manufacturer's recommendations. Amplification and detection of HCV RNA were performed using the Cobas TaqMan 48 analyser (Roche). The final viral load was reported in international units (iu)/mL. Genotyping was performed using the HCV Genotyping ASR kit (Abbott Molecular Diagnostics, Abbott Park, IL, USA).

A 5 µL sample of the extracted RNA was added to 11 µL of each master mix. Amplification and detection were performed using an ABI 7900 real-time polymerase chain reaction (PCR) system (Applied Biosystems, Foster City, CA, USA). Data analysis was performed using the Celera sequence genotyping software (SGS v2.0).

The methodology described above identified the first Saudi blood donor who was seronegative but positive for HCV RNA. This is the first case out of 21,306 donors tested. The HCV genotype found in this patient was genotype 4, and the initial HCV viral load result obtained was 51,303 iu/mL. When repeated six weeks after the initial test, screening serology for HCV was positive and the viral load was 2,052,120 iu/mL. Clearly, more blood donors need to be tested in order to have a better idea about the prevalence of NAT-positive, seronegative donors. The HCV genotype 4 is consistent with the known HCV genotype in Saudis and is the predominant genotype in the Saudi population.[6]

Anti-HCV antibodies are usually detected three to 20 weeks after initial exposure to the virus, but this can be delayed from six to nine months in rare cases.[7,8] There are examples in the literature of blood donors who have remained viraemic for up to five years without developing anti-HCV antibodies.[9,10] This was attributed in part to the presence of subgenomic HCV in the plasma of infected patients as a result of the absence of immunological pressure and the presence of a high viral load.[11]

Immunosilent donors can be responsible for transfusion-related infections, although the introduction of NAT methodology in blood banks has achieved a reduction in the level of risk. However, even when NAT methods are negative, there remains a small risk of contracting HCV from blood donations.[12]

The introduction of NAT in the authors' hospital has reduced the incidence of transfusion-related HCV and HIV infections. Clearly, the introduction of this methodology in all blood banks in the region is recommended.

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Comparison of clustered, regularly interspaced short palindrome repeats (CRISPRs) in viridans streptococci (*Streptococcus gordonii*, *S. mutans*, *S. sanguinis*, *S. thermophilus*) and in *S. pneumoniae*

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Clustered, regularly interspaced short palindrome repeats (CRISPRs) have been described in several archaea and bacteria. An *in silico* analysis of pneumococcal and viridans group streptococci (VGS) demonstrated the presence of

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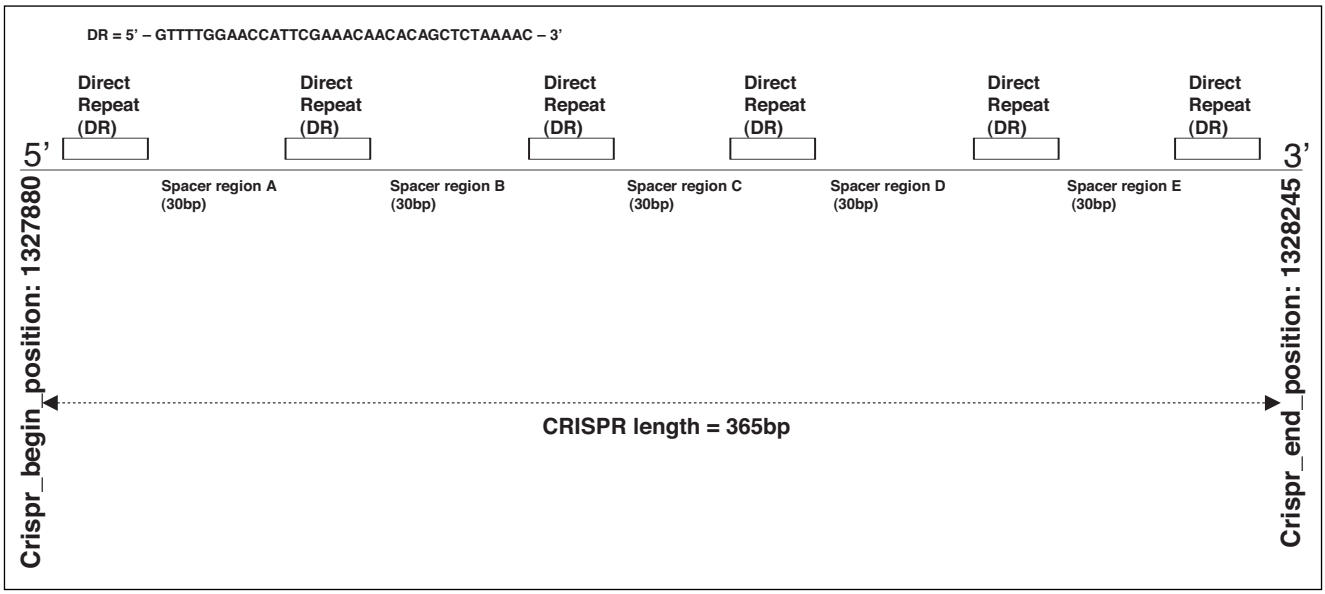

