Skin microbiome of children with cystic fibrosis

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Abstract

Introduction Infection is the main source of morbidity and mortality in cystic fibrosis (CF). In recent years, understanding of infection in CF patients has increased dramatically due to the advent of next-generation sequencing technology. This has allowed a culture-independent approach to studying entire communities of bacteria, the microbiome, living within the CF lung, and thus avoiding bias of culture-based techniques. Microbiome studies have revealed associations between microbial community structures in the CF lung and clinical outcome measures of the patients, such as pulmonary function and exacerbation status. In addition, microbiome studies in CF have enabled identification of potential pathogens which are difficult to cultivate. Though the lung microbiota in CF is gaining recognition, the role of other bacterial communities in CF remains unchartered. This study aims to assess feasibility of isolating bacterial DNA from the skin, nare, and oropharynx of children with CF and provide pilot data for a study into the culture-based and culture-independent microbiomes of children with CF compared to healthy controls.

Methodology 18 CF patients, 17 healthy controls, and 1 subject with equivocal diagnosis were recruited into a study exploring the bacterial communities in the antecubital fossa, forearm, axilla, nare, and oropharynx. Culture swabs and swabs for DNA extraction were collected from patients. In addition, clinical information was collected from each subject.

Results DNA was isolated from swabs and libraries prepared for sequencing of hypervariable region 4 in the 16S gene showing significant overlap in all sites between both CF patients and healthy controls. Culture-swabs of both CF subjects and healthy controls demonstrated frequent isolation of *Pseudomonas* in all sites examined. *Pseudomonas* was isolated from the skin in 11.1% and nare or oropharynx in 22.2% of CF subjects (n=18), and was also isolated on the skin in 23.5% and nare or oropharynx in 11.8% of healthy subjects (n=17). In addition, *Staphylococcus aureus* was isolated from the skin of some CF patients but no healthy controls.

Discussion This study is the first step in a feasibility study to determine the role of the microbiome in CF infection and can charter transmission events. Unexpectedly, a high proportion of healthy controls were colonised with *Pseudomonas* and only CF patients were colonised with *S. aureus* which merits further exploration.

Contents

List of Figures

List of Tables

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Glossary

ACF	Antecubital fossa
ARISA	Automated ribosomal intergenic spacer analysis
ASL	Airway surface liquid
CF	Cystic fibrosis
CFTR	Cystic fibrosis transmembrane conductance regulator
CFPE	Cystic fibrosis pulmonary exacerbation
DGGE	Denaturing gradient gel electrophoresis
DEPC	Diethylpyrocarbonate
DNA	Deoxyribonucleic acid
ENaC	Epithelial sodium channel
HMP	Human Microbiome Project
MRSA	Methicillin-resistant *Staphylococcus aureus*
MSA	Mannitol salt agar
MALDI-TOF	Matrix-assisted laser desorption/ionization time of flight
NBS	Newborn screening
NGS	Next-generation sequencing
NTM	non-*Tuberculosis mycobacteria*
OTU	Operational taxonomic unit
PCR	Polymerase chain reaction
PBS	Phosphate-buffered saline
PCL	Periciliary liquid layer
PFGE	Pulsed-field gel electrophoresis
RISA	Ribosomal intergenic spacer analysis
SDW	Sterile Distilled Water
TGGE	Temperature gradient gel electrophoresis
T-RFLP	Terminal restriction fragment length polymorphism
QC	Quality check
TE	Tris-EDTA
TE-Tween	Tris-EDTA with 1% Tween-20
μ	Mean
σ	Standard deviation

Chapter 1

Introduction

1.1 Cystic fibrosis

Cystic fibrosis (CF) is an autosomal recessive disorder caused by mutations in the *Cystic Fibrosis Transmembrane Conductance Regulator* (CFTR) gene which encodes a chloride channel. Recurrent chest infections are the primary cause of morbity and mortality in CF though it is a systemic disease with other pertinent manifestations in the gastrointestinal tract. CF has an incidence of 1 in 2000-3000 births in caucasian populations, as such approximately 1 in 26 caucasians carry a CF-causing mutation[1]. It is the most common life-threatening genetic disorder in the Western world and carries a median predicted survival of 41.1 years as of 2012[2].

1.2 Epidemiology

CF is strongly associated with European descent, however its incidence varies substantially across different regions, with some having population-specific mutations[1].

This high incidence of a severe genetic disease has lead to speculation surrounding a selective advantage for carrier status in preventing dehydration during diarrhoeal diseases such cholera[3] and typhoid[4], though this has been challenged[5]. It is estimated F508del originated 11,000 to 34,000 years ago, likely in a pre-Neolithic era and predating modern humans, although a place of origin has not been determined[6, 7].

1.3 CFTR

The CFTR gene is found on the long arm of chromosome 7, encoding a 1.7kDa chloride ion within the ATP-binding-cassette transporter superfamily and comprises two

membrane-spanning domains, two nucleotide-binding domains, and a regulatory domain[8], see figure 1.1. Over 1800 CFTR variants have been characteristed to date (http://www.cftr2.org/), most of which are known to cause CF. The most common CF-causing mutation is deletion of phenylalanine at postion 508 (F508del), causing two thirds of cases worldwide[1].

CF-causing mutations can be divided into six classes, depending on the mechanism in which they cause disease. Class I mutations prevent initial synthesis of CFTR; Class II mutations, such as F508del, result in defective processing of CFTR within the cell; Class III mutations cause defects in the regulation of CFTR though it is able to reach the apical membrane; Class IV mutations lead to decreased conductance of CFTR; and Class V mutations result in decreased synthesis of CFTR[9]. A sixth class was added later, encompassing CF mutations where there is increased turnover of CFTR[10]. Mutation classes I-III are regarded as causing severe disease since there is nearly no functional CFTR present[11], however genotype only partially determines disease severity with other factors being implicated such as genes regulating immune response and susceptibility to infection, as well as environmental factors[12].

1.4 Pathogenesis

1.4.1 CFTR ion channel

The apical surface of epithelial cells in the airway are coated in airway surface liquid (ASL), which can be divided into the upper mucus layer and the periciliary liquid layer (PCL)[14]. The PCL forms an interface between the upper mucus layer and epithelial cells, enabling the mucocilliary escalator to function in a low viscosity liquid while allowing the mucus layer to act as a physical barrier against inhaled pathogens[15]. In cystic fibrosis, the lack of CFTR not only prevents chloride ions being transported through the apical membrane into the PCL, but also leads to upregulation of the epithelial sodium channel (ENaC) through mechanisms that remain obscure[16]. This results in increased sodium ion uptake into epithelial cells through ENaC and chloride paracellular transport, and thus water follows from the ASL into the epithelial cells. Dehydration of the ASL leads to a decreased volume of ASL with more viscous and

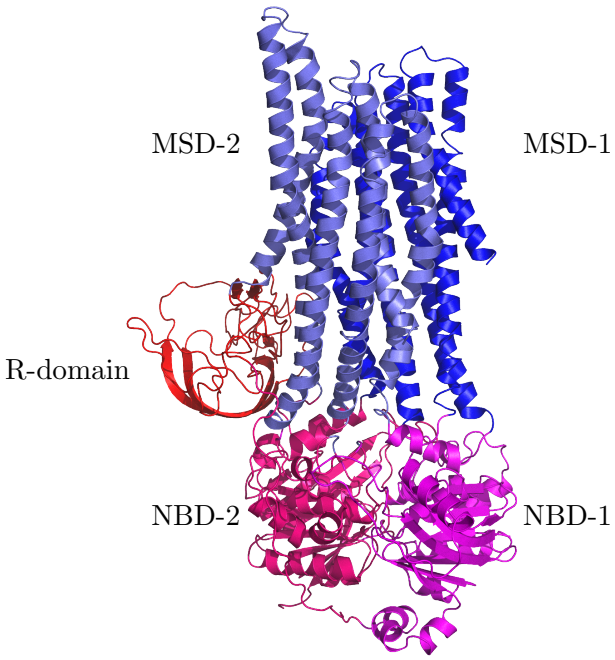


Figure 1.1: CFTR protein
R-domain indicates regulatory domain, MSD indicates membrane spanning domain, NBD indicates nucleotide-binding domain. Loops indicate α-helices while ribbons indicate βsheets. Modelled on Sav1826[13], rendered with Pymol.

adherent mucus in the airways, which coupled with a lack of mucocilliary function leads to bacterial colonisation[17].

A similar pattern of disease is seen in other mucous lined luminal organs within CF patients, as such common complications include exocrine pancreatic insufficiency, vitamin deficiencies, diabetes mellitus, liver disease, meconium ileus and its adult equivalent, distal intestinal obstructive syndrome[18].

1.4.2 Secondary functions of CFTR

CFTR has been demonstrated to act as a receptor to internalise smooth *Pseudomonas aeruginosa*, a dominant CF pathogen, with intact pili, into epithelial cells[19] but not other common respiratory pathogens[20]. The impact of *P. aeruginosa* internalisation is debated, with some studies finding increased bacterial clearance[19], while others show mutant CFTR leads to increased uptake of *P. aeruginosa* with increased survival and replication of some strains[21].

Furthermore, CFTR has been found on non-polarized cells. In macrophages from CFTR

knock-out mice, there is reduced killing of *P. aeruginosa* compared to wild-type due to a less acidic environment within the phagosome; likely because mutant CFTR is unable to transport Cl^- which provides a counterion effect to increase H^+ within the phagosome[22]. Similar defects have been found in phagosomes from neutrophil-like cells[23]. Dysfunctional innate immune responses are consistent with inability to clear inhaled pathogens in CF despite high numbers of neutrophils in the airways[24].

1.4.3 Immune responses

Dysfunctional immune response plays a key role in damage of CF airways. Defects may be caused by CFTR directly, as discussed previously, or acquired through exposure of the CF lung to pathogens. Neutrophilic infiltration is a hallmark of the CF lung[24], likely due to activation of respiratory epithelial cell Toll-like receptors by respiratory pathogens, leading to potent neutrophil influx via interleukin-8 (IL-8)[25]. In addition, the *P. aeruginosa* flagellum can bind to the glycolipid asioloGM1 on the surface of epithelial cells, eliciting a strong nuclear factor-κB mediated inflammatory response. This enables greater IL-8 production, thus leading to greater neutrophil recruitment[25].

In the presence of persistently high levels of proinflammatory cytokines such as IL-8, or bacterial molecules such as lipopolysaccharide, neutrophils have a propensity to degranulate causing damage to the host.[25] Moreover, with high levels of neutrophil infiltration and degranulation, neutrophil serine proteases are released, which are detrimental to the phagocytic and bacterial killing functions of nearby neutrophils through cleavage of the complement 5a receptor [26] and CXCR1 (IL-8 receptor, alpha)[27] on the cell surface. The extent to which immune changes in CF are directly mediated by CFTR as opposed to acquired changes in the airway environment is not yet clear.

1.5 Natural history

1.5.1 Screening and diagnosis

Previously, children with CF would present either with meconium ileus at birth (13-17% of patients) or in early childhood usually with gastrointestinal symptoms, initially lacking respiratory symptoms[28, 29]. Presentation later in life is associated with pancreatic sufficiency, and diagnosis is typically prompted by recurrent chest infections

or infertility[29]. Infants with CF are increasingly identified by newborn screening (NBS), exploiting high levels of immunoreactive trypsinogen (IRT) found in the serum of CF infants in the first days of life. However, this test lacks sensitivity, meaning most NBS protocols adopt a multi-tier approach incorporating DNA testing prior to diagnostic pilocarpine iontophoresis (commonly known as the sweat test)[30]. Two or more sweat tests of greater than 60mmol/L of chloride in sweat is the gold standard for diagnosis[29]. Due to the lack of sensitivity, NBS can identify carriers and infants with equivocal diagnosis whose natural history remains unclear[31]. These are previously unrecognised groups of infants with at least one CF mutation of unclear clinical consequence or one CF-causing mutation and an intermediate sweat chloride level (30-59mmol/L chloride on pilocarpine electrophoresis)[31]. These new challenges underpin the heterogenous nature of CF.

There is evidence from infants identified by NBS that structural lung damage may be present from as early as 3 months of age in addition to colonisation with CF pathogens, such as *P. aeruginosa*[32], although these may be independent events. This highlights the importance of early respiratory interventions to curtail development of lung damage and consideration of infection with significant CF pathogens early. Over recent years, life expectancy and quality of life in CF have improved dramatically which is thought to be largely related to more proactive measures to prevent and treat infection, in addition to closer monitoring of nutritional status[33]. Despite this, most patients with CF develop chronic chest infections and die of respiratory failure before their fifth decade[2].

1.5.2 CF pulmonary exacerbation

CF pulmonary exacerbation (CFPE) is characterised by sudden increase in symptoms experienced by patients and is associated with detrimental long-term outcomes, particularly progressive lung disease and bronchiectasis in both children[34, 35] and adults[35]. Although CFPE is associated with significant clinical outcomes, little is known about the underlying mechanism although there is association with both host immune changes and changes in lung microbiology[36]. Price *et al*[37] find adults with CF have no change in the diversity or bacterial genera present during CFPE, and no change in bacterial

load. This is consistent with a study in adults suggesting there is no acquisition of new *P. aeruginosa* strains during CFPE[38]. Viral infection has been associated with CFPE in addition to colonisation with pathogens[39], although viral infection in CF has not been well studied and the impact of these will likely be revealed in well-designed metagenomic studies. Moreover, high levels of pro-inflammatory cytokines have been found in airways during CFPE[36], and although the reasons behind this have yet to be elucidated, this provides an explanation for long-term damage of airways due to CFPE.

1.6 Typical CF pathogens

Evaluation of CF patients for pathogens is driven by culture-based techniques. There are limitations on groups of organisms which can be cultured using standard microbiological techniques. In addition, children with CF are often unable to expectoriate sputum, particularly prior to chronic *P. aeruginosa* infection. As such, oropharyngeal swabs are used instead, however throat swabs do not accurately reflect the lower airways. Armstrong *et al* found oropharnyngeal swabs to have a poor negative predictive value for CF pathogens when compared with bronchoalveolar lavage in the same patient group[40]. Furthermore, some of the major CF pathogens, notably *Staphylococcus aureus* and non-typable *Haemophilus influnenzae*, can colonize the upper respiratory tract in health, thus their roles as pathogens when found in the lower respiratory tract is not clear.

There is a trend for CF lung infections to start with *S. aureus* and *H. influenzae* either on their own or together as a coinfection in early childhood, and with progressive lung damage *P. aeruginosa* and other significant pathogens become dominant[41, 42].

1.6.1 *Staphylococcus aureus*

S. aureus is a Gram-positive coccus, typically associated with large golden-yellow colonies, β-haemolysis on blood agar, and grape-like clusters on microscopy[43]. Though it is widely regarded as aerobic, it may also grow as a facultative anaerobe and can produce biofilms[44]. It is a skin commensal, usually residing in the anterior nares[45], but also found in moist areas of the skin such as those dominated by apocrine glands in

the axilla and groin[46]. It is also a pathogen responsible for skin infections and early chest infections in CF patients[43]. Williams[46] and Kluytmans[47] postulate there are three distinct groups of the population - those who persistently carry *S. aureus* (10-20%), those who intermittently carry (20-75%) and those who rarely carry (5-50%). Gamblin *et al* present a more recent study from a UK population, estimating a 27% nasal carriage rate[48]. Prevalence of *S. aureus* in the nares of children with cystic fibrosis taking anti-staphylococcal prophylactic antibiotics is 29%, while those without prophylaxis carry at 57%, much higher than the general population[49].

Airway inflammation and damage associated with *S. aureus* infection is postulated to create a niche for *P. aeruginosa* infection later in life[41, 42]. As such, in the UK anti-*Staphylococcal* antibiotics are given from diagnosis in attempt to prevent this infection, and although some have argued this leads to an increase in *P. aeruginosa* infection, overall there is clinical benefit[50]. Methicillin-resistant *Staphylococcus aureus* (MRSA) infection, previously thought to only occur as a nosocomial infection, is an emerging problem in CF chest infections[42].

1.6.2 *Haemophilus influenzae*

H. influenzae is a non-motile Gram-negative coccobacillus which is a facultative anaerobe and can form biofilms[51, 52]. It is generally divided into encapsulated strains for which there are six serotypes, and unencapsulated strains[53]. *H. influenzae* was also the first complete bacterial genome to be sequenced[54]. It is a normal commensal of the upper respiratory tract and is known only to cause disease in humans; primarily otitis media and upper respiratory tract infections in children, in addition to lower respiratory tract infections in CF[55]. Serotype b is particularly virulent, causing meningitis, however this has been largely eradicated in the Western world since routine immunisation against this serotype with the Hib vaccine.

1.6.3 *Pseudomonas aeruginosa*

P. aeruginosa is a Gram-negative aerobic bacillus which is usually motile with a single flagellum, although non-motile isolates are often found in clinical isolates from CF sputum[56]. Though traditionally considered aerobic, it is a faculative anaerobe and

has the ability to form biofilms[57]. Its morphology can be broadly categorised into non-mucoid and mucoid due to excessive alginate production, the latter being typical of clinical isolates from CF patients with chronic *P. aeruginosa* infection[56]. Moreover, *P. aeruginosa* can secrete a variety of pigments which may stain solid media, ranging from blue (pyocyanin) through to black (pyomelanin). These pigments are thought to have important roles in virulence, cell adhesion, and siderophore activity[58–60]. It is an opportunistic pathogen in animals and plants[61]. In humans, it is primarily associated with infection in burn wounds and immunocompromised patients in a variety of sites, ranging from chest, urinary tract, and gastrointestinal infections, through to sepsis, and is widely regarded to be the most dominant pathogen in the CF lung[56].

1.6.4 *Burkholderia cepacia complex*

Burkholderia cepacia complex are a group of bacteria belonging to the *Burkholderia* genus, which through next-generation sequencing, is estimated to comprise at least 17 species, however routine biochemical testing cannot currently distinguish these adequately[62, 63]. It is ubiquitous in the environment and was initially identified as a plant pathogen but many species have subsequently been demonstrated to be beneficial to plants[63]. In CF patients, it may be carried asymptomatically, lead to chronic infection, or lead to the fatal "cepacia syndrome" where a sudden decrease in clinical status followed by sepsis is seen, unlike other CF pathogens[63].

