQFQIGYKGFIQLAQRSGQFKRLVALPVYK-KQLI--------K------------   132

Query       65  -E----DVYQRLT-------S--L--------I--------P----R----K--P--S-G  81
ZP_05985602 128 -E----DVYQRLT-------S--L--------I--------P----R----K--P--S-G  144
ZP_00135136 137 -E----DPINGFE-------F--D--------W--------K----Q----K--P--A-K  153
ZP_06066245 135 -E----NPLLGYE-------F--D--------W--------S----V----K--P--N-G  151
ZP_05992197 134 -E----DPINGYE-------F--D--------W--------K----Q----K--P--A-K  150
ZP_08250835 133 -K----DFINGFE-------F--D--------W--------E----Q----E--PEQN-E  151

Query       82  ----Q----I-IGYIAYFQLLNGYEANLTMTMEELEAH--AKRYSQT-----Y-------  118
ZP_05985602 145 ----Q----I-IGYIAYF            QLLNGYEANLTMTMEELEAH--AKRYSQT-----Y-------  181
ZP_00135136 154 DE--K----P-IGYYAYFKLINEFTAELYMSTQDVYDH--AARYSQT-----Y-------  192
ZP_06066245 152 ----N----P-IGYVAFFKLINGFTAELYMSKEEVMKH--ANKYSQT-----A-------  188
ZP_05992197 151 DE--K----P-IGYYAYFKLINEFTAEIYMTTQEVHDH--ANRYSQT-----Y-------  189
ZP_08250835 152 ----N----P-IGYYAYFKLVNDFSAELYMSHDDK14(1)5219-----E--PEQN-E--Y-------  189
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References

1. Machuca P, Daille L, Vinés E, Berrocal L, Bittner M (2010) Isolation of a novel E D F W H U L R S K D J H V S H F L ¿ F I R U W K H S H U L R G R Q W D O S D W K R J H Q) X V R E D F W H U L X P Q X F O H D W X P Appl Environ Microbiol 76: 7243-7250.

2. + L W F K * 3 U D W W H Q - 7 D \ O R U 3 : , V R O D W L R Q R I E D F W H U L R S K D J H V I U R P W K H R U D cavity. Lett Appl Microbiol 39: 215-219.

3. Rylev M, Kilian M (2008) Prevalence and distribution of principal periodontal S D W K R J H Q V Z R U O G Z L G H - & O L Q 3 H U L R G R Q W R O

4. 3 D O P H U 5 - - U * R U G R Q 6 0 & L V D U - 2 . R O H Q E U D Q G H U J 3 (& D mediated interactions of streptococci and actinomyces detected in initial human G H Q W D O S O D T X H - % D F W H U L R O

5. Ryan EM, Gorman SP, Donnelly RF, Gilmore BF (2011) Recent advances in bacteriophage therapy: how delivery routes, formulation, concentration and W L P L Q J L Q À X H Q F H W K H V X F F H V V R I S K D J H W K H U D S 1204.

6. Delisle AL, Rostkowski CA (1993) Lytic Bacteriophages of Streptococcus mutans. Curr Microbiol 27: 163-167.

7. + R X V E \ - 1 0 D Q Q 1 + 3 K D J H W K H U D S \ ' U X J ' L V F

8. 7 D Q J . + < X V R I I . 7 D Q : 6 ' L V S O D \ R I K H S D W L W R Q E D F W H U L R S K D J H 7 D Q G L W V S R W H Q W L D O L Q J H Methods 159: 194-199.

9. + D X E H N ' : L O O L . 3 R X O V H Q . 0 H \ H U - . L O L D Q

bacteriophage Aa phi 23 correlates with the population genetic structure of $FWLQREDFLOOXV DFWLQRP\FHWHPFRPLWDQV (XU 2UDO 6FL

10. Hiroki H, Shiki A, Totsuka M, Nakamura O (1976) Isolation of bacteriophages VSHFL¿F IRU WKH JHQXV 9HLOORQHOOD $UFK 2UDO %LRO

11. $O -DUERX $1 *HQRPLF /LEUDU\ 6FUHHQLQJ IRU 9LUXVHV IURP WKH +XPDQ 'HQWDO 3ODTXH 5HYHDOHG 3DWKRJHQ 6SHFL¿F /\WLF 3KDJH 6HTXHQFHV &XUU Microbiol 64: 1-6.

12. Chanishvili N, Chanishvili T, Tediashvili M, Barrow PA (2001) Phages and their DSSOLFDWLRQ DJDLQVW GUXJ UHVLVWDQW EDFWHULD - &KHP 7HFKQRO %LRWHFKQRO 699.

13. Cars O, Högberg LD, Murray M, Nordberg O, Sivaraman S, *et al*. (2008) 0HHWLQJ WKH FKDOOHQJH RI DQWLPLFURELDO UHVLVWDQFH %0- D

14. Bachrach G, Leizerovici-Zigmond M, Zlotkin A, Naor R, Steinberg D (2003) Bacteriophage isolation from human saliva. Lett Appl Microbiol 36: 50-53.

15. &DR - 6XQ < %HUJOLQGK *et al.* (2000) Helicobacter pylori-