Fig 1. CRISPR arrangement in *Streptococcus mutans* UA159 showing a highly conserved direct repeat region (DR), interspaced by five highly variable spacer regions (A–E).

several confirmed and putative new CRISPR regions. The sharing of CRISPR sequence homology between the pneumococci suggests the presence of a unique signature-sequence diagnostic marker for potential aided identification and genotyping of the pneumococci.

Streptococcus pneumoniae is the most frequent cause of bacterial pneumonia across all age groups, as well as an important cause of meningitis and otitis media in children.[1] If only those cases serious enough for admission to an intensive care unit are considered, *S. pneumoniae* is still the most frequent pathogen and shows a mortality rate of 75%.[2] It causes between 500,000 and one million cases of pneumonia in the USA each year,[3] and an estimated one million deaths per year in children under the age of five years in developing countries.[2]

The annual financial burden to the National Health Service of managing 17.9 million episodes of lower respiratory tract infections has been estimated to cost approximately £1311 million (1992–03 prices). A study conducted in Nottingham from 1984 to 1985 evaluated 4027 patients in the community with lower respiratory tract infections treated with antibiotics. Thirty-six per cent of infections were attributable to *S. pneumoniae*. Combining these two studies, the estimated annual cost of treating pneumococcal lower respiratory tract infections in the UK would be £400 million.

In the USA, where pneumonia is the sixth leading cause of death, the estimated cost of treatment was $23 billion in 1994 ($14 billion in direct patient care costs and $9 billion in lost wages). The increased number of nursing homes residents, in parallel with the ageing of the population and an increased numbers of immunocompromised patients, will only serve to increase these costs.

Oral streptococci largely comprise members of the viridans streptococci, which currently encompasses 20 species, and are commensal inhabitants of the oropharyngeal cavity and the gastrointestinal and genital tracts in mammals.[4] On the basis of 16S ribosomal RNA (rRNA) sequence homology, these bacteria are categorised in four groups: the salivarius rRNA homology group (including *S. thermophilus, S. vestibularis* and *S. salivarius*), the mitis group (including *S. cristatus, S. gordonii, S. oralis, S. mitis, S. pneumoniae, S. sanguinis* and *S. parasanguinis*), the anginosus group (including *S. anginosus, S. constellatus* and *S. intermedius*) and the mutans group (including *S. mutans, S. criceti, S. downei, S. ferus, S. macacae, S. ratti* and *S. sobrinus*).

Recently, several CRISPRs have been described in the genomes of various bacteria and archaea. These repeats ranged in size from 23 to 47 bp, usually showed some dyad symmetry but may not have been be truly palindromic, and where separated by spacers of similar length.[5,6]

Horizontal gene transfer (HGT) may be an important mechanism for the exchange of essential genetic material between VGS in the mouth and upper respiratory tract and *S. pneumoniae*. Therefore, this study aims to determine whether or not these motifs exist in VGS and pneumococci, and, if so, whether or not the direct repeat sequences share homology.