1.6.5 non-*Tuberculosis mycobacteria*

non-*Tuberculosis mycobacteria* (NTM) have been implicated in CF infections increasingly over recent decades[64], especially *Mycobacerium abscessus*. It has emerged that there are likely to be three subspecies of *M. abscessus* identified through whole-genome sequencing; subsp *abscessus*, subsp *massiliense* and subsp *bolletii*, although currently they cannot be distinguished by standard techniques[65]. NTM were thought to be acquired from the environment, however recently it was discovered subsp *massiliense* likely undergoes person-to-person transmission[66]. Patients infected with NTM have an unclear clinical outlook, with some patients carrying at low levels asymptomatically, while others experience a drastic decline in lung function associated with this

group of pathogens[64]. It is currently debated whether treatment of this pathogen is necessary[64].

1.6.6 *Achromobacter xylosoxidans*

Achromobacter xylosoxidans is a ubiquitous environmental species regarded as an opportunistic pathogen, especially in CF where it has been reportedly been increasing in prevalence of CF patients infected[67]. The clinical importance of colonisation with *A. xylosoxidans*, like NTM, is debated[67]. Isolates of *A. xylosoxidans* from CF patients typically have high levels of antibiotic resistance, with some genomic determinants of resistance (specific resistance integrons) being linked to greater biofilm formation[68]. Though there have been evidence of outbreaks of *A. xylosoxidans* in hospitals through disinfectant solutions, saline, and dialysis fluid, there is little evidence of person-to-person transmission[69].

1.7 Microbiomes

The human body is populated by a rich plethora of microorganisms including archaea, bacteria, viruses and fungi. It is estimated bacterial cells outnumber human cells by a factor of ten[70]. These microorganisms reside on the topological surface of the human which can be divided into distinct niches within the gut, respiratory tract, urogenital tract, and integumentary system[71]. The community of microorganisms is defined as the "microbiota" and this alongside the environment they reside is termed the "microbiome"[72]. The human microbiota encompasses a community with complex interactions between each other and their host, contributing to host immunity, metabolism, and disease[73]. Molecular techniques to study microbial communities have become the standard approach, with increasing recognition that a significant proportion of the bacterial kingdom cannot be cultured under standard laboratory conditions[74]. As next-generation sequencing technology has decreased in cost, it has provided the primary means of investigating the microbiome[75].

Interest in the human microbiome culminated in the Human Microbiome Project (HMP), exploring the microbiota of 300 heathy adults[71]. The project involved sampling 15 and 18 specific niches, from males and females respectively, in each subject

and in some subjects multiple samples were taken to study temporal variation in the microbiome[76]. Next-generation sequencing technology was exploited to determine community members and structure, in addition to functional metabolic components of each microbiome[77]. This important project resulted in an understanding of the variabtion between normal microbiomes in adults and a strong contribution of reference genomes, providing a foundation for study of the microbiome in disease settings.

1.8 Community ecology methods

Microbiome analysis involves not only surveying abundance of members of the community present, but also the structure of the community; how diverse the community is and the degree of evenness in distribution of abundance[78]. An ecological approach is important to understanding microbiota in disease states, for example antibiotic therapy leads to community disturbances and after discontinuation of therapy, the community reassembles[79]. Ecological approaches to the microbiome have stemmed largely from plants and animals with subtle differences in the way in which this ecosystem is observed; usually employing DNA sequencing or community fingerprinting techniques[80]. These techniques will delineate taxonomic units within a sample, termed operational taxonomic units, which represent individuals at a species or genus level, depending on level of genetic distance from the rest of the community.

1.8.1 Alpha diversity

Alpha diversity is a commonly used metric in microbiome studies to describe the culmination of richness (the number of different species present) and evenness (distribution of abundance of species) within a sample[80]. Commonly used metrics for alpha diversity include the Shannon index[81]:

$$H' = -\sum_{i=1}^{S}(p_i \ln(p_i))$$

and Simpson index[81]:

$$D = \sum_{i=1}^{S} p_i^2$$

where p_i is the fraction of the community made up by abundance of species i and S is the total number of species in the community.

These metrics are calculated with OTU approximations of species-level taxa present.[80]

Alpha diversity is thought to reflect ecological flux and stability in a community; it has been speculated that high alpha diversity protects from invasion of pathogenic bacteria[82]. This is of great clinical consequence as antibiotic therapy has been shown to reduce alpha diversity, and it is postulated this may create a niche for CF pathogens[80]. Many of the factors contributing to alpha diversity in microbiome studies, however, have not yet been elucidated[80], and many published microbiome studies do not demonstrate causation between environmental parameters and alpha diversity.

1.8.2 Beta diversity

Beta diversity compares overlap in taxa present in different microbiomes, and is usually given as a measure of dissimilarity between them, the Bray-Curtis index[81]:

$$BC_{ij} = \frac{S_i + S_j - 2C_{ij}}{S_i + S_j}$$

where S_i is the number of OTUs found in site i, S_j is the number of OTUs found in site j, and S_{ij} is the sum of abundance of shared OTUs, using the value from the site of least abundance.

Beta diversity relates to one of the initial questions of the HMP, as to whether adults had a core microbiome between sites and between each other[70]. It is now postulated a core microbiome is likely not to exist as high beta diversity is observed both within the individual microbiomes and even higher beta diversity between microbiomes from different individuals[80].

1.9 16S ribosomal RNA gene

16S ribosomal RNA (rRNA) is an integral part of the bacterial 30S subunit and its nucleic acid sequence carries phylogenetic signal[83]. As such, the gene encoding 16S rRNA is a widely-used target to study microbial communities, in identifying community members and determining the phylogenetic distributions of prokaryotes. The 18S rRNA gene is the eukaryotic equivalent[84].

The 16S rRNA gene is approximately 1.5kb and comprises nine hypervariable regions (V1-V9) interspaced with conserved regions[85]. Each bacterial genome carries

multiple copies of 16S, which may need to be accounted for in downstream analyses[86]. Each hypervariable region carries a distinct specificity for detection of particular taxonomic lineages[87] so the choice of hypervariable region is dependent on the expected taxonomic composition of the microbial community. In addition, intragenomic heterogeneity within 16S genes in each taxon must be considered[88] as this would artificially increase the diversity of taxa observed. This is particularly important for extremophiles where large insertions in 16S may be adaptive to render some copies of 16S non-functional[88]. V4 and V5 regions display the least intragenomic heterogeneity[88], supporting use of these regions in studies comparing microbial community structures.

A variety of methods have employed the 16S gene to study microbial communities, from electrophoresis-based methods to next-generation sequencing.

1.10 Community fingerprinting techniques

Community fingerprinting techniques provide a snapshot at one particular time of a microbial community structure. They all start with extracting total DNA from directly from the sample without culture, and then proceed to amplify a particular gene product using the polymerase chain reaction (PCR), usually within the 16S region for microbial ecology[89]. The most commonly used techniques have been described, though they have largely been superseded by next-generation sequencing approaches.

1.10.1 Temperature and Denaturing gradient gel electrophoresis

Temperature gradient gel electrophoresis (TGGE) is performed by running PCR products of the 16S region on a polyacrylamide gel moving up a gradient of temperatures, and relying on differences between taxa in GC content of 16S; as the DNA melts, it forms a band through use of a GC clamp[90]. This method has been refined using a denaturant, formamide, to melt DNA rather than a temperature gradient. It has been criticised for lack of reproducibility, its qualitative nature, and inability to determine phylogeny[91].

1.10.2 Terminal restriction fragment length polymorphism

Terminal restriction fragment length polymorphism (T-RFLP)is a technique based on PCR with a 5' fluorescently labelled primer for a specific region, in this case usually the 16S gene or another gene with strong phylogenetic signal, followed by treatment with restriction endonuclease enzymes[92]. After size selection for the terminal fragments (the 5' end of sequence containing the fluorescent probe, each assumed to represent an OTU), the fluorescent intensity can be measured and plotted against fragment length, producing an electropherogram[92].

1.10.3 Automated ribosomal intergenic spacer analysis

Ribosomal intergenic spacer analysis (RISA) takes advantage of the heterogeneity observed in both length and sequence, even at strain level, in the intergenic region between 16S and 23S regions on the rRNA operon[93]. RISA initially consisted of performing PCR on the intergenic region followed by polyacrylamide gel electrophoresis, however due to the time-consuming nature of this protocol, an automated technique (ARISA) was develope[93], whereby similar to T-RFLP, a fluorescent primer is used enable measurement of fluorescence intensity and produce an electropherogram. Although some bacteria may have multiple heterogeneous copies of the intergenic spacer region, falsly increasing estimates of diversity, ARISA demonstrates greater resolution for rare taxa compared to T-RFLP[94].

1.11 Next-generation sequencing

Next-generation sequencing has rapidly changed the way microbial communities are explored. With decreased sequencing costs, microbiome studies are becoming increasingly commonplace. Pyrosequencing was introduced by Roche with the 454 GS-FLX (454 Life Sciences) as the first high-throughput sequencing technology[95], followed by Illumina sequencing. Both technologies perform "sequencing by synthesis," whereby nucleotides are incorporated into a DNA strand from the sample and sequence generated based on either release of pyrophosphate in the case of pyrosequencing[95] or incorporation of a fluorescent base-pair in the case of Illumina[96]. Illumina platforms are

currently the most commonly used technology for microbiome studies as the readlength is adequate for hypervariable region sequencing (up to 2×300bp if using a dual index approach, where the amplicon is sequenced from both 3' and 5' ends), the error rate is low compared to other platforms, and many samples can be run in parallel by applying unique barcodes to each sample[96].

Next-generation sequencing in microbiology has been applied to amplicon sequencing of 16S hypervariable regions, as mentioned previously. In addition, it has been applied to performing metagenome analysis, whereby the entire DNA composition of a microbial community is sequenced to provide a collection of full genomes[97]. However, this approach requires large quantities of DNA, is largely prohibitive in terms of cost and provides unique computational issues in terms of assembling short reads from the sequencing platform together to form individual microbial genomes[77]. It has the advantage of a more in depth analysis of the genomic structures within the microbial community and a full overview of viral, bacterial and fungal elements.

1.12 Development of the microbiome

Development of the human microbiome begins with colonisation of a previously uninhabited niche. Traditionally it was thought amniotic fluid is sterile prior to rupture of membranes. Bearfield et al[98] found in a sample of 48 women with intact membranes admitted for elective caesarian section that 34 had evidence of bacteria in amniotic fluid by universal bacterial PCR, 20 of which had *Streptococcus* species, and 7 had *Fusibacterium nucleatum*, both common oral commensals. This study postulated that oral commensals can translocate into the amniotic fluid in response to increased levels of prostaglandins. The presence of bacteria in amniotic fluid is supported by Hitti et al[99], where 5 of 14 patients admitted with premature labour and intact membranes had positive universal bacterial PCR despite culture-negative amniotic fluid. Unfortunately without culture-based methods it is difficult to comment on levels of bacterial load present in healthy amniotic fluid and whether they are viable. During birth, babies are first exposed to maternal vaginal and faecal microbiota in the case of vaginal delivery and to skin microbiota in the case of Caesarian section. Newborns delivered

vaginally initially carry a microbiome dominated by their maternal vaginal and faecal microbiota, which shows little variation between body site[100]. A similar pattern is seen with maternal skin microbiota in those infants born by Caesarian section[100]. The initial microbiome may impact development of atopic conditions in infants, as an association has been shown between Caesarian section and risk of eczema and asthma, which may be mediated by *Clostridium difficile* colinisation[101].

The infant faecal[102] and skin microbiomes[103] increase in diversity within the first months of life and show evidence of ecological succession, in the skin microbiome there is differentiation towards specific microbiota for different skin sites, as is seen in adults[71]. In the faecal microbiome of healthy and CF infants, significant alterations correspond with environmental events, such as changes in feeding and health[102, 104]. Moreover in children with CF, respiratory microbiota increases in diversity over time at a greater rate than the faecal microbiota and changes in community membership coincide with one another[104]. A firm theory of how the healthy microbiome develops is still lacking, as the patterns of microbiomes observed do not enable speculation surrounding the roles of dispersal, immigration of new microorganisms, and selective pressures in the differentiation of microbiomes.

1.13 Paranasal sinus microbiome

Nearly all CF patients have inflammatory paranasal sinus disease which tends to be more widespread and severe than non-CF patients with sinusitis[105]. The paranasal sinuses have mucus thickening as elsewhere in CF, however it differs to the CF lung in its weaker immune response, decreased antibiotic bioavailability, and the different nutritional resources available for bacteria[106]. The paranasal sinus may mimic the hypoxic conditions found in the CF lung[107] and is postulated to afford pathogens protection from the host immune system and antibiotic therapy[108]. *P. aeruginosa* undergoes substantial diversification and evolution in the sinus, migrating to and from the respiratory tract, conferring the sinus a pivotal role in the progression of chronic lung infections in CF[106, 109].

The microbiota of paranasal sinuses in CF have received little attention, although

typical CF pathogens including *S. aureus*, *P. aeruginosa*, and *H. influenzae* have been cultured in the CF sinus with many other aerobic and anaerobic pathogens[110–116]. Furthermore, correlations have been observed between pathogens in sinuses and bronchoalveolar lavage washings, particularly *S. aureus*[112, 115]. The concordance between pathogens isolated in sinus and airways increases with age, and on pulsed-field gel electrophoresis (PFGE) the same strains are found in sinus and airways concomitantly[114]. Culture-independent study of the paranasal sinus microbiota would advance understanding of this niche and the biological plausibility of interventions such as sinus surgery which are currently being explored in CF patients[117–119].

1.14 Nasal microbiome

Nasal mucosal microbiota in CF has largely been explored in terms of a niche for *S. aureus*. It is postulated *S. aureus* adheres to the nasal mucosa prior to respiratory tract colonisation and infection[45]. In healthy children, *S. aureus* is cultured at a similar frequency from nare and oropharynx, though *S. aureus* is found more frequently in the oropharynx than nares in children with CF[120, 121]. This contradicts dogma of the nare as the most important site for colonisation by *S. aureus* preceding infection. Transmission of *S. aureus* between CF patients and household members has been reported[121], though it is rare and host-host transmission is more likely in MRSA[122].

1.15 Airway microbiome

1.15.1 Development of lung microbiota

The airway microbiome in CF is a field of growing interest though still in its infancy. Initially the Human Microbiome Project excluded the lung as a priority organ since the lungs were presumed to be sterile[123], though it is now suspected that healthy lungs are colonised with commensal bacteria[124]. The temporal progression of infant microbiota migrating to distinct compartments remains unclear although in CF, one study suggests lung microbial communities are acquired from the GI tract[104]. This is consistent with current theories that the mouth acts as a reservoir for infection in CF through microaspiration, evidenced by similar microbiota in mouth washings and airways of individual patients with cystic fibrosis[125].

During early childhood, the CF airway microbiota diversifies before declining and phylogenetically clustering in later life[126]. Decreased alpha diversity is associated with age[126, 127], decreased FEV1[127], and the presence of both *P. aeruginosa* and long-term antibiotic therapy[127, 128]. The cause of decreased diversity over time in the CF airway remains to be elucidated though it may represent development of an ecological niche with increased lung damage. The confounding factor of antibiotic treatment cannot be eliminated until prospective studies examine acquisition of *P. aeruginosa*[129].

1.15.2 Respiratory microbiome in CF

Studies on CF airway microbiota have focussed on the impact of typical CF pathogens, relationships with clinical parameters, and comparison of stable patients to those with an exacerbation. Distinct patterns of CF airway microbiota have been described in relation to presence of *Pseudomonas* and *Streptococcus*, in addition to antibiotic exposure. Microbiota can be divided into core and satellite taxa based on their relative abundance and persistence in time and space[130]. There is discordance over whether members of core taxa, particularly *P. aeruginosa*, disturb the wider microbial community structure varying from reports of decreased number of taxa associated with *P. aeruginosa*[128] to no effect on background microbiota[125].

The CF airway microbiota in individual patients appears to be stable over time, only decreasing in diversity during antibiotic therapy and returning to baseline within one month[127, 131]. Interestingly, there seems to be no increase in bacterial density during CF exacerbation[132] but increased *P. aeruginosa* virulence factors have been documented[133]. The underlying mechanism of CF exacerbation is yet to be elucidated though the decrease in diversity appears to be pivotal.

Pseudomonas predominance in a patient sample has been suggested to correlate with patient outcome but not related to CF genotype, while *Streptococcus* abundance, regardless of *Pseudomonas* fraction may indicate clinical stability[129]. The species found in clinically stable patients by Filkins *et al* were chiefly oral *Streptococcus* and *Streptococcus milleri* group (SMG)[129], contradicting Sibley *et al* who suggest SMG is associated with exacerbations in CF[134]. Considering *Streptococcus* is an abundant taxa in the CF airway microbiome[129, 130, 135] and its pathogenicity is unclear, further studies should elucidate the underlying mechanisms.

1.15.3 Pathogen discovery in microbiome studies

In the era of culture-independent methods, many bacterial genera, particularly anaerobes, are emerging as potential CF pathogens. Though anaerobes have received much attention in adult airway microbiomes, they tend to be more abundant in younger patients[126]. Tunney *et al.* demonstrates the anaerobic genera *Prevotella* and *Veillonella* can form the predominant bacterial fraction in CF patient sputum, predominating 18 of 40 and 13 of 40 patients studied respectively[136]. Supernatant from *Prevotella intermedia* grown in an anaerobic environment has been shown to be more virulent than supernatant from *P. aeruginosa* grown in anaerobic conditions, both *in vitro* and *in vivo*, however murine lung infection using an agar beads model did not result in mortality[137]. This is likely due to limitations of the model and possibly inoculation of a sub-infectious dose; 10^6 CFUs were used in the model compared to an upper limit of 10^7 found in patient sputum. Further studies investigating the role of anaerobic species in the CF airway are necessary to understand these previously overlooked members of the community.

1.15.4 Lung allograft microbiome

The sinus may not act only as a hiding place for pathogens during antibiotic treatment, but also as a reservoir for infection of the allograft following lung transplant[138, 139]. Remarkably, there appears to be little association between colonisation with typical CF pathogen and outcome after lung transplant, apart from *B. cepacia* genomovar III which carries a high mortality rate following transplantation[139]. Studies on CF lung transplant microbiomes are limited, however one conference abstract reports *Proteobacteria* being the dominant phylum, with phylogenetic diversity increasing for the first nine months before decreasing[140]. These findings in the microbial communities of CF lung allografts deserve further investigation, especially in the context of the potential role for allograft microbiome in the development of bronchiolitis obliterans syndrome[139, 141], one of the most lethal complications of lung transplant.

1.16 Gastrointestinal microbiome

The gastrointestinal tract can be split into several components, each with a distinct microbiome. The oral cavity, distal colon and faeces have received the most attention due to ease of sampling[142]. Moreover, these sites likely attract interest due to suspicion of more diverse and complex microbial communities than elsewhere in the gastrointestinal tract. It is thought bacteria associated with the intestinal mucosa may have more important interactions with the host, though unfortunately faecal samples cannot distinguish these bacteria from non-adherent bacteria simply passing through from higher in the gastrointestinal tract[142].

Bacterial communities in CF faecal samples from both children and adults appear to be dependent on CFTR genotype; patients with more severe genotypes harbour a greater abundance of potentially pathogenic bacteria[143]. In young children with CF, metagenomic sequencing of faecal microbiota found higher levels of *Escherichia coli* compared to healthy age-matched controls, with no specific features of virulence, suggesting *E. coli* could be involved in an inflammatory relationship with the host[144].

The community structure of CF faecal microbiota has been determined by DGGE, revealing CF patients have greater inter-individual variation and greater variation in

faecal microbiota over time compared to their siblings[145]. Though there is also vast inter-individual variation in the CF airway microbiome, the fluctuation in composition of faecal microbiota over time is discordant with the relative stability in the CF airway microbiome. Since no clinical data is presented in this paper, it is difficult to speculate on the reasons behind these striking differences, although antibiotic tissue penetration and biofilm formation in the lungs may play a role in determining the community structure of these different niches.

A link between gastrointestinal and respiratory microbiomes has been reported in infants, whereby particular bacterial genera increase in abundance sequentially in the gastrointestinal tract followed by the respiratory tract, and gaining greater phylogenetic diversity[104]. It is therefore not surprising that infant gut microbiota is also strongly associated with mode of delivery[146].

Metagenomic sequencing of faecal microbiota from healthy volunteers reveals people can be grouped into enterotypes, which is likely dependent on diet, and this diverse community may have a significant symbiotic relationship with healthy hosts through digestion of carbohydrates and production of vitamins[147]. In addition, this paper speculates metabolic processes of bacterial communities in the gastrointestinal tract may have an effect on health, specifically obesity. Despite efforts to demonstrate acquisition of a particular enterotype causes obesity in a mouse model[148], it does not demonstrate a temporal relationship between enterotype and obesity can occur naturally, albeit reinforcing the pivotal role of gut microbiota metabolic symbiosis. *Clostridium* cluster XIVa has been demonstrated to be the most common mucin-adherent bacterial species in models of the human gut[149]. Exploring dysbiosis in CF gut, Duytschaever *et al.* found CF patients had decreased abundance of *Clostridium* cluster XIVa, likely due to antibiotic therapy, and that abundance of this species correlates with patient weight for height[150]. This demonstrates an association between gut microbiota and nutritional status in CF, although the underlying reasons have not been elucidated.

Antibiotic exposure is another major extrinsic factor influencing gut microbiota. In healthy adults given short-term antibiotics, bacterial abundance in faecal samples declined markedly, though returned to normal following discontinuation of antibiotic

therapy[151]. The effects of long-term prophylactic antibiotics and additional antibiotic therapy administered during exacerbation in cystic fibrosis would therefore have a pivotal role in the composition of CF gut microbiota. Gut microbiota also has interactions with the host immune system, and in CF there is a reduction in abundance of *Bifidobacterial* species, a collection of bacteria involved in maturation of the host mucosal immune system[150]. This could have far-reaching effects in augmenting lung inflammation.

Therapeutic interventions to change gut microbiota, such as faecal transplantation and probiotic products, have received some interest. Recently, a small clinical trial demonstrated faecal transplant cured 15 of 16 patients with recurrent *Clostridium difficile* infection, being significantly more effective than standard treatment[152]. Probiotic products have largely escaped regulation as pharmaceutical products, although there is evidence that they may be effective in controlling symptoms in patients with irritable bowel syndrome, however studies have failed to demonstrate efficacy in inflammatory bowel disease[142].

1.17 Aims of this study

The skin microbiome has received no attention in CF, apart from limited studies on hand hygeine[153, 154]. These studies have demonstrated carriage of CF pathogens on the hands of CF patients, however did not sample other sites or compare to any control groups. The CF skin has a higher salt level as evidenced by the role of pilocarpine iontophoresis in diagnosis and may harbour a transient pathogenic community. In addition, given the differences in the CF skin conditions, there may be a different community structure present. The aims of this study are to:

- Assemble and identify a collection of bacterial and fungal isolates from skin, nare, and oropharynx from children with CF and healthy controls.
- Optimise DNA extraction methodology for use on swabs from skin, nare, and oropharynx.
- Characterise the microbiomes of skin, nare, and oropharynx in children with CF and healthy controls.

Identification and assembly of a collection of bacterial and fungal isolates is undertaken with the intention of providing a culture-based overview of microbiota on the skin, nare, and oropharynx of children with CF and healthy controls. These data will be used to characterise each niche and provide comparison between sites within CF patients and also comparisons between each niche in CF patients and healthy controls. Optimisation of DNA extraction for swabs from skin, nare and oropharynx is necessary to ensure adequate yields and quality for next-generation sequencing given the paucity of papers describing DNA extraction methods from low biomass environments, for example skin.

Examining the microbiome using 16S sequencing of skin, nare, and oropharynx of CF patients and healthy controls intends to identify to genus level bacteria present in these niches which, to include bacteria which are difficult to isolate using traditional microbial culture techniques, and provide relative quantity data for each niche. In addition to culture-based data this could provide further insights into differences between each

niche in CF patients and healthy controls and any overlap present which may suggest transmission routes of pathogens. This study intends to study the feasibility of investigating microbiomes of skin, nare and oropharynx in children with CF and healthy controls, and may provide potential routes of transmission. Furthermore, these bacterial samples can be assembled to provide a basis for investigation in further studies.

Chapter 2

Methodology

2.1 Research governance

The study protocol and patient documentation was designed. Research ethics committee approval was sought from the Liverpool Central National Ethics Committee to conduct this study and for any subsequent substantial amendment required.
Sponsorship and insurance were sought from the University of Liverpool to conduct this study and approval sought for any subsequent amendments to the study protocol.
A research passport was sought from the University of Liverpool to collect samples and clinical data for this study.
Approval from the Research and Development department at Alder Hey Children's NHS Trust was sought to conduct the study in their Trust.
Funding applications for the project were made to Alder Hey Children's Charity and the University of Liverpool Technology Directorate voucher scheme.

The approval letters from the research ethics committee and the Research and Development department at Alder Hey Children's NHS Trust can be found in the appendix.

2.2 Patient samples

Children with CF 12 years or younger were recruited from the CF clinic and healthy age and sex matched subjects were recruited from the surgical clinic at Alder Hey Children's NHS Trust. Subjects were sex and age-matched within two years. This is to ensure that the development of the microbiome of each subject is comparable during analysis.

Clear inclusion and exclusion criteria were set as follows for both groups.
Inclusion:

1. 12 years or younger
2. In the CF group only: diagnosis of CF confirmed by genotype

Exclusion:

1. Significant concomitant medical condition unrelated to CF
2. Antimicrobials taken within the past month (in addition to any anti-staphylococcal prophylaxis for the CF group).
3. Viral illness within past month.
4. Atopic eczema or other skin condition affecting sampling areas

Two swabs were taken from each site on each subject; one Catch-All swab (MoBio) followed by one culture swab in Amies media (Sterillin). Sites swabs were taken from:

1. Entire forearm and hand or only antecubital fossa.
2. Both axillae (same swab)
3. Both nares (same swab)
4. Oropharynx

Entire forearm swabs were performed in a uniform manner, with the swab (for both culture and culture-independent analysis) immersed in sterile distilled water (SDW), and rubbed vigorously along each surface of the forearm five times before rotating the swab 90°. After covering the forearm and the dorsal side of the hand in this manner, the palmar surface of the hand was rubbed five times in addition to the interdigital spaces. The dominant hand and arm was swabbed first, followed by the non-dominant hand and arm with the same swab. In all further chapters, swabs from hand and forearm will be referred to as "forearm" intended to mean inclusion of hand as well.

Swabs from the antecubital fossa were performed in a uniform manner whereby the swab (for both culture and culture-independent analysis) was immersed in SDW and

rubbed across the antecubital fossa (inner elbow) vigorously ten times and then rotated 90°, and this repeated four times across this area.

Nasal swabs were taken with a dry swab whereby the swab was rubbed on the inside of the left anterior nare ten times, followed by the right anterior nare ten times. This was performed for both culture swab and culture-independent swab.

Oropharyngeal swabs were taken by inserting the swab into the mouth of the subject, asking the subject to cough and then as they cough, rubbing the swab along the soft palate including the oropharynx. This was performed for both the culture swab and culture-independent swab. This was a pragmatic sampling method chosen to enable sampling of small children and infants in the same manner as the older subjects where it is not possible to gain a pure cough swab and to enable greater bacterial yields.

Although further sampling from the groin for example would have been desirable, particularly to detect *S. aureus*, this was balanced against the dignity of subjects and their likely willingness to partake in the study. In addition, it would have been desirable to use more vigorous methods to collect skin samples, for example scraping or rubbing skin with a sterile brush though this also had to be weighed against subject discomfort and their willingness to participate in the study.

For all swabs, great care was taken to avoid contamination (gloves were worn, swabs were only removed from packaging immediately prior to sample collection, and care was taken to avoid contact with and other surface). The culture-dependent swabs were cut dry into small sterile autoclaved tubes with sterile disposable scissors (Instrapac) immediately after collection while culture swabs were placed directly into the Amies media. All swabs were transported to the laboratory within four hours and culture-independent swabs frozen in the tubes at -20°C immediately on arrival. This is in line with published work finding storage at room temperature for prolonged periods makes negligible differences to sample integrity and microbial community structure[155]. Culture swabs were inoculated into media within 18 hours.

2.3 Patient data

Patient data collected included CFTR genotype, clinical state at sampling (stable or exacerbation), previous medical history, drugs currently taken by patients at the time of sampling, and hygiene routines.

Data collected from healthy controls included any previous medical history, and hygiene routines.

All data collected was current at time of sampling, however the ethical approval included the possibility to record elements of medical history from case notes during the study period (initially set at two years) but after sampling.

2.4 DNA extraction

DNA extraction prior to library preparation was carried out using MoBio Ultraclean DNA Isolation kit, according to the manufacturer's instructions with the following deviations from the protocol:

1. Catch-All swab was incubated for 18 hours at 37°C, shaking at 300rpm, in a 1.5mL Eppendorf tube with 300μL Microbead solution (MoBio) and 4μL Ready-Lyse Lysosyme (Epicentre).

2. Contents of the Eppendorf, including the swab, was transferred to the MoBio beadtube.

3. Following addition of MD1 solution (MoBio), the beadtube was subject to heating at 70°C for 10 minutes followed by beadbeating (MP Bio) at 6.5 m/s for 30s twice with a 5 minute interval on ice between each cycle.

4. Elution of DNA was performed in DEPC water (Ambion) and water left in the spin column for ten minutes prior to centrifugation. The elute was then placed back into the spin column and left for a further ten minutes prior to a second centrifugation step at 10,000G.

Other DNA extraction kits used in optimisation were PowerSoil DNA Isolation kit (MoBio), BiOstic Bacteremia kit (MoBio), and QIAmp DNA minikit (Qiagen). Any deviations from the manufacturer's protocol are stated.

2.5 DNA concentration methods

DNA concentration was performed with three methods (Ampure magnetic beads, ethanol precipitation, and vacuum method) for optimisation, after which a vacuum method was decided upon, using the SpeedVac.

2.5.1 Ampure XP beads

100μL (1:1 ratio by volume) of Ampure XP magnetic beads (Beckman) were added to the sample which was then vortexed briefly and incubated for 10 minutes. The tube was then placed in a magnetic rack and left for 5 minutes for the DNA to bind to the magnetic beads. The supernatant was then removed and the beads eluted in 13μL DEPC water (Ambion). This was vortexed briefly and left for the DNA to elute in the smaller volume for 5 minutes. The tube is then returned to the magnetic rack and supernatant of water with DNA eluted removed.

2.5.2 Ethanol precipitation

10μL (1:10 ratio by volume) of sodium acetate (Sigma) were added to the DNA elute, followed by addition of 300μL of 100% molecular grade ethanol (Sigma). This was incubated for 18 hours at -20°C, followed by centrifugation at 15000G at room temperature for 15 minutes. The supernatant was then discarded and the remaining pellet washed with 70% molecular grade ethanol (Sigma), before centrifugation at 15000G at room temperature for 15 minutes. The supernatant was then discarded and pellet eluted in 13μL DEPC water (Ambion).

2.5.3 Vacuum

Tubes with DNA eluted in water were placed into the SpeedVac machine for 1-2 hour on medium setting and left for water to evaporate. Following this, the DNA is eluted in 13μL DEPC water (Ambion) and left for 18-24 hours at 4°C.

2.6 Library preparation

Miseq dual index amplicon library preparation was performed according to the Illumina preparation protocol, as follows:

1. First PCR (adaptors)
2. Clean up with magnetic beads
3. Second PCR (barcodes)
4. Clean up with magnetic beads
5. Library assessment

2.6.1 PCR

PCR was carried out on the Rotogene 6000 qPCR machine (Corbett Life Science), according to the protocol and conditions specified below. The primers used for the first adaptor PCR are listed in Table 2.2. The forward barcode primers are listed in Table 2.3. The reverse barcode primers are listed in Table 2.4.

- 1μL Forward primer
- 1μL Reverse primer
- 12.5μL NEB-Next High Fidelity 2× Master Mix(New England Biolabs)
- 10.5μL Sample including up to 5ng DNA with DEPC water (Ambion) to complete volume

Cycling conditions were as follows:

Stage	Temperature (°C)	Time (s)
Denaturation	95	120
Denaturation	98	20
Annealing	60	15
Elongation	72	40
Elongation	72	60

Table 2.1: Cycling conditions for both PCR cycles in library preparation. The first PCR ran for 10 cycles while the second ran for 15 samples

Name	Forward N5	N5	V4 region annealing site
N5_DR_Knight515	CTACACTCTTTCCCTACACGACGCTCTTCCGATCT	NNNNN	GTGCCAGCMGCCGCCGTAA
DRknight806rev	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT		GACTACHVGGGTWTCTAAT

Table 2.2: The above Illumina adaptor -N5 forward and reverse primers were used for the first PCR

Name	5' adaptor	i5 index	Pad/linker
DI_N501For	ATTGATACGGCGACCACCGAGATCTACAC	TAGATCGC	ACACTCTTTCCCTACACGACG
DI_N502For	ATTGATACGGCGACCACCGAGATCTACAC	CTCTCTAT	ACACTCTTTCCCTACACGACG
DI_N503For	ATTGATACGGCGACCACCGAGATCTACAC	TATCCTCT	ACACTCTTTCCCTACACGACG
DI_N504For	ATTGATACGGCGACCACCGAGATCTACAC	AGAGTAGA	ACACTCTTTCCCTACACGACG
DI_505For	ATTGATACGGCGACCACCGAGATCTACAC	GTAAGGAG	ACACTCTTTCCCTACACGACG
DI_N506For	ATTGATACGGCGACCACCGAGATCTACAC	ACTGCATA	ACACTCTTTCCCTACACGACG
DI_N507For	ATTGATACGGCGACCACCGAGATCTACAC	AAGGAGTA	ACACTCTTTCCCTACACGACG
DI_N508For	ATTGATACGGCGACCACCGAGATCTACAC	CTAAGCCT	ACACTCTTTCCCTACACGACG

Table 2.3: List of forward barcode primers for second PCR in library preparation

Name	5' adaptor	i5 index	Pad/linker
DI_N701Rev	CAAGCAGAAGACGGCATACGAGAT	TCGCCTTA	GTGACTGGAGGTCAGACGTGTGCTCTTCCGATCT
DI_N702Rev	CAAGCAGAAGACGGCATACGAGAT	CTAGTACG	GTGACTGGAGGTCAGACGTGTGCTCTTCCGATCT
DI_N703Rev	CAAGCAGAAGACGGCATACGAGAT	TTCTGCCT	GTGACTGGAGGTCAGACGTGTGCTCTTCCGATCT
DI_N704Rev	CAAGCAGAAGACGGCATACGAGAT	GCTCAGGA	GTGACTGGAGGTCAGACGTGTGCTCTTCCGATCT
DI_N705Rev	CAAGCAGAAGACGGCATACGAGAT	AGGAGTCC	GTGACTGGAGGTCAGACGTGTGCTCTTCCGATCT
DI_N706Rev	CAAGCAGAAGACGGCATACGAGAT	CATGCCTA	GTGACTGGAGGTCAGACGTGTGCTCTTCCGATCT
DI_N707Rev	CAAGCAGAAGACGGCATACGAGAT	GTAGAGAG	GTGACTGGAGGTCAGACGTGTGCTCTTCCGATCT
DI_N708Rev	CAAGCAGAAGACGGCATACGAGAT	CCTCTCTG	GTGACTGGAGGTCAGACGTGTGCTCTTCCGATCT
DI_N709Rev	CAAGCAGAAGACGGCATACGAGAT	AGCGTAGC	GTGACTGGAGGTCAGACGTGTGCTCTTCCGATCT
DI_N710Rev	CAAGCAGAAGACGGCATACGAGAT	CAGCCTCG	GTGACTGGAGGTCAGACGTGTGCTCTTCCGATCT
DI_N711Rev	CAAGCAGAAGACGGCATACGAGAT	TGCCTCTT	GTGACTGGAGGTCAGACGTGTGCTCTTCCGATCT
DI_N712Rev	CAAGCAGAAGACGGCATACGAGAT	TCCTCTAC	GTGACTGGAGGTCAGACGTGTGCTCTTCCGATCT

Table 2.4: Reverse barcode primers for second PCR in library preparation

2.6.2 Clean up with magnetic beads

Clean up was performed on both first and second PCR products using AMPure XP magnetic beads (Beckman) as follows:

1. 10μL AMPure XP added
2. Vortex and incubate for 10 minutes
3. Place tubes in magnetic rack and wait 5 minutes to bind
4. Remove supernatant
5. Wash beads twice with 80% ethanol
6. Elute in 13μL of DEPC water (Ambion)
7. Place tubes in magnetic rack and wait 5 minutes to bind
8. Transfer supernatant to a new tube

The magnetic beads bind high molecular weight DNA and leave behind small fragments. Smaller fragments can be recovered with decreasing volume ratios of AMPure XP to PCR product, see Figure 2.1. As such, 0.8μL AMPure: 1μL PCR product was chosen to capture the amplicon without any small DNA fragments.