Streptococcal whole and completed bacterial genomes were obtained from searching the online genome search engine (www.genomesonline.org), which revealed that completed genomes were available for three pneumococcal genomes and four viridans streptococcal genomes (including *S. gordonii, S. mutans, S. sanguinis* and *S. thermophilus)* (Table 1). Complete genomes were converted *in silico* from GenBank format to FASTA format commencing >gi|15902044|ref|NC_003098.1| *Streptococcus pneumoniae* R6, complete genome TTG AAA GAA AAA CAA TTT TGG… and examined in conjunction with the CRISPRfinder analysis software (http://crispr.u-psud.fr/Server/CRISPRfinder.php).

Resulting data were tabulated to identify confirmed or suspected CRISPRs in each genome examined, as well as CRISPR length, direct repeat consensus sequence and number of spacers identified (Table 1). Analysis demonstrated that all streptococci examined contained at least one suspected or confirmed CRISPR region, which varied from species to species up to a maximum of three suspected and three confirmed CRISPR regions in *S. thermophilus*.

Table 1. Description of CRISPR regions in *Streptococcus pneumoniae* and viridans group streptococci.

Organism	GenBank accession number	Size of genome (bp)	Number of CRISPRs	CRISPR start - CRISPR end positions
Viridans streptococci				
Streptococcus gordonii Challis CH1	NC_009785	2,196,662	1 confirmed 1 suspected	1422480–1424232 564850–564930
Streptococcus mutans UA159	NC_004350	2,030,921	1 confirmed	1327880–1328245
Streptococcus sanguinis SK36	NC_009009	2,388,435	2 confirmed 3 suspected	1277797–1279292 1279382–1280717 1280790–1280891 1326987–1327105 2097702–2097927
Streptococcus thermophilus LMD-9	NC_008532	1,856,368	3 confirmed 3 suspected	649125–650217 897070–897328 1377229–1377794 395404–395575 775648–775793 1602067–1602159
Pneumococci				
Streptococcus pneumoniae D39	NC_008533	2,046,115	3 suspected	96673–96768 1388016–1388137 1698114–1698220
Streptococcus pneumoniae R6	NC_003098	2,038,615	3 suspected	96673–96768 1380485–1380606 1690583–1690689
Streptococcus pneumoniae TIGR4	NC_003028	2,160,842	2 suspected	102124–102219 1715175–1715260

Subsequent BLAST analysis of the direct repeat sequences demonstrated that only one of these sequences was shared by other streptococci. This was a 95 bp putative CRISPR, which was shared by all pneumococci, where the 5′ end of the CRISP was adjacent to a transcriptional regulator belonging to the PadR family of proteins, and the 3′ end was adjacent to a transporter protein. In addition, a 121 bp putative CRISPR was shared by *S. pneumoniae* D39 and *S. pneumoniae* R6.

In the single confirmed CRISPR in *S. mutans* UA159, five CRISPR spacer regions are shown (Table 2 and Fig. 1), flanked by a highly conserved 36 bp direct repeat region (5′–GTT TTG GAA CCA TTC GAA ACA ACA CAG CTC TAA AAC–3′), all of which lay with a 365 bp CRISPR region (Fig. 1). Subsequent analysis of the direct repeat showed that this sequence was not unique to *S. mutans*, but was shared with *S. agalactiae* (AL766848), and showed 97% homology with *S. pneumoniae* (AE005672) (TIGR4).

The recent description of CRISPRs in the archaea and bacteria has caused much excitement in terms of microbial evolution. To date, 183 CRISPRs have been identified in 46 archaea genomes sequenced, and 594 CRISPRs have been located in the 535 bacterial genomes fully sequenced. To date, the function of CRISPRs is not entirely clear.

Table 2. BLAST analysis of five CRISPR spacer regions located in *Streptococcus mutans* UA159.

CRISPR spacer	CRISPR spacer sequence (5′–3′)	CRISPR spacer sequence position	CRISPR spacer length (bp)
A	TGTGGCAAGTCATGTCCGAATACATAGGCA	13279117–13279147	30
B	GTTTGTCTATTGTCTAATTGAAAATATTTA	1327982–1328012	30
C	CTAACTATGATGACACAACAGCTTTTAGCG	1328048–1328348	30
D	AGAGCACTAACTGCGCTAGCTGGTTCAATC	1328114–1328144	30
E	TCACCATATTAATTAATGGCGTTTCCTTTT	1328180–1328210	30

BLAST: basic local alignment search tool.