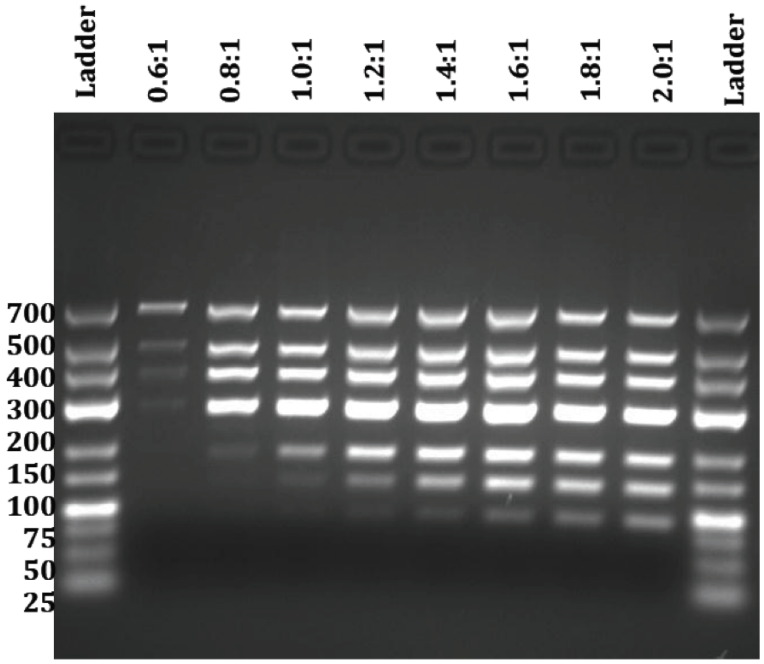


Figure 2.1: AMPure ratio for size selection
The gel demonstrates size of PCR fragments remaining after each volume ratio of AMPure beads to PCR product (Reproduced with permission from Dr Jonathan Keats, TGen, Phoenix)

2.6.3 Library assessment

DNA was quantified from individual samples by fluorometry using a Qubit High Sensitivity Assay Kit (Life Technologies). Library assessment was carried out by the Centre for Genomic Research, University of Liverpool using a Bioanalyzer 2100 (Agilent)

2.7 Culture swab processing

2.7.1 Initial processing

All culture swabs taken in Amies media were initially inoculated onto all of the following solid media, in this order, within 24 hours:

1. Blood Columbia × 2
2. Pseudomonas-specific (Oxoid)
3. Staphylococcus-specific (Oxoid)
4. Sabouraud-Dextrose (Oxoid)

One blood Columbia plate was incubated at 37°C in a microaerophillic environment for 48 hours. Sabouraud Dextrose plates were incubated at 37°C for three weeks. All other media was incubated at 37°C overnight.

2.7.2 Growth conditions and storage

Unless otherwise stated, all bacterial isolates were grown according to their original isolation conditions.

When broth media was necessary, LB-Lennox broth was used and incubated at 37°C overnight shaking at 70-300rpm.

Growth was streaked on the same media to produce single colonies or single colonies picked from the original plate. Molds were always sampled from the edge of the mycelium.

Bacterial and fungal isolates were stored at 4°C on the respective medias for short term storage. Bacteria and yeasts, segregated based on colony morphology were stored at -80°C in 20% LB-glycerol for longterm storage. Molds were stored at 4°C on Sabouraud-dextrose slants for longterm storage.

2.7.3 Media

All media was prepared by autoclaving at 121°C for 15 minutes and stored at 4°C until use. Ingredients which cannot be autoclaved due to breakdown at high temperatures were filtered through syringe filters with a 0.2μm diameter (Appleton Woods) after any base materials had been autoclaved and cooled.

2.8 Identification of bacteria

2.8.1 Gram stain

Gram stain procedure was applied to all bacterial and yeast isolates. The procedure used entailed inoculating small quantities of fresh colonies onto a glass slide using a pipette tip, and smeared in sterile water. Slides were heat fixed by briefly flaming with a Bunsen burner. Slides were washed with crystal violet for 1 minute, and then gently washed with tap water. Slides were then immersed in Lugol's iodine for 1 minute before being gently washed again with tap water. Slides were counterstained with safranin for

30 seconds before gently washing with tap water and leaving to dry.

Microscopy was undertaken and Gram stain reaction, shape of cells, size of cells, and arrangement of cells was noted.

2.8.2 Oxidase testing

A fresh inoculum of each isolate was rubbed with a sterile loop onto oxidase strips and observed for colour change immediately. A positive reaction was given as an immediate colour change to dark purple. *P. aeruginosa* was used as a positive control and *S. aureus* (Oxford strain) was used as a negative control.

2.8.3 Catalase testing

A fresh inoculum of each isolate was rubbed on a sterile plastic plate and flooded with 3% hydrogen peroxide (Sigma), and then observed for evidence of catalase immediately. A positive reaction was given as immediate and widespread effervescence. *S. aureus* (Oxford strain) was used as a positive control and *Streptococcus pneumoniae* as a negative control.

2.8.4 Haemolysis

Presence of α- and β- haemolysis was noted qualitatively on blood-Columbia media by spotting 3μL of growth from overnight LB broth cultures in 96 well plates over two solid media plates. This was performed in duplicate. In the event of any uncertainty or widespread haemolysis, individual isolates were streaked onto blood Columbia solid media. α-haemolysis was determined by an olive green halo around colonies in the absence of pigment secretion on Columbia media. β-haemolysis was given as a transparent zone of clearance surrounding colonies.

2.8.5 Gelatinase activity

Gelatin solid media was stab innoculated with 3μL of growth from overnight LB broth cultures in 96 well plates over four solid media plates and incubed for up to one week. These were checked daily for halos indicative of gelatinase activity. After one week, the plate was flooded with saturated ammonium sulphate solution to precipitate protein and reveal subtle gelatinase activity. Gelatinase activity was considered present if a

transparent halo appeared around the stab site. This was performed in duplicate and in the case of any discrepency and with any large areas of gelatinase activity disrupting interpretation repeated on individual plates.

2.8.6 Urease activity

Isolates were incubated overnight in LB broth and 3μL innoculated into 96 well plates containing solid Christensen's urea agar. Evidence of slow urease reaction was noted after 18 hours and final results of urease testing noted after 48 hours. Any change in the colour of media from yellow to pink or red was considered a positive test. If the colour change was only in part of the media closest to the bacterial growth, this was recorded as a slow urease reaction. For each isolate, this was repeated in duplicate and in the case of any descreptency, individual isolates were streaked onto solid media plates.

2.8.7 Mannitol salt agar

Isolates were incubated overnight in LB broth and 3μL innoculated into 96 well plates containing mannitol salt agar (Oxoid). Evidence of any growth and mannitol fermentation noted after 18-24 hours. Mannitol fermentation was considered positive if there was any colour change in the media from pink to yellow. This was repeated in duplicate and in the case of any descreptency, individual isolates were streaked onto solid media plates. *S. aureus* (Oxford strain) was used as a positive control and *P. aeruginosa* was used as a negative control.

2.8.8 Carbohydrate fermentation tests

Carbohydrate broths were prepared with dextrose, maltose, fructose, and lactose with a phenol red indicator. Carbohydrate broth was alliquoted into 96 well plates and innoculated with a 5μL loop of overnight growth from Columbia media. These were incubated at 37°C overnight. This was performed in duplicate and any discrepancy was repeated in triplicate. Acid production, indicated by any colour change from pink broth to yellow, and any deposits in the tubes were noted. Alkalki response was noted by a change towards a darker red or pink colour. Positive controls used for lactose and glucose fermentation were *Klebsiella pneumonia* ATCC 43816 and *Escherichia coli* K12

strain. *E. coli* K12 strain was also used as a positive control for fructose fermentation. *S. aureus* (Oxford strain) was used as a positive control for maltose, fructose, and sucrose. *P. aeruginosa* PAO-1 was used as a negative control for all fermentation tests. In addition, blank wells were used as negative controls.

2.8.9 Computational identification

Pibwin[156], a probabilistic algorithm based on published matrices of identification resulting from biochemical tests was used to identify isolates from biochemical tests. This was used to provide a consistent approach to identification and to ensure the power of identification battery used. Scores are given in the appendix of the likelihood of each identified isolate.

2.9 Statistical analysis

All statistical analysis was carried out in R version 3.10[157]. Comparisons between continuous distributions first employed the Shapiro-Wilk test[158] to determine determine normality of the distribution; $p<0.05$ indicates a likely non-normal distribution. Comparisons of similarity in normal distributions were undertaken using the Welch unpaired t-test. In non-normally distributed data, similarity was determined using the Mann-Whitney U-test. Standard deviation and mean are stated alongside use of these tests.

2.10 Microbiome analysis

Initial analysis of microbiome data was conducted by the Centre for Genomic Research, University of Liverpool. Analysis of sequence data from 16S amplicon sequencing initially underwent quality filtering using QIIME[159]. This included trimming and removing illumina adaptors and further trimming to remove low quality bases. Sequences were aligned to produce a readpare that should cover the 250bp V4 region. Sequences outside of the range 200bp-350bp were excluded as these likely do not represent the V4 region. Chimeras were then excluded using both reference-based detection (using a 16S database, Greengenes, to find potential chimera sequences which may match) and de-novo chimera calling. OTU picking was then undertaken using QIIME. After this stage, we were given the microbiome data as OTU tables for further analysis. This was carried out in QIIME to produce alpha-diversity statistics using the chao1 measure, a proxy of the rare species count[159] and all further OTU-based analysis was conducted in R 3.10[157].

Chapter 3

Biochemical identification of isolates

3.1 Introduction

The skin is a complex organ which spans 1.8m^2 in the average adult and host to a diverse microbial community which varies greatly according to topography[160]. The skin functions to provide defence against infection, a covering to underlying tissue, temperature regulation, and enables vitamin D synthesis. The skin microbiome varies greatly according to site, largely due to intrinsic structural differences which create distinct niches[160]. Intrinsic differences in the structure of skin include density of glands, presence of hair, and folds due to anatomic site. For example, the axilla provides a humid, unexposed environment with many apocrine sweat glands. As such, the axilla is thought to harbour a microbiome of relatively low diversity compared to dry sites such as the forearm[160]. There is also a difference noted between the "resident" microbiota of the skin, indicated by consistent identification of these commensals over time, and "transient" microbiota of the skin, indicated by inconsistent identification over time in the same subject[161]. The resident microbiota is largely comprised of *Staphylococcus* and *Cornynebacteria*[162]. The skin microbiome exhibits greater variability over time compared to other human niches, rendering the transient microbiota of interest as a potential influencing factor. Although the transient microbiota has not been explored in great depth[160], there is some evidence that transient species can include pathogens such as *S. aureus*, *S. pyogenes*, and *P. aeruginosa*[161, 163]. Extrinsic influences on skin microbiota are wide-ranging, from cosmetics used to exposure to UV light[160]. It

is clear that there is a great deal of inter-individual variation in skin microbiota and that there is likely not to be a shared common "healthy microbiome"[71].

Bacterial identification has been carried out using culture-based techniques for over 100 years, which focused on selective isolation of bacterial colonies followed by identification by staining and biochemical testing for differentiating characteristics of bacterial taxonomy[164]. More recently, automated versions of biochemical testing have been used including strips embedded with many tests specific to a suspected genus or general testing have been established to streamline the diagnostic microbiology laboratory. A prominent example of this product includes the API system[165]. Mass spectrometry-based identification[166], matrix-assisted laser desorption/ionization time of flight (MALDI-TOF), has streamlined the process of identifying bacterial cultures, however this is not currently a widely used technology outside of large tertiary centres and does still rely on selective isolation of colonies for culture, meaning overnight incubation of clinical samples. In addition, MALDI-TOF requires a comprehensive database of reference spectra to accurately identify isolates[167]. More recently, there has been a move towards next-generation sequencing for diagnostic identification of clinical samples, and this has been applied liberally to track outbreaks through identification of single nucleotide polymorphisms in the whole genome sequence[164]. Incorporation of this technology into diagnostic microbiology would enable culture-independent assessment of clinical samples, however cost is currently prohibitory to employing this in routine clinical practice.

This chapter aims to provide an overview of the culture-based microbiota present in CF patients and healthy subjects on a variety of sites at a single time of sampling. Biochemical identification techniques have been employed as this approach remains the gold standard for differentiation of bacterial species[63].

3.2 Patient characteristics

18 CF patients were recruited in total, all during stable disease. As stated in the introduction, CF exacerbation can be described as a sudden increase in symptoms[34] however there is no uniform interpretation of this, so to enable standardised recruitment in this study, patients who were taking back-up antibiotics were excluded. Subjects 1 to 10 were swabbed from ACF, axilla, nare and oropharynx with both culture swabs and Catch-All swabs for DNA extraction. Subjects 14 to 18 were swabbed from forearm, axilla, nare and oropharynx with both culture swabs and Catch-All swabs for DNA extraction. Subjects 11, 12, and 13 were swabbed from ACF and forearm during separate clinic visits in addition to axilla, nare and oropharynx using both culture swabs and Catch-All swabs for DNA extraction. Their characteristics can be seen in Table 3.1.

				Pathogens isolated in previous year						
Subject ID	Sex	CFTR Genotype	Age at sampling	*Staphylococcus*	*H. influenzae*	*P. aeruginosa*	*Streptococcus*	*Candida*	Other significant	
1	M	F508del/F508del	4		x		x		x	
2	M	F508del/G551D	5		x	x				
3	M	5F08del/F508del	6							
4	M	F508del/F508del	9							
5	F	F508del/F508del	10	x	x					
6	F	F508del/exon 10 duplication	12			x		x		
7	F	F508del/F508del	12			x		x		
8	F	F508del/1154insTC	9		x					
9	M	F508del/F508del	4						x	
10	M	F508del/F508del	10		x					
11	F	F508del/G551D	6			x	x		x	
12	F	F508del/3007delG	5		x	x	x		x	
13	M	F508del/F508del	12			x				
14	F	F508del/F508del	3		x	x		x		
15	M	F508del/F508del	0			*				
16	M	F508del/F508del	9	x		x			x	
17	M	F508del/F508del	3	x					x	
18	F	F508del/F508del	7							
36†	M	F508del/R117H (7T)	0							

Table 3.1: CF patients recruited during study period with CFTR genotype, age at sampling and pathogens identified in oropharyngeal swabs or sputum during the previous year (CF Trust data).
∗ indicates cultured during the previous year, however no annual review has taken place.
† indicates subject with equivocal diagnosis.

13 of 18 CF subjects recruited have homozygous F508del CFTR genotype, 2 of 18 CF subjects have F508del/G551D CFTR genotype, one CF subject had F508del with a large exon 10 duplication, one CF subject had F508del/1154insTC, and one had F508del/3007delG. All CF patients were taking anti-staphylococcal antibiotic prophylaxis. None of the patients with G551D mutation were taking ivacaftor as part of treatment. The age of subjects varied between infants (subject 15) and 12 years old (subjects 6, 7 and 13). Culture results from oropharyngeal swabs and sputum samples collected in outpatient clinics and hospital in patient stays during the previous year are displayed. Other significant pathogens cultured include *Haemophilus parainfluenzae*, *Streptococcus pyogenes*, and *Escherichia coli* in subject one; *Pseudomonas putida* in subject nine; *Serratia* in subject 11; and *Streptococcus pnemoniae* and *H. parainfluenzae* in subject 12.

17 age-matched healthy controls were recruited from the surgical clinic at Alder Hey Children's NHS trust. These patients are all aged within two years of their respective CF patients for the microbiome study analysis. These controls had often had previous surgical problems and were followed up in an outpatient clinic so they will have had contact with the hospital environment prior to being recruited. All healthy control subjects were swabbed with culture swabs and Catch-All swabs from forearm, axilla, nare and oropharynx. The differences in swab technique through the study were due to difficulties with low quantities of DNA from swabs of only ACF during the start of the study and this was remedied by using the entire forearm including hand.

One patient (subject 36) was recruited with equivocal diagnosis. This infant had a borderline sweat test following positive newborn screening and was found to have F508del/R117H with a 7 thymidine repeat, a genotype with variable clinical consequence[168].

Table 3.2 shows a summary of CF patients and age-matched controls. 17 age-matched healthy controls were recruited including one subject to pair with subject 36, the patient with equivocal diagnosis.

Cystic fibrosis group				Age-matched healthy controls			
Subject ID	Sex	Age	Genotype	Subject ID	Sex	Age	Sex-match
1	M	4.12	F508del/F508del				
2	M	5.59	F508del/G551D	30	M	6.10	Yes
3	M	6.88	F508del/F508del	33	F	6.27	No
4	M	9.31	F508del/F508del	29	F	8.67	No
5	F	10.02	F508del/F508del	22	F	9.01	Yes
6	F	12.64	F508del/exon 10 duplication	27	M	11.42	No
7	F	12.06	F508del/F508del	35	F	10.34	Yes
8	F	9.06	F508del/1154insTC	34	F	8.25	Yes
9	M	4.35	F508del/3007delG	23	F	5.30	No
10	M	10.74	F508del/F508del	28	F	9.48	No
11	F	6.34	F508del/F508del	21	M	6.19	No
12	F	5.52	F508del/F508del	26	F	5.96	Yes
13	M	12.76	F508del/F508del	24	M	12.60	Yes
14	F	3.84	F508del/F508del				
15	M	0.47	F508del/F508del	31	F	0.21	No
16	M	9.12	F508del/F508del	19	M	8.62	Yes
17	M	3.56	F508del/F508del	25	M	4.83	Yes
18	F	7.95	F508del/F508del	20	M	6.35	No
36†	M	0.65	F508del/R117H(7T)	32	M	0.30	Yes

Table 3.2: Characteristics of CF patients and their age-matched controls.
† indicates subject with equivocal diagnosis.

Table 3.2 shows similar average ages of CF patients and healthy controls, though slightly younger healthy controls and similar ratios of male:female. There is no significant difference of age (t-test, p=0.73) or sex (χ^2, p=0.24).

	CF patients	Healthy controls
Mean age (SD)	7.46 (3.51)	7.05 (3.37)
Median age	7.41	6.35
% male	55.60%	47.10%

Table 3.3: Summary statistics of subjects in CF and control group.

Data on personal hygiene was gathered from 15 of 18 CF patients and 12 of 17 healthy controls recruited. This information could not be collected from some patients due to lack of time given pressures of space for clinical work in the outpatient department, and was not possible to gain through telephone contact. Table 3.2 demonstrates similarities between the CF patients and healthy controls recruited for their use of deodorant and preference for showering or bathing. Frequent use of alcohol gel was reported in 3 of 18 CF patients and not at all in the control group.

	CF patients	Healthy controls
Collected	15 (83%)	12 (71%)
Alcohol gel	3 (20%)	0 (0%)
Deodorant	4 (26%)	3 (25%)
Shower	2 (13%)	2 (16%)
Bath	11 (73%)	9 (75%)
Shower/Bath	2 (13%)	1 (8%)

Table 3.4: Summary statistics of CF patients vs healthy controls where data could be collected on hygeine, proportion using alcohol gel and deodorant.
It also shows the proportions who prefer to shower or bath, and those who bath and shower equally.

The frequency of bathing or showering also appears to be higher in CF patients compared to healthy controls, shown in figure 3.1, however is not statistically significant (χ=5.63, p=0.34). A similar pattern in shown in frequency of handwashing in figure 3.2 where although it appears higher in CF patients it is not statistically significant (χ=4.24, p=0.37). The categories for handwashing frequency were chosen in response to difficulty of families interviewed to quantify handwashing, and many responding with these categories.

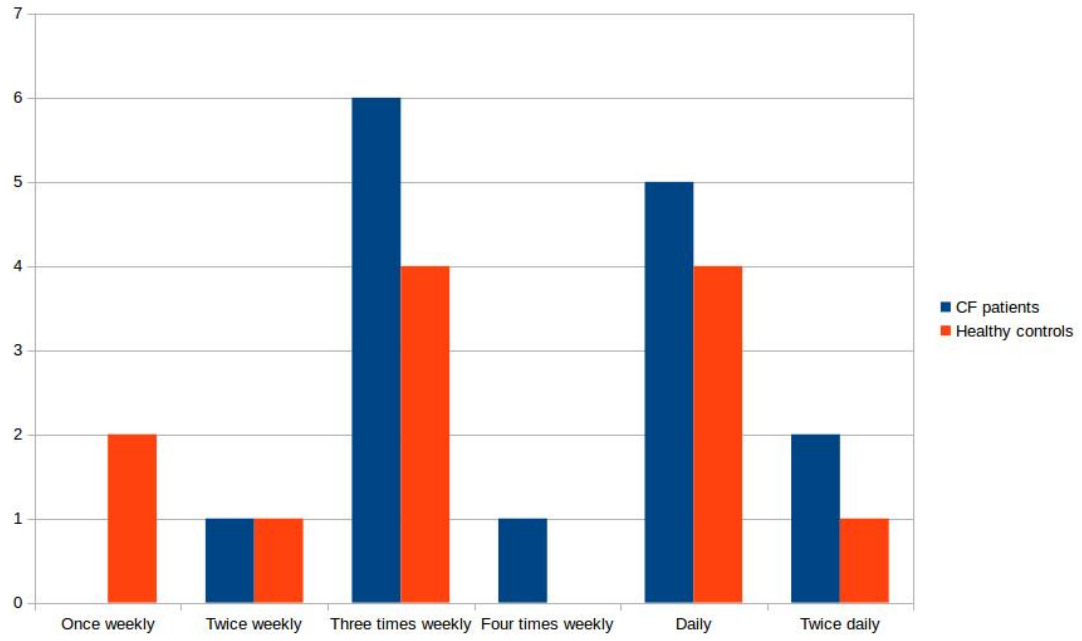


Figure 3.1: Frequency of bathing or showering in CF patients recruited compared to healthy controls.

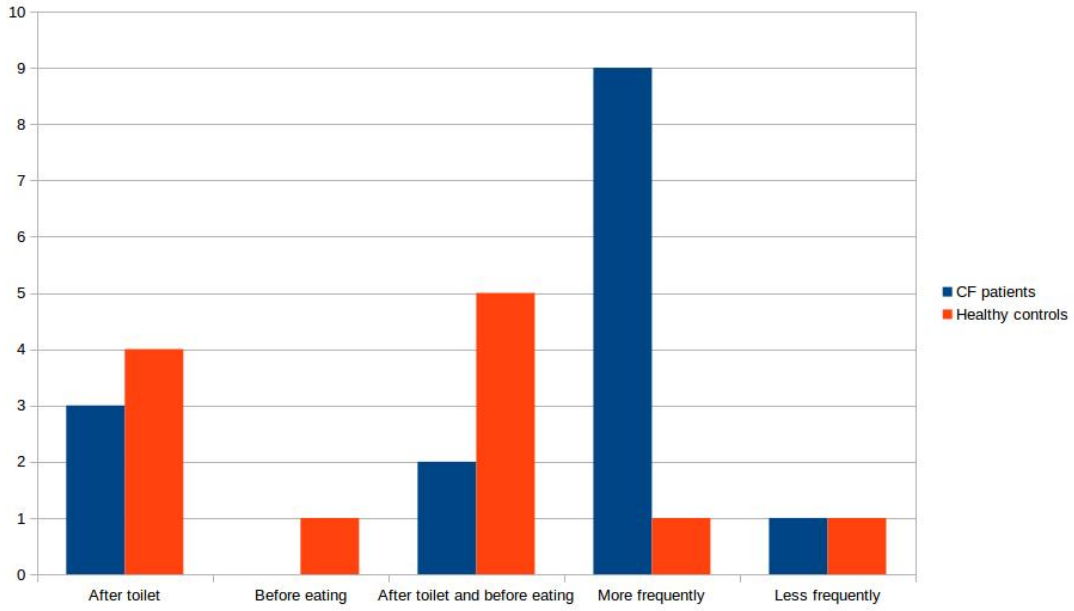


Figure 3.2: Frequency of handwashing in CF patients compared to healthy controls. "Less frequently" refers to less frequently than after toilet or before eating and "more frequently" refers to more frequently than before eating and after toilet.

3.3 Results

3.3.1 Bacterial culture results from CF patients

This chapter contains the bacterial culture results from healthy subjects and subjects with CF. Although many *Staphylococcus* isolates were identified to species level, all apart from *S. aureus* are presented only as the *Staphylococcus* to ease interpretation. Moreover, not all isolates from subjects with forearm, axilla, nare, and oropharynx have been identified given time pressures to complete laboratory work. As such, from these subjects, only aerobic cultures were identified. A full list of bacterial culture and biochemical test results can be found in Appendix D.

Table 3.5 shows culture results from ACF, axilla, nare, and oropharynx in CF patients. *Staphylococcus* was universally present in all samples. *S. aureus* was found in the ACF and axilla of subject 4, and the nare of subject 10.

Subject ID	Anatomic site	*Micrococcus*	*Staphylococcus* (non-*aureus*)	*S. aureus*	*H. influenzae*	*Pseudomonas*	*Streptococcus*	*Candida*	Other significant
1	ACF		x						
1	Axilla		x						
1	Nare		x						
1	Oropharynx		x		c		c		c
2	ACF		x						
2	Axilla		x						
2	Nare		x						
2	Oropharynx		x		c	c			
3	ACF		x						
3	Axilla		x						
3	Nare		x						
3	Oropharynx		x						
4	ACF		x	x					
4	Axilla		x	x					
4	Nare		x						
4	Oropharynx		x						
5	ACF		x						
5	Axilla		x						
5	Nare		x						
5	Oropharynx		x c		c				
6	ACF	x	x				x		
6	Axilla		x						
6	Nare	x	x			x			
6	Oropharynx	x	x			x c		c	x
6	Sput					x c			
7	ACF		x						
7	Axilla		x						
7	Nare		x						
7	Oropharynx		x			c		c	
8	ACF		x						
8	Axilla	x	x						
8	Nare		x						
8	Oropharynx		x		c				
9	ACF		x				x		
9	Axilla		x						
9	Nare		x						
9	Oropharynx		x						c
10	ACF		x						
10	Axilla		x						
10	Nare		x	x			x		
10	Oropharynx		x		c				

Table 3.5: Bacterial culture results from CF patients swabbed from ACF, axilla, nare and oropharynx. "x" indicates isolated on culture during study period while "c" indicates isolated during a clinic visit in the year prior to the study.
Other significant pathogens included: *S. pyogenes*, and *E. coli* isolated from subject one during a clinic visit; *Acinetobacter* and *Arthrobacter* isolated from subject six during the study period. Subject five was found to have MRSA during a clinic visit.

Subject ID	Anatomic site	*Micrococcus*	*Staphylococcus* (non-*aureus*)	*S. aureus*	*H. influenzae*	*Pseudomonas*	Other significant
14	Forearm		x	x			
14	Axilla		x	x			
14	Nare		x				
14	Oropharynx		x		c	c	c
15	ACF		x				
15	Axilla		x				
15	Nare		x				
15	Oropharynx		x				
16	Forearm		x				
16	Axilla		x				
16	Nare		x				
16	Oropharynx	x	x	c		x c	c
17	Forearm	x	x				
17	Axilla		x				
17	Nare		x				
17	Oropharynx		x	c			c
18	Forearm	x	x			x	
18	Axilla		x				
18	Nare		x				
18	Oropharynx		x				
36*	Forearm		x				
36*	Axilla		x				
36*	Nare		x				
36*	Oropharynx		x				

Table 3.6: Bacterial culture results from CF patients swabbed from entire forearm, axilla, nare and oropharynx. "x" indicates isolated on culture during study period while "c" indicates isolated during a clinic visit in the year prior to the study.
Other significant pathogens included: *Candida* isolated from subject 14 during a clinic visit; *M. catarrhalis* and MRSA isolated from subject 16 during a clinic visit; and a coliform isolated from subject 17 during a clinic visit.
$*$ indicates patient with equivocal diagnosis.

Table 3.6 shows culture results from the entire forearm, nare, and oropharynx of CF patients. This distribution of results does not differ greatly from those obtained from just the ACF, and it is clear that *Staphylococcus* is present in all the samples. *P. aeruginosa* was isolated from the oropharynx of patient 16 during the study and on culture during clinic visits in the year prior to the study. Of note, *Pseudomonas* was isolated from the forearm of subject 18, who had no growth of respiratory pathogens from oropharyngeal swabs in the previous year.

Subject ID	Anatomic site	*Micrococcus*	*Staphylococcus* (non-*aureus*)	*S. aureus*	*H. influenzae*	*Pseudomonas*	*Streptococcus*	Other significant
11(1)	ACF	x	x					
11(1)	Axilla		x	x				
11(1)	Nare		x					
11(1)	Oropharynx		x			c	x c	c
11(2)	Forearm		x					
11(2)	Axilla		x					
11(2)	Nare		x					
11(2)	Oropharynx		x			c	c	c
12(1)	ACF		x					
12(1)	Axilla		x					
12(1)	Nare		x					
12(1)	Oropharynx		x		c	c	c	c
12(2)	Forearm	x						
12(2)	Axilla		x	x				
12(2)	Nare		x	x		x		
12(2)	Oropharynx		x		c	c	c	c
13(1)	ACF		x					
13(1)	Axilla		x					
13(1)	Nare		x					
13(1)	Oropharynx	x	x			c		
13(2)	Forearm		x			x		
13(2)	Axilla		x					
13(2)	Nare		x			x		
13(2)	Oropharynx		x			x c		

Table 3.7: Bacterial culture results from CF patients swabbed from ACF and entire forearm at separate clinic visits, in addition to axilla, nare and oropharynx. "x" indicates isolated on culture during study period while "c" indicates isolated during a clinic visit in the year prior to the study.
Other significant pathogens included: *Serratia* isolated from subject 11 during a clinic visit and *S. pneumoniae* isolated from subject 12 during a clinic visit.

Table 3.7 shows culture results obtained from patients initially recruited with swabs of only ACF of the arm taken in addition to axilla, nare and oropharyngeal swabs, and then seen in a further clinic visit with forearm swabs taken in addition to another set of axilla, nare and oropharyngeal swabs. Although subject 11 had cultured *P. aeruginosa* in the previous year, this was not detected on the oropharyngeal swabs obtained during the study. In addition, subject 11 was initially found to have *S. aureus* in the axilla and *Streptococcus* in the oropharynx but neither were isolated at a later date. Subject 12 had cultured *P. aeruginosa* during the previous year, and although this was not cultured in the oropharynx during the study visit, *Pseudomonas* was found in the nare the second time this subject was swabbed in addition to *S. aureus* in the axilla and nare which was absent during the first visit. Subject 13 had cultured *P. aeruginosa* during the previous year and had no growth of *Pseudomonas* from any site during the first study visit, but on the second visit *Pseudomonas* was found on the forearm, nare, and oropharynx.

3.3.2 Bacterial culture results from healthy subjects

Tables 3.8 and 3.9 demonstrate culture results obtained from healthy subjects where the skin swabs are from the ACF and forearm, respectively, and both sets with axilla, nare, and oropharynx. Three healthy controls overall were found to have *Pseudomonas* colonisation (see table 3.9): Subject 22 was found to be colonised in the ACF, axilla and nare; Subject 25 was found to be colonised in the axilla, nare and oropharynx; and subject 26 was found to be colonised in the axilla only. Subject 20 was found to be colonised with *Streptococcus* in the axilla and nare, while subject 26 was found to be colonised with *Acinetobacter* in addition to *Pseudomonas* in the axilla.

Interestingly, similar proportions of CF patients and healthy controls were found to be colonised with *Pseudomonas* during the study period; 5 of 18 CF subjects compared to 3 of 17 healthy controls. The subject with equivocal diagnosis was only found to be colonised with *Staphylococcus* at all sites.

Subject ID	Patient site	*Micrococcus*	*Staphylococcus* (non-*aureus*)	*S. aureus*	*Pseudomonas*	Other significant
14	Forearm		x	x		
14	Axilla		x	x		
14	Nare		x			
14	Oropharynx		x			
15	Forearm		x			
15	Axilla		x			
15	Nare		x			
15	Oropharynx		x			
16	Forearm		x			
16	Axilla		x			
16	Nare		x			
16	Oropharynx	x	x		x	
17	Forearm	x	x			
17	Axilla		x			
17	Nare		x			
17	Oropharynx		x			
18	Forearm	x	x		x	
18	Axilla		x			
18	Nare		x			
18	Oropharynx		x			
19	ACF		x			
19	Axilla		x			
19	Nare		x			
19	Oropharynx		x			
20	ACF		x			
20	Axilla		x			x
20	Nare	x	x			x
20	Oropharynx		x			
21	ACF		x			
21	Axilla	x	x			
21	Nare		x			
21	Oropharynx	x	x			
22	ACF	x	x		x	
22	Axilla	x	x		x	
22	Nare	x	x		x	
22	Oropharynx		x			
23	ACF		x			
23	Axilla		x			
23	Nare		x			
23	Oropharynx		x			
24	ACF		x			
24	Axilla		x			
24	Nare		x			
24	Oropharynx		x			
25	ACF	x	x			
25	Axilla		x		x	
25	Nare	x	x		x	
25	Oropharynx	x	x		x	
26	ACF		x			
26	Axilla		x		x	x
26	Nare	x	x			
26	Oropharynx	x	x			
27	ACF		x			
27	Axilla		x			
27	Nare		x			
27	Oropharynx		x			

Table 3.8: Bacterial culture results from healthy subjects swabbed from ACF, axilla, nare and oropharynx. "x" indicates isolated on culture during study period.
Other significant results included: *Streptococcus* from subject 20 in the axilla and nare and *Acinetobacter* from the axilla of subject 26 during the study period.

Subject ID	Patient site	*Micrococcus*	*Staphylococcus* (non-*aureus*)
28	Forearm		x
28	Axilla	x	x
28	Nare		x
28	Oropharynx		x
29	Forearm		x
29	Axilla		x
29	Nare		x
29	Oropharynx		x
30	Forearm	x	
30	Axilla		x
30	Nare		x
30	Oropharynx		x
31	Forearm		x
31	Axilla		x
31	Nare		x
31	Oropharynx		x
32	Forearm	x	x
32	Axilla		x
32	Nare		x
32	Oropharynx	x	
33	Forearm		x
33	Axilla		x
33	Nare		x
33	Oropharynx		x
34	Forearm	x	x
34	Axilla		x
34	Nare		x
34	Oropharynx		x
35	Forearm		x
35	Axilla		x
35	Nare		x
35	Oropharynx		x
36	Forearm		x
36	Axilla		x
36	Nare		x
36	Oropharynx		x

Table 3.9: Bacterial culture results from healthy subjects swabbed from entire forearm, axilla, nare and oropharynx. "x" indicates isolated on culture during study period.

3.3.3 Culture results by anatomic site

Subject ID	CF	*Micrococcus*	*Staphylococcus (non-aureus)*	*S. aureus*	*Pseudomonas*	*Streptococcus*
1	Y		x			
2	Y		x			
3	Y		x			
4	Y		x	x		
5	Y		x			
6	Y	x	x			x
7	Y		x			
8	Y		x			
9	Y		x			x
10	Y		x			
11(1)	Y	x	x			
12(1)	Y		x			
13(1)	Y		x			
19	N		x			
20	N		x			
21	N		x			
22	N	x	x		x	
23	N		x			
24	N		x			
25	N	x	x			
26	N		x			
27	N		x			

Table 3.10: Bacterial culture results from the ACF of both CF patients and healthy controls. "x" indicates isolated on culture during study period. (1) indicates the isolates were obtained during the first study visit of these subjects.

Bacterial culture results from only the ACF (see table 3.10) indicate a slight increase in *Streptococcus* colonisation in CF patients compared to healthy controls (2 of 13 CF subjects compared to 0 of 13 healthy subjects). However, the only *Pseudomonas* colonisation was found to be in one healthy subject.