CRISPR length (bp)	Direct repeat consensus	Direct Repeat length (bp)	No. spacers
1752	GTTGTACAGTTACTTAAATCTTGAGAGTACAAAAAC	36	26
80	AAGAGGGTCCTTCTCTATCTGAAC	24	1
365	GTTTTGGAACCATTCGAAACAACACAGCTCTAAAAC	36	5
1495	GTTTCCGTCCCCTCCCGAGGTGACTGGGG	29	20
1335	GTTTCCGTCCCCTCCCGAGGTGACTGGGG	29	18
101	GTTTCCGTCCCCTCCCGAGGTGACTGGGG	29	1
118	AGCTCGAACTGCATCAATGGTCCTAAATTCAACT	34	1
225	TCAGTCTGCATCAATGCCTCTAAATTCGACTGCGCCCACGGCTTGTCTCATTAAG	55	1
1092	GTTTTTGTACTCTCAAGATTTAAGTAACTGTACAAC	36	16
258	GATATAAACCTAATTACCTCGAGAGGGGACGGAAAC	36	3
565	GTTTTGGAACCATTCGAAACAACACAGCTCTAAAAC	36	8
171	AGTTGTATTTCCACCAGTTGTATTT	25	3
145	CAACTGATGTAACCACTACAGATAATGAAGAGGAAACACTTGGCTCAGAA	50	1
92	CTTTTCTTTACGATATCAATTTTAACATCTTT	32	1
95	AATGTGTAAGATTTTTATATATAA	24	1
121	ACTTCTGGTGTCGGTACATTTGGTGTTGG	29	1
106	TTCAACCCACTACAGTTGACAAAGAGCCAAAAAA	34	1
95	AATGTGTAAGATTTTTATATATAA	24	1
121	ACTTCTGGTGTCGGTACATTTGGTGTTGG	29	1
106	TTCAACCCACTACAGTTGACAAAGAGCCAAAAAA	34	1
95	AATGTGTAAGATTTTTATATATAA	24	1
85	TTTTTTGAAACGTTTCATTTTTTT	24	1

The present CRISPR examination of the *S. mutans* genome demonstrates that spacers C and D share high homology with a streptococcal bacteriophage genome (M102) (see Table 2). This finding is consistent with recent work by Barrangou *et al.*,[5] who suggested that the presence of bacteriophage genetic material within the spacer regions in bacteria acts as a defence mechanism against phage predation of the bacteria. Indeed, pneumococcal bacteriophages have been described where they were first discovered in 1975 in Tomasz's laboratory. However, the present study did not identify any pneumococcal bacteriophage genetic material occupying spacer regions within the putative CRISPRs in *S. pneumoniae* R6, D39 or TIGR4, but the description of pneumococcal phage is interesting as a potential means of transduction of antibiotic resistance determinants within this species.

In conclusion, this *in silico* analysis of pneumococcal streptococci and VGS demonstrated the presence of several confirmed and putative new CRISPR regions. The sharing of CRISPR sequence homology between the pneumococci suggests the presence of a unique signature-sequence diagnostic marker for potential aided identification and genotyping of the pneumococci.

BLAST similarity index [GenBank accession number] (% similarity showing top two hits)
Shewanella baltica OS185, [CP000753] complete genome; (56%) *Shewanella baltica* OS155, [CP000563] complete genome; (56%)
Mus musculus chromosome 1, clone RP23–103C13, complete sequence; (77%) *Mus musculus* chromosome 1, clone RP23–242G7, complete sequence; (77%)
Streptococcus phage M102 ORF DNA [AM749121](100%) *Mus musculus* clone IgKVk9–135 immunoglobulin kappa-like gene [AY591744] (63%)
Streptococcus phage M102 ORF DNA [AM7479121] (67%) *Medicago truncatula* chromosome 7 BAC clone mth2–58i19 [AC186135] (57%)
Drosophila melanogaster chromosome X, complete sequence [AE014298] (63%) *Drosophila melanogaster* X BAC RP98–48G8 [AC104625] (63%)

This work was supported financially by a project grant from the HPSS (NI) Research & Development Office commissioned Research in Antimicrobial Resistance Action Plan (AMRAP) (COM/2730/04).

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