Subject ID	CF	*Micrococcus*	*Staphylococcus* (non-*aureus*)	*S. aureus*	*Pseudomonas*	Other significant
1	Y		x			
2	Y		x			
3	Y		x			
4	Y		x	x		
5	Y		x			
6	Y		x			
7	Y		x			
8	Y	x	x			
9	Y		x			
10	Y		x			
11(1)	Y		x	x		
11(2)	Y		x			
12(1)	Y		x			
12(2)	Y		x	x		
13(1)	Y		x			
13(2)	Y		x			
14	Y		x	x		
15	Y		x			
16	Y		x			
17	Y		x			
18	Y		x			
19	N		x			
20	N		x			x
21	N	x	x			
22	N	x	x		x	
23	N		x			
24	N		x			
25	N		x		x	
26	N		x		x	x
27	N		x			
28	N	x	x			
29	N		x			
30	N		x			
31	N		x			
32	N		x			
33	N		x			
34	N		x			
35	N		x			
36	N		x			

Table 3.11: Bacterial culture results from the axilla of both CF patients and healthy controls. "x" indicates isolated on culture during study period. (1) indicates the isolates were obtained during the first study visit while (2) indicates the isolates were obtained during the second study visit.
Other results found include *Streptococcus* from subject 20 and *Acinetobacter* from subject 26.

Bacterial culture results from only the axilla (see table 3.11) indicate distinct differences between colonisation of the CF subjects and healthy subjects. *S. aureus* is only seen in the CF subjects, and in observing subjects 11, 12, and 13 where sequential samples were obtained, it does not appear to be constantly present in the samples. *Pseudomonas* is only found in the axilla of healthy subjects in this study, and in addition *Acinetobacter* and *Streptococcus* were isolated from the axilla of healthy subjects.

Bacterial culture results from the nare (see table 3.12) indicate marked differences between CF and healthy subjects. Like in the axilla, *S. aureus* is only present in the CF subjects and in the subjects with sequential samples (subjects 11, 12, and 13), was not present in both. *Pseudomonas* was present in both CF subjects and healthy controls, and like *S. aureus*, was not seen on both occasions where sequential isolates were obtained. *Streptococcus* was seen in one subject in each group. There appears to be a slightly higher proportion of healthy subjects who have nasal colonisation with *Micrococcus* compared to CF subjects (4 of 17 compared to 1 of 18).

Subject ID	CF	*Micrococcus*	*Staphylococcus* (non-*aureus*)	*S. aureus*	*Pseudomonas*	Streptococcus
1	Y		x			
2	Y		x			
3	Y		x			
4	Y		x			
5	Y		x			
6	Y	x	x		x	
7	Y		x			
8	Y		x			
9	Y		x			
10	Y		x	x		x
11(1)	Y		x			
11(2)	Y		x			
12(1)	Y		x			
12(2)	Y		x	x	x	
13(1)	Y		x			
13(2)	Y		x		x	
14	Y		x			
15	Y		x			
16	Y		x			
17	Y		x			
18	Y		x			
19	N		x			
20	N	x	x			x
21	N		x			
22	N	x	x		x	
23	N		x			
24	N		x			
25	N	x	x		x	
26	N	x	x			
27	N		x			
28	N		x			
29	N		x			
30	N		x			
31	N		x			
32	N		x			
33	N		x			
34	N		x			
35	N		x			
36	N		x			

Table 3.12: Bacterial culture results from nares of both CF patients and healthy controls. "x" indicates isolated on culture during study period. (1) indicates the isolates were obtained during the first study visit while (2) indicates the isolates were obtained during the second study visit.

Bacterial culture results from the forearm (see table 3.13) demonstrated carriage of potential pathogens only in CF patients. *S. aureus* was isolated from subject 14, while *Pseudomonas* was isolated from subject 13 on the second study visit and subject 18.

Bacterial culture results from oropharyngeal swabs and sputum samples of CF subjects and healthy controls (see table 3.14 demonstrate colonisation with *Pseudomonas* in two subjects with CF and two healthy subjects. In subject 6, *Pseudomonas* was cultured from both the oropharyngeal swab and a sputum sample and subject 13 was found to have *Pseudomonas* following the second study visit but not the first. In addition to colonisation with *Pseudomonas*, subject 6 was found to have *Acinetobacter* and *Arthrobacter*. Subject 11 was found to have *Streptococcus* colonisation during the first study visit but not the second.

Subject ID	CF	*Micrococcus*	*Staphylococcus* (non-*aureus*)	*S. aureus*	*Pseudomonas*
11(2)	Y		x		
12(2)	Y	x			
13(2)	Y		x		x
14	Y		x	x	
15	Y		x		
16	Y		x		
17	Y	x	x		
18	Y	x	x		x
28	N		x		
29	N		x		
30	N	x			
31	N		x		
32	N	x	x		
33	N		x		
34	N	x	x		
35	N		x		
36	N		x		

Table 3.13: Bacterial culture results from forearms of both CF patients and healthy controls. "x" indicates isolated on culture during study period. (2) indicates the isolates were obtained during the first study visit from these subjects.

Subject ID	CF	*Micrococcus*	*Staphylococcus* (non-*aureus*)	*S. aureus*	*Pseudomonas*	Other significant
1	Y		x			
2	Y		x			
3	Y		x			
4	Y		x			
5	Y		x			
6	Y	x	x		x	x
6*	Y				x	
7	Y		x			
8	Y		x			
9	Y		x			
10	Y		x			
11(1)	Y		x			x
11(2)	Y		x			
12(1)	Y		x			
12(2)	Y		x			
13(1)	Y	x	x			
13(2)	Y		x		x	
14	N		x			
15	N		x			
16	N	x	x		x	
17	N		x			
18	N		x			
19	N		x			
20	N		x			
21	N	x	x			
22	N		x			
23	N		x			
24	N		x			
25	N	x	x		x	
26	N	x	x			
27	N		x			
28	N		x			
29	N		x			
30	N		x			
31	N		x			
32	N	x				
33	N		x			
34	N		x			
35	N		x			
36	N		x			

Table 3.14: Bacterial culture results from the oropharynx and sputum of both CF patients and healthy controls. "x" indicates isolated on culture during study period. (1) indicates the isolates were obtained during the first study visit while (2) indicates the isolates were obtained during the second study visit.
Other significant results include *Acinetobacter* and *Arthrobacter* in the oropharyngeal swab of subject six, and *Streptococcus* from the first study visit of subject 11.
$*$ indicates sputum sample.

3.3.4 Overall summary of bacterial culture results

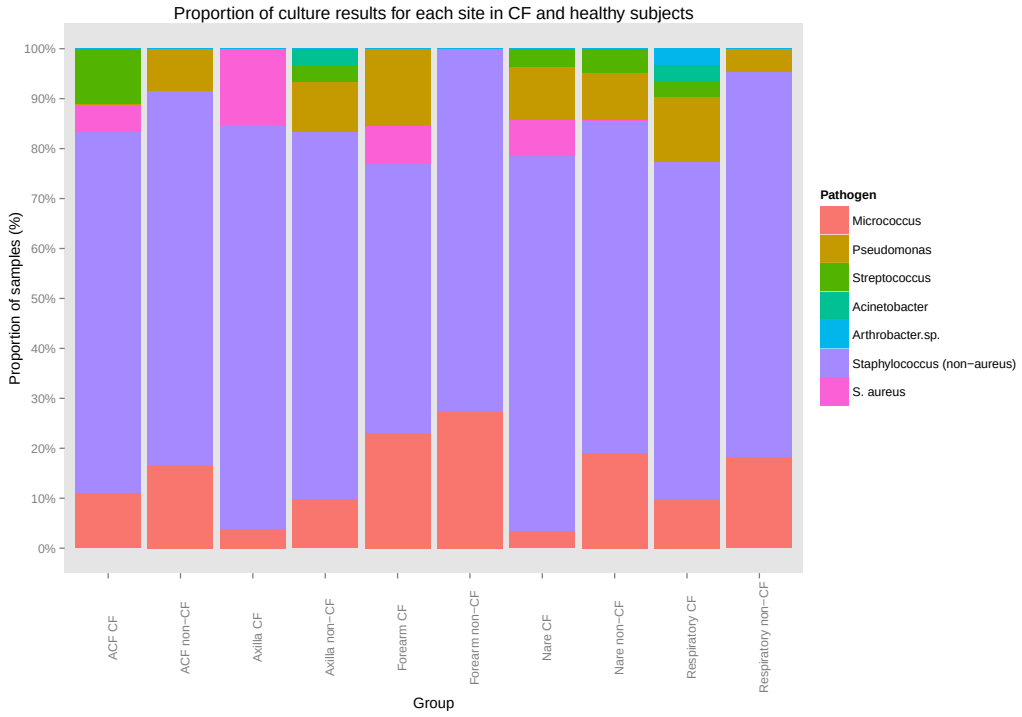


Figure 3.3: 100% stacked bar chart demonstrating the proportion of culture results by site in both CF and healthy subjects. This data includes sequential samples from the same patients.

Figure 3.3 demonstrates the differences between commensals isolated in CF and healthy subject sites. Although there appears to be a general background of *Staphylococcus* and *Micrococcus*, there appears to be some differences between CF and healthy subjects for those genera isolated less frequently. There were significant differences between CF patients and healthy controls in the axilla (χ=7.98, p=0.02) but not in ACF (χ=3.67, p=0.45), nare (χ=3.95, p=0.27), forearm (χ=2.92, p=0.40), or respiratory samples (χ=1.70, p=0.43).

Interestingly,*S. aureus* was isolated from the skin and nares of some CF patients in this study but not from any healthy controls. *S. aureus* was not isolated from the oropharynx or sputum of any subject in this study.

3.4 Discussion

This study has demonstrated a very similar microbiota between healthy children and children with CF, both demonstrating transient colonisation with potential pathogens. All sites were shown to be similar between groups apart from the axilla, however these findings should be viewed with caution given the low sample numbers used in these Chi-squared tests. This study demonstrated some CF patients carried *S. aureus* on their skin and nare whereas no healthy controls did. Given that this population of CF patients is constantly being treated with *S. aureus* antibiotic prophylaxis, this finding merits further investigation. Unfortunately due to the high cost of rabbit plasma the gold-standard test for *S. aureus*, the tube coagulase test[169], could not be used and *S. aureus* was presumptively identified by microscopy and other biochemical methods. This limits the specificity of identification and could mean some mannitol-fermenting Gram-positive cocci identified in the CF population might not be *S. aureus*. Larger numbers of participants and more appropriate methods for identification of *S. aureus* would be needed to confirm this potentially important finding.

It has been generally accepted that pathogens transiently colonise human skin[161], and that direct contact between CF patients is the most likely route of transmission[170]. However, these views are only given as a result of culture-based studies on bacteria isolated from the hands of CF patients without any healthy controls[153, 154]. This study demonstrates that not only is *Pseudomonas* sometimes present alone in skin sites such as the axilla, without being present on the hands, but also that a very similar pattern is seen in healthy children which has not been demonstrated previously. It is clear from this study that in the few subjects where sequential isolates were taken, these are transient microbiota as they are not always present in the same site, however a much greater sample size would be needed to determine this with certainty.

This study could not accurately identify many isolates to species level, detracting from the ability to accurately determine the commensal bacteria of CF patients and healthy controls on culture. Moreover, it is clear from inspection of the biochemical tests that many more isolates than suspected by the probabalistic model (Pibwin) are likely to be *S. aureus* (only identified to their genus), as evidenced by Gram-positive

fermenting cocci which turn the phenol-red indicator in mannitol salt agar yellow. In addition, identification matrices do not take into all common indicators, for example, colony morphology and haemolysis were not included. These are clear disadvantage of using a computational model based on published matrices, however it does detect that the culture-based biochemical tests undertaken for identification are likely to be underpowered and that further tests are necessary for species level identification.

This approach, however, may be useful in identifying a representative number of suspected pathogens which could be further identified and characterised for this study and does enable a consistent approach to be taken across all isolates. A more comprehensive approach to biochemical testing for this study would have been too burdensome given the number of samples and of limited value in providing conclusions about the skin microbiota given that culture-based studies underestimate the bacterial diversity present[160]. The benefits of limited identification, does however allow for putative identification of pathogens and builds a large collection of strains for further characterisation.

This study made use of selective media (*Pseudomonas*-specific and *Staphylococcus*-specific) which increases the sensitivity of culture-based studies in identifying pathogens[171], and also eased identification of cultures likely to be *P. aeruginosa*.

Definitions of species still largely revolve around biochemical tests to differentiate from other similar genera[63], and as such the biochemical approach to bacterial identification still remains the gold standard compared to newer high-throughput methods, such as mass spectroscopy-based methods[166]. In addition for a large number of samples such as this, it is possible to undertake a large number of biochemical tests simultaneously using 96 well plates. An issue in doing so is the propensity for contamination, especially with spore-forming bacteria, when compared to performing biochemical tests in Durham tubes, for example. The issues of contamination, can however be controlled with blank wells as a negative control. In addition, this method of testing still remains more cost-effective compared to use of biochemical test strips, such as the API system, or MALDI-TOF for a large number of samples where only putative identification of pathogens is necessary.

MALDI-TOF could form part of further work to fully characterise these strains but unfortunately the local facility was prohibitively expensive for this study. If it were available it would be the preferred method for performing high-throughput identification of bacterial cultures. Due to the high proportion of commensals, however it would be necessary to ensure the reference spectra include the expected skin flora for accurate identification[167] as pathogens would be expected to comprise the predominant fraction of the reference database. Studies on fungal isolates were not completed, and this would form part of later work to fully characterise the entire microbial landscape of CF skin, in addition to further work examining the characteristics of the bacterial isolates, not only to identify them but demonstrate any differences in behaviour. Bacterial characteristics of interest would include quorum sensing to determine how they are acting in the community[172], antibiotic resistance profiling, and virulence determination using a high-throughout model such as *Galleria*[173] to determine if there are differences between pathogens isolated in different sites, a marker of evolution within the host[174].

Chapter 4

Optimisation of DNA extraction from swabs

4.1 Introduction

Bacterial DNA extraction protocols can be broadly split into those that employ a kit, those that use buffers which can be prepared in any laboratory, and those that employ a phenol-chloroform method. Due to thick cell wells, particularly of Gram-positive bacteria, extraction of DNA from bacteria often requires enzymatic and mechanical disruption[175].

In microbiome studies, a significant degree of bias can be introduced during the DNA extraction as this can influence the community composition markedly. There are many commercial kits available for DNA extraction, each using a slightly different method which can impact on community composition, and as such, it is important to use the same kit throughout the study[175]. In addition, commercial kits often have a microbiome of their own which means it is important to have negative controls included in the sequencing run[176]. Even if negative controls are included, it is not clear how best to handle this data in the analysis; although those taxa appearing in the kits could be excluded from the samples, the bacterial sequences detected in the kit are likely to be ubiquitous in the environment as well. Phenol-chloroform methods do not suffer this issue since phenol is a harsh environment for bacteria to survive, however any DEPC water used downstream can also carry contaminants. Beadbeating is a mechanical process whereby the sample is placed in a tube with small beads in, which is then shaken vigorously by a beadmill, a device designed to perform beadbeating.

Beadbeating leads to better representation of the community structure of microbiomes and improves yield through greater disruption of cell walls, however it has been criticised for increasing shearing of DNA, which can be reduced by beadbeating for shorter time periods[175]. As such, beadbeating is included in the DNA extraction.

Skin microbiota is notoriously difficult to extract DNA from[160], and as such optomisation of the method is necessary. Through quantitative culture, it has been demonstrated that moist areas of skin, such as the axilla, have a higher bacterial load compared to dry areas, such as the volar forearm[160]. This suggests that the lowest DNA yields in this study will be from the ACF and forearm.

This chapter aims to determine the most appropriate method for DNA extraction from swabs of different skin sites, nare, and oropharynx, so that results of amplicon sequencing are deemed comparable. Swabs tested were obtained from healthy adults within the laboratory group. All comparisons were made between groups with at least three swabs from each. It will determine a balanced protocol between yields given from each kit and deviations from the kit protocols investigated for each site to enable pooling of these samples. Only commercial kits are used in this study due to safety issues surrounding use of phenol-chloroform methods.

4.2 Results

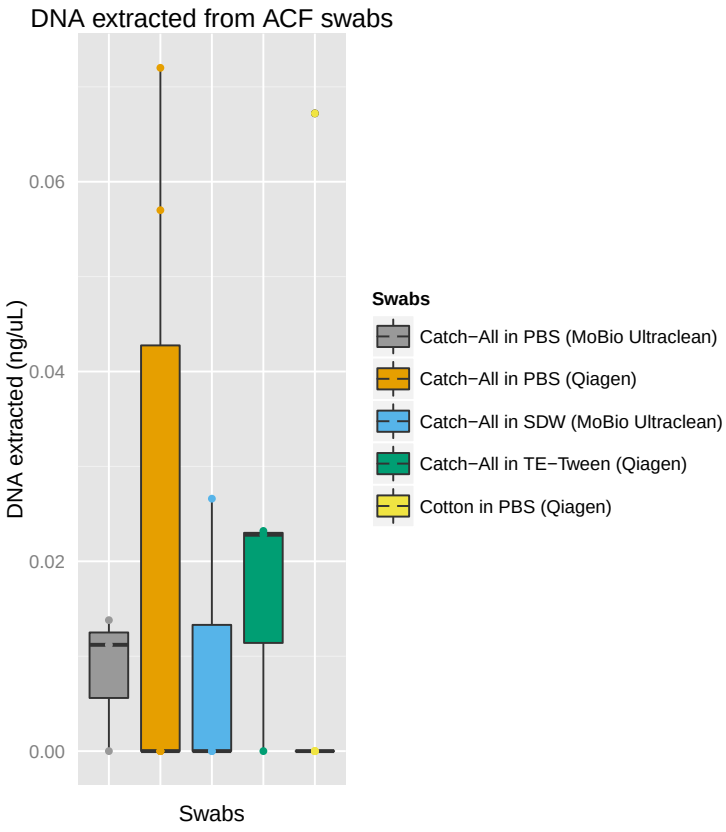


Figure 4.1: Comparison of DNA extraction kits, swab types and swab moisening reagents on DNA yields from swabs of antecubital fossa.
Comparison includes Catch-All in PBS (MoBio Ultraclean) (n=3, mean (μ)=0.008ng/μL, standard deviation (σ)=0.007), Catch-All in PBS (Qiagen) (n=6, μ=0.022ng/μL, σ=0.034), Catch-All in SDW (MoBio Ultraclean) (n=3, μ=0.009ng/μL, σ=0.015), Catch-All in TE-Tween-20 (Qiagen) (n=3, μ=0.015ng/μL, σ=0.013), and Cotton in PBS (Qiagen) (n=5, μ=0.013ng/μL, σ=0.030).
Qiagen refers to QIAamp DNA minikit.
MoBio Ultraclean refers to MoBio Ultraclean Microbial DNA Isolation kit.

Figure 4.1 demonstrates differences in DNA quantity yielded from different kits using different buffers to moisten the swabs prior to rubbing on the skin. After each swab is obtained, the manufacturer's instructions as per the kit (for the Qiagen QIAamp kit, the Tissue protocol was followed) were followed with all additional steps suggested in the manufacturer's protocol which increase DNA yields.

Although figure 4.1 demonstrates some samples using Catch-All swabs moistened

with PBS in the Qiagen kit gained some of the highest DNA yields, the majority of these samples yielded DNA quantities beneath the detectable range (66.6%, n=6). 33.3% (n=3) of Catch-All swabs moisened with PBS in the MoBio Ultraclean kit were beneath detectable range. 66% (n=3) of Catch-All swabs moisened in SDW in the MoBio Ultraclean kit had were beneath detectable range. 33.3% (n=3) of Catch-All swabs moisened in TE-Tween-20 buffer and processed in the Qiagen kit were beneath detectable range, and 80% (n=5) of cotton swabs in PBS processed in the Qiagen kit were beneath detectable range of DNA. There are no significant differences in this comparison (Mann-Whitney U-test: W=8, p=0.888 between distributions with greatest differences in mean).

As such, it appears that the two approaches which give provide a balance between DNA yield and reproducibility for extracting DNA from ACF swabs are Catch-All swabs moistened in PBS using the MoBio Ultraclean Microbial DNA Isolation kit and Catch-All swabs moistened in TE-Tween-20 using the Qiagen QIAamp DNA Extraction kit.

Figure 4.2 demonstrates differences in DNA yields between different moistening agents and swab types for DNA extraction of axilla swabs with a Qiagen QIAamp kit following the Tissue protocol otherwise. It appears that the most acceptable yields are obtained from Catch-All swabs moistened in PBS or TE-Tween-20, however the plot of Catch-All swab moistened with TE is skewed by one data point; 66.6% (n=3) had DNA yield below detectable limits. Only 20% (n=5) of Catch-All swab in PBS were beneath detectable DNA limits. 66.6% (n=3) cotton swabs moistened with PBS were beneath detectable limits and 100% (n=3) of cotton moistened with TE-Tween were beneath detectable limits. There were significant differences between Catch-All swabs in PBS (Qiagen) and both cotton in PBS (Qiagen) and cotton in TE-Tween-20 (Qiagen) (Student t-test: t=-3.58, p=0.016 and t=-2.58, p=0.036 respectively) but not Catch-All in TE-Tween (Qiagen) (Student t-test: t=-0.394, p=0.723).This suggests that for swabs from the axilla, Catch-All swabs moistened with PBS give the most acceptable results when using the Qiagen QiaAMP kit.

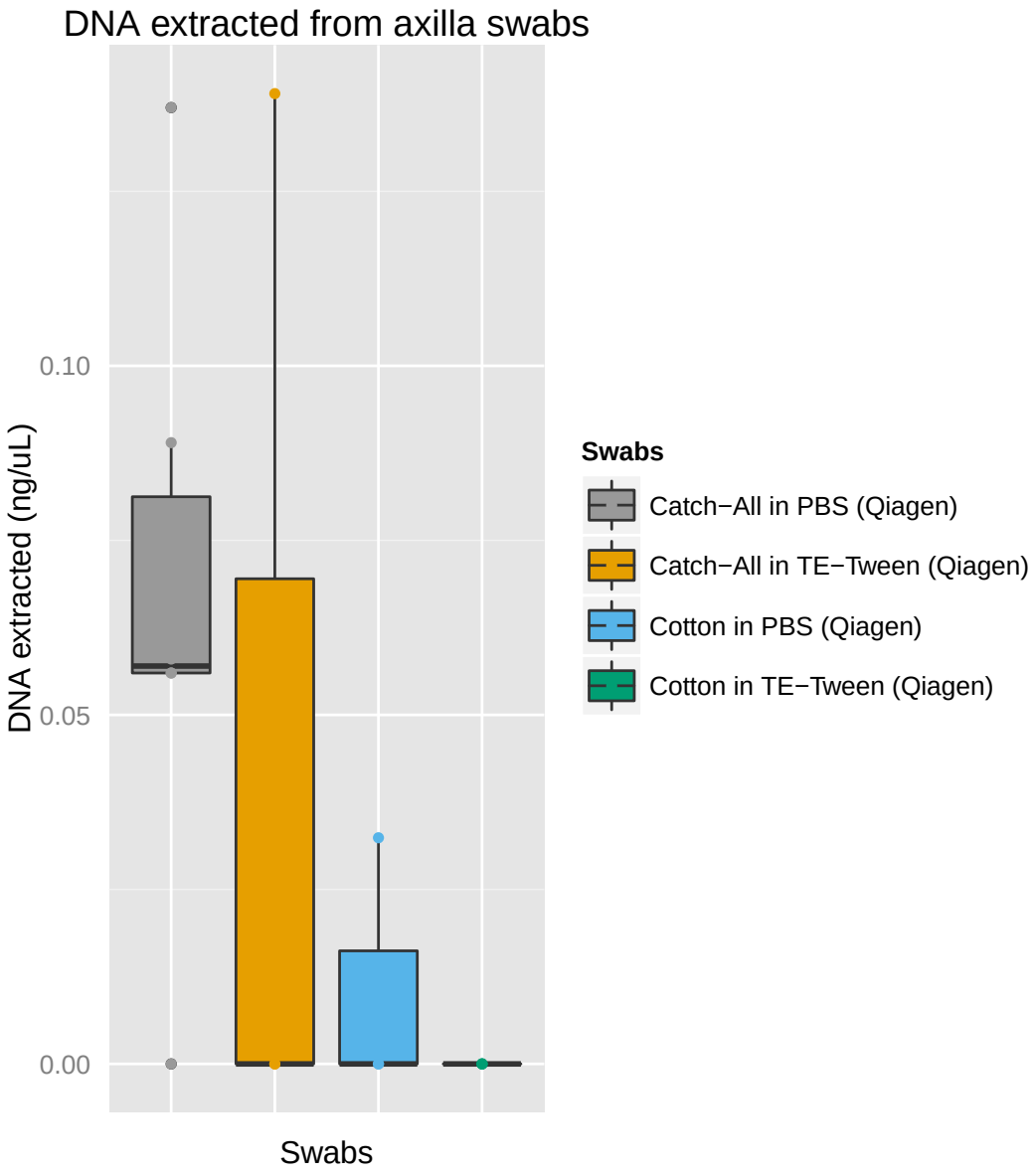


Figure 4.2: Comparison of swab types and swab moisening reagents on DNA yields from axilla swabs.
Comparison includes Catch-All in PBS (Qiagen) (n=5, μ=0.066ng/μL, σ=0.045), Catch-All in TE-Tween-20 (Qiagen) (n=3, μ=0.046ng/μL, σ=0.080), Cotton in PBS (Qiagen) (n=3, μ=0.011ng/μL, σ=0.019), and Cotton in TE-Tween-20 (Qiagen) (n=3, μ=0ng/μL, σ=0).

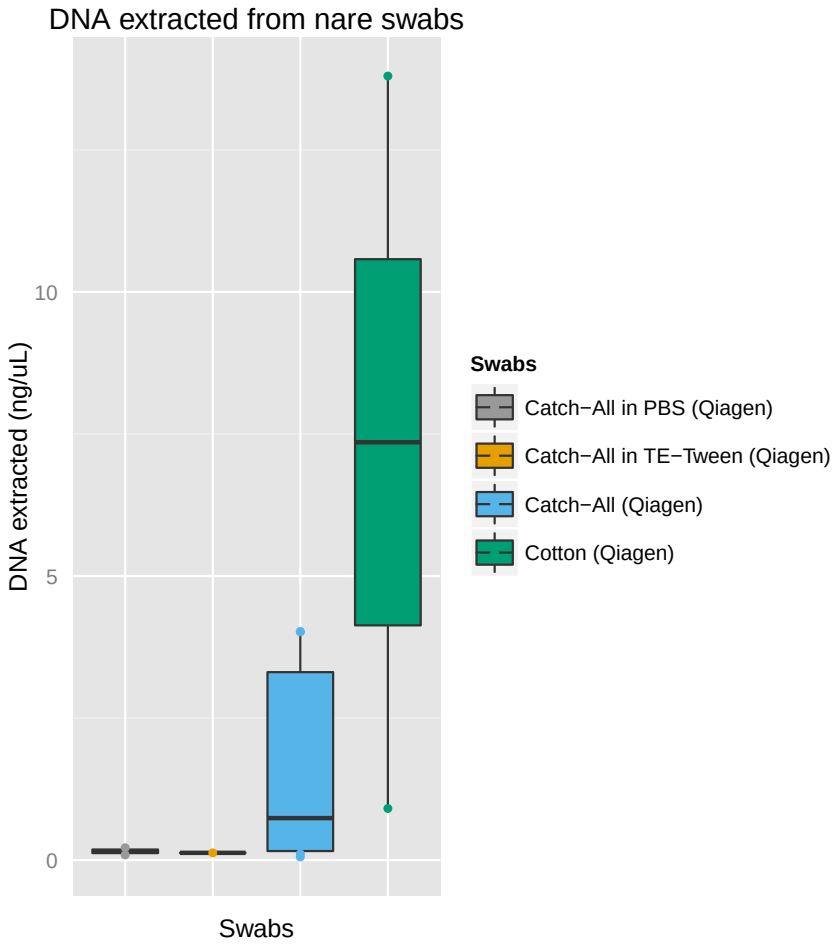


Figure 4.3: Comparison of DNA extraction kits and swab types on DNA yields for nare swabs.
Comparison includes Catch-All in PBS (Qiagen) (n=3, μ=0.154ng/μL, σ=0.090), Catch-All in TE-Tween-20 (Qiagen) (n=3, μ=0.127ng/μL, σ=0.015), Catch-All dry (Qiagen) (n=6, μ=1.61ng/μL, σ=1.91), and Cotton dry (Qiagen) (n=2, μ=7.35ng/μL, σ=9.12).

Figure 4.3 demonstrates differences in DNA yields between different moistening agents and swab types for DNA extraction of nare swabs with a Qiagen QiaAMP kit following the Tissue protocol otherwise. There were no significant differences between this comparison (Mann-Whitney U-test: W=9, p=0.0765 between distributions with greatest differences in mean) although there is a trend to suggest that the most acceptable yields are obtained from the dry cotton and Catch-All swabs, compared to using a moistening agent for the nare. All swab samples tested had DNA levels above the

detectable range. Samples from the nare gave higher DNA yields compared to the skin swabs, with no issues of variability.

Figure 4.4 demonstrates differences in DNA yields between different swab types and DNA extraction kits for oropharyngeal swabs. 40% (n=5) of Catch-All swabs using the MoBio Ultraclean kit had undetectable levels of DNA, 11.1% (n=9) of Catch-All swabs using the Qiagen kit had undetectable levels of DNA, 0% (n=3) of the cotton swabs using MoBio Ultraclean kit had undetectable DNA levels, and 0% (n=3) of cotton swabs using the Qiagen kit had undetectable DNA levels. Catch-All (Qiagen) had a significantly higher yield than Catch-All (MoBio Ultraclean) (Mann-Whitney U-test: W=44.5, p=0.0432) but it did not have a significantly higher yield compared to cotton (MoBio Ultraclean) or cotton (Qiagen) (Mann-Whitney U-test: W=22, p=0.146 and W=17, p=0.6 respectively). It appears that the Catch-All swab using a Qiagen kit gives the greatest DNA yield for oropharyngeal swabs. Samples from the oropharynx gave a higher DNA yield compared to skin swabs.

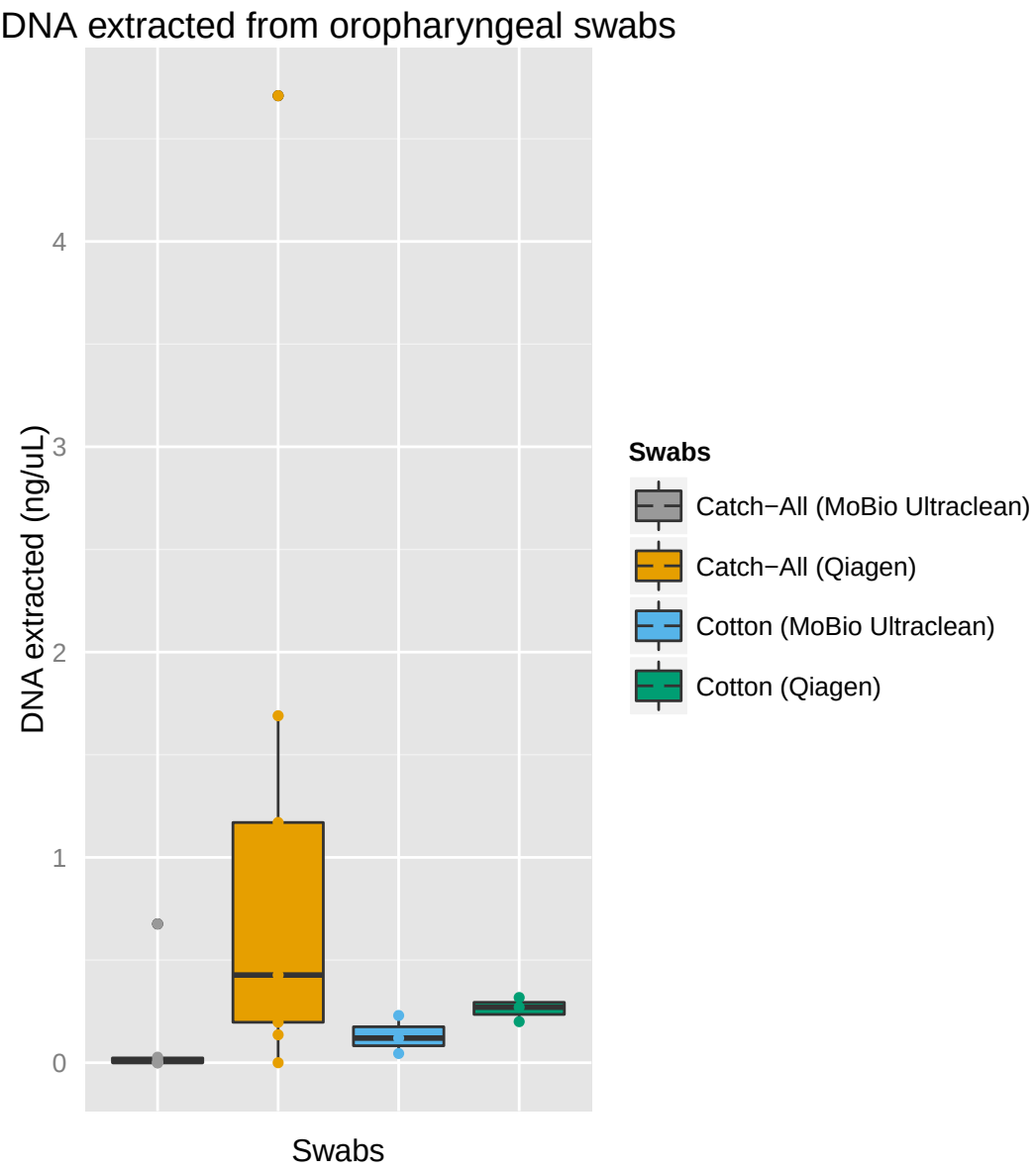


Figure 4.4: Comparison of DNA extraction kits and swab types on DNA yields for oropharyngeal swabs.
Comparison includes dry swabs of Catch-All (MoBio Ultraclean) (n=5,μ=0.119ng/μL, σ=0.273) Catch-All (Qiagen) (n=9, μ=1.03ng/μL, σ=1.48), Cotton (MoBio Ultraclean) (n=3, μ=0.132ng/μL, σ=0.093), Cotton (Qiagen) (n=3, μ=0.263ng/μL, σ=0.059).

The protocols from Qiagen for the QiaAMP minikit include a Buccal swab protocol, in addition to the Tissue protocol. Previously, we have used the Tissue protocol with success for processing oropharyngeal swabs. The major difference in the buccal swab protocol surrounds using greater quantities of buffer AL to ensure sufficient cell lysis in the larger sample volume. There were no significant differences on DNA yields between buffer volumes for ACF (Mann-Whitney U-test: W=11, p=0.195), axilla (Mann-Whitney U-test: W=8, p=0.082), nare (Mann-Whitney U-test: W=9, p=0.275), and oropharynx (Mann-Whitney U-test: W=23, p=0.536). Greater volumes of buffer AL lead to greater DNA yields using the Qiagen kit (see figure 4.5).

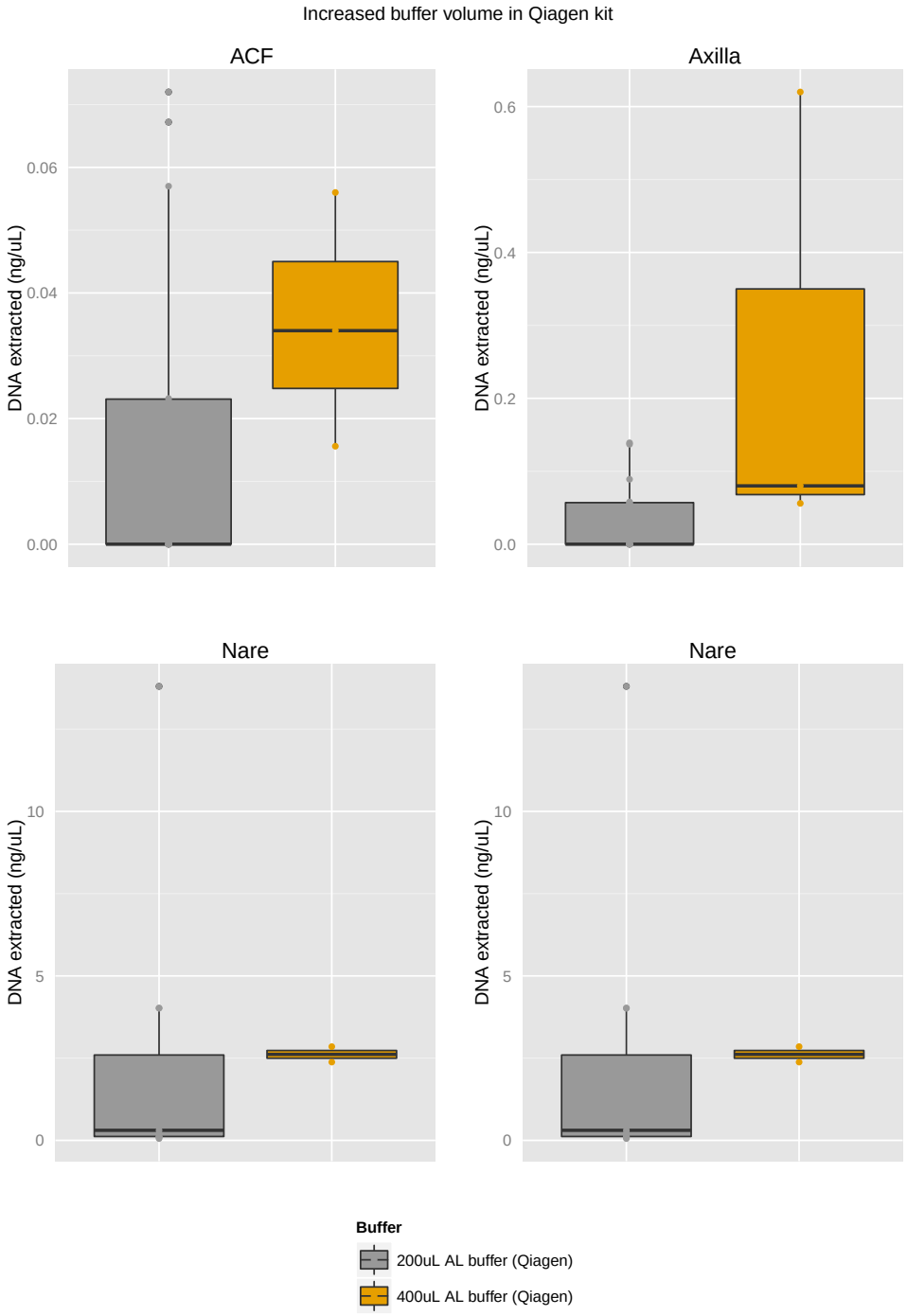


Figure 4.5: Effect of increased buffer volumes in Qiagen kits on DNA yield. Comparison is of swabs all processed using the Qiagen kit. Swabs were from ACF with 200μL AL (n=8, μ=0.017ng/μL, σ=0.027), ACF with 400μL AL (n=3, μ=0.035ng/μL, σ=0.020), axilla with 200μL AL (n=9, μ=0.038ng/μL, σ=0.050), axilla with 400μL AL (n=3, μ=0.252ng/μL, σ=0.319), nare with 200μL AL (n=10, μ=2.257ng/μL, σ=4.112), nare with 400μL AL (n=3, μ=2.490ng/μL, σ=0.320), oropharynx with 200μL AL (n=9, μ=0.842ng/μL, σ=1.311), and oropharynx with 400μL AL (n=3, μ=0.265ng/μL, σ=0.125).

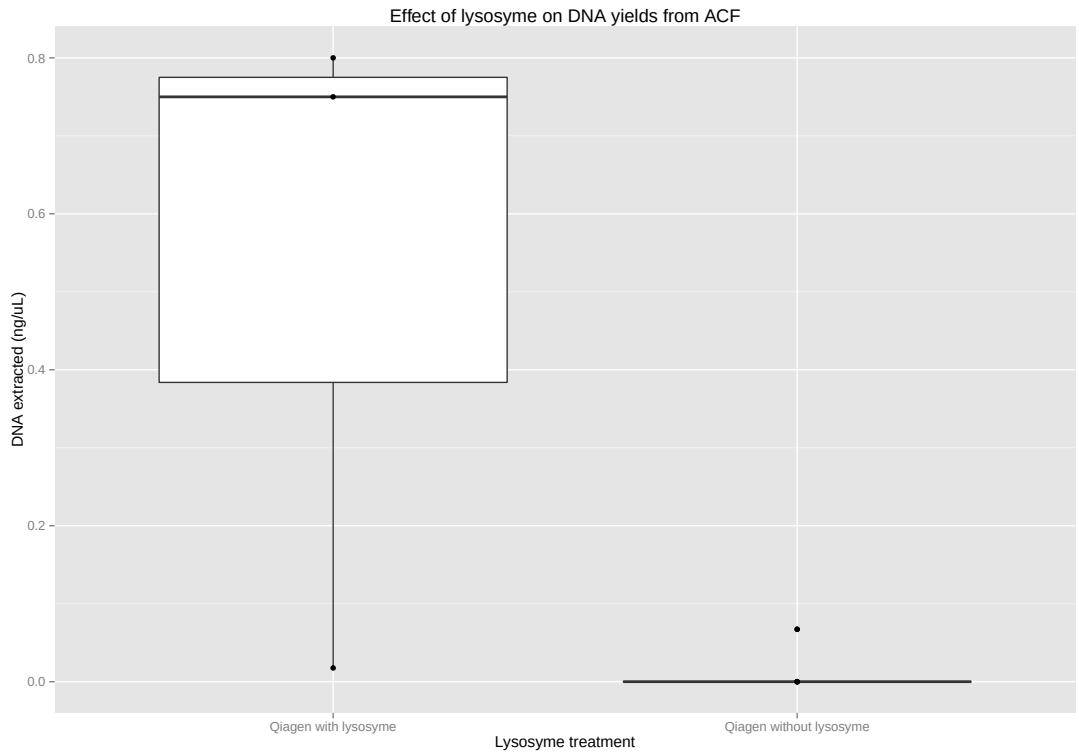


Figure 4.6: Effect of 4μL ReadyLyse lysosyme (MoBio) on DNA yields.
Comparison includes ACF swabs with lysosyme (n=3, μ=0.523ng/μL, σ=0.438) and ACF swabs without lysosyme (n=5, μ=0.013ng/μL, σ=0.030). All swabs moistened with PBS.

DNA yields from ACF swabs with 18 hour incubation with 4μL of ReadyLyse lysosyme (MoBio) prior to extraction following manufacturer's instructions ensures much greater DNA yield than without lysosyme in ACF swabs using the Qiagen kit (see figure 4.6). There were no significant differences in DNA yields between ACF swabs treated with lysosyme and those not treated with lysosyme (Mann-Whitney U-test: W=14, p=0.057).

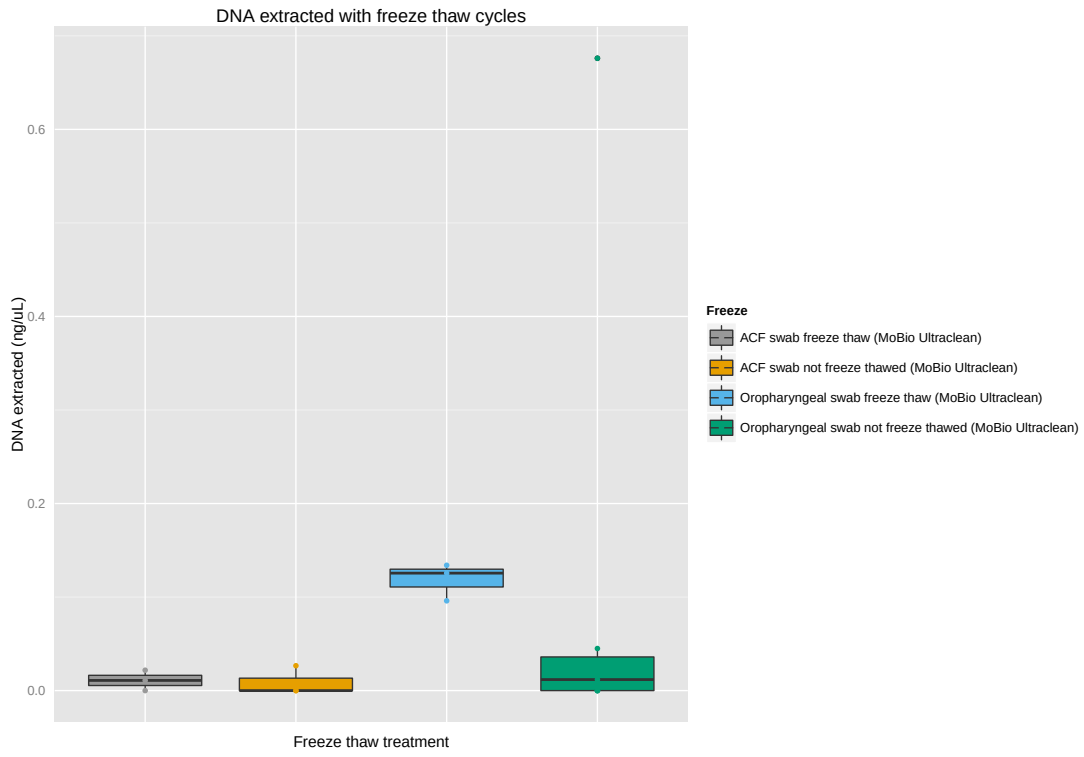


Figure 4.7: Effect of three cycles of freeze thawing on DNA yields from swabs. Snap freezing performed in ethanol-dry ice slurry followed by thawing in a 37°C heating block for 10 minutes.
Comparison includes ACF swabs freeze thawed (n=3, μ=0.011ng/μL, σ=0.011) and not freeze thawed (n=3, μ=0.009ng/μL, σ=0.015), and oropharynx swabs freeze thawed (n=3, μ=0.119ng/μL, σ=0.020) and not freeze thawed (n=5, μ=0.109ng/μL, σ=0.251). All swabs moistened with PBS.

Figure 4.7 demonstrates that freeze thawing decreases the yield from MoBio Ultraclean kits for both ACF swabs (representing a low DNA yielding sample type) and oropharyngeal swabs (representing a high DNA yielding sample type). There were no significant differences in DNA yield between samples freeze-thawed and those not freeze-thawed in both ACF and oropharynx swabs (Mann-Whitney U-test: W=5, p=1 and W=18, p=0.106 respectively).

Figure 4.8 demonstrates the DNA yields possible from swabs of the entire forearm with different MoBio kits using the Catch-All swab moistened in different reagents. There were no significant differences between yields in forearm DNA extraction methods (Student t-test: t=-2.48, p=0.134 when distributions with greatest difference in means were compared). However, the DNA yield possible with entire forearm swabs moistened

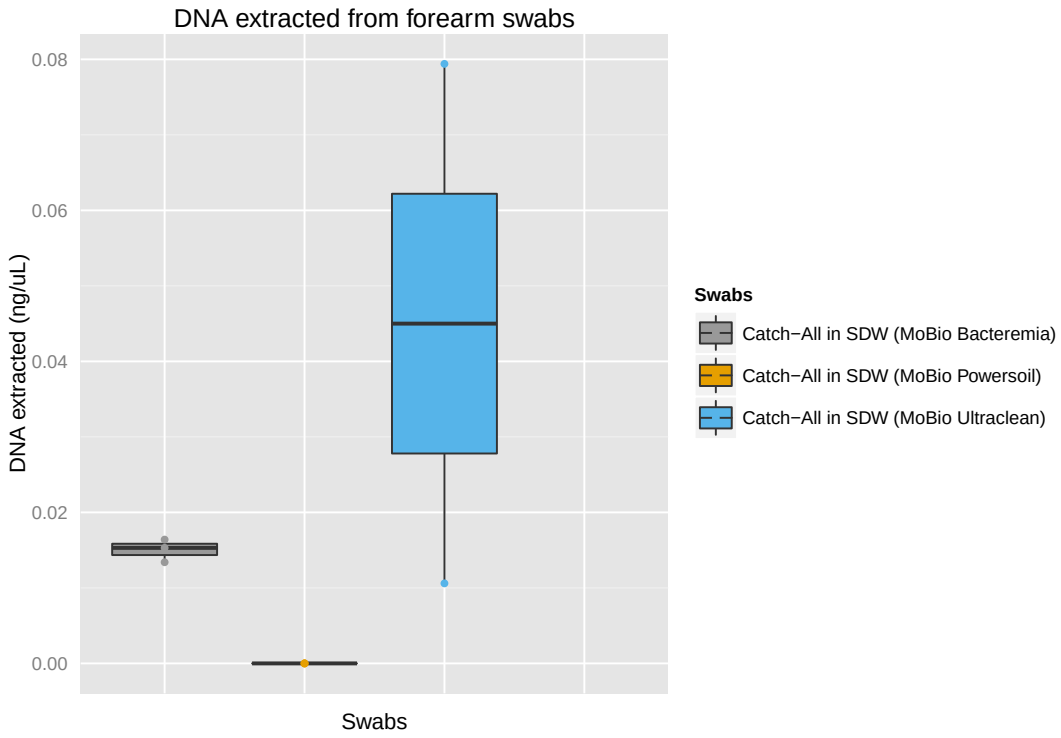


Figure 4.8: Comparison of different DNA extraction methods for forearm swabs Comparison includes forearm Catch-All swabs moistened with SDW processed with MoBio Bacteremia kit (n=3, μ=0.015ng/μL, σ=0.002), MoBio Powersoil kit (n=3, μ=0ng/μL, σ=0), and MoBio Ultraclean kit (n=3, μ=0.045ng/μL, σ=0.005).

with SDW and processed in the MoBio Ultraclean kit is higher than that achieved with ACF swabs, as seen in figure 4.1.

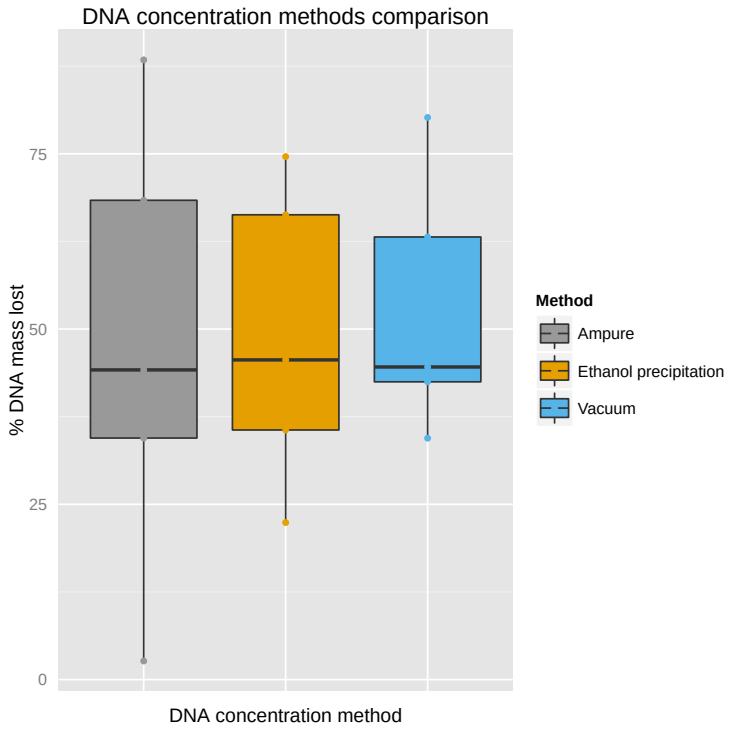


Figure 4.9: Comparison of different methods of concentrating DNA (percent loss of ng/μL.
Comparison includes samples concentrated with Ampure XP beads (n=5, μ=47.6%, σ=32.8), ethanol precipitation (n=5, μ=48.9%, σ=21.5), and vacuum using SpeedVac (n=5, μ=53.0%, σ=18.5).

Due to the small quantities of DNA recovered from skin swabs, a greater elute was employed of 100μL to gain more DNA, although in a larger volume. Concentrating samples was necessary to provide sufficient DNA quantities for library preparation (1-5ng in 10.5μL). DNA was concentrated using each of the methods detailed in figure 4.9. There were no significant differences in percent DNA concentration loss between different methods (Student t-test: t=0.318, p=0.761). It is evident that concentrating DNA using the SpeedVac leads to the least loss in DNA quantity, in addition to being the least costly and technically challenging.

4.3 Discussion

Measures of DNA quality resulting from DNA extractions were not systematically recorded as the library preparation process involves cleaning of DNA, removing compounds which are problematic in downstream sequencing and the quantities of DNA obtained would not result in a visible result on an ethidium bromide gel until pooled in a higher concentration. For quantification, qPCR may have been more successful in detecting low copy number of 16S and could discriminate between bacterial and eukaryotic DNA using universal primers for 16S, as used in the library preparation efforts in this study (F515Knight/806Reverse). The disadvantage of this approach would be a larger volume of sample employed in performing qPCR in triplicate. Qubit quantification through fluorometry still remains highly replicable[177], and is as such suitable for a study investigating differences between DNA extraction approaches where eukaryotic DNA burden is likely to be minimal due to use of swabs.

In addition, sampling from the entire forearm and hand means that a wide range of niches are sampled meaning that it could be argued that community ecology measures are not representative of a single population (for example, the ACF and hand provide distinct niches for different bacterial communities)[160]. Though this is a weakness, and individual samples of each niche would have been more fitting, it must be balanced against cost and the time subjects spend being sampled. Furthermore, although the samples taken are not representative of specific communities, the pooled communities sampled are comparable to one another in this study. Improvements to this study could have included taking multiple swabs from the same subject on the same occasion and pooling multiple DNA extracts, which would have likely increased the final quantity of DNA yielded. Other approaches could include performing a greater number of cycles within the PCR steps of library preparation, however this may introduce greater error which already comprise the greatest proportion of errors observed in 16S sequencing[75]. An approach which may reduce these errors while still enabling greater amplification of the library has been taken by Grice *et al.*[178] whereby replicate samples are taken for PCR in library preparation and in one sample, Sybr Green is used (providing a fluorescent signal) to determine on an individual sample basis how many cycles can be

obtained on the parallel samples with Taq polymerase (suitable to provide products for downstream sequencing) without exceeding the linear range of the reaction. The downsides of this are the greater quantities of DNA required to monitor progression of the PCR and the intensive nature of monitoring individual reactions. Solutions further downstream may include pooling larger numbers of samples with lower quantities of DNA, however it is difficult to ascertain the necessary read-depth to draw accurate conclusions from a microbiome study[77].

The method of sampling is important in terms of DNA yield. In this study, it was noted that Catch-All swabs performed best for DNA yield in all sites apart from nare where cotton swabs performed better. Catch-All swabs are soft foam brushes that appear to be generally more reliable in transferring bacterial populations from site to tube. Cotton swabs may have performed better in the nare due to greater possibility of removing organic debris since these brushes are thicker. Initially, the human microbiome project used blunt scalpels to scrape skin, however this was later changed to MoBio Catch-All swabs due to high levels of eukaryotic DNA which lead to problems performing metagenenomic sequencing[160]. Since this study performs PCR for bacterial 16S regions, leading to bacterial DNA amplicons, high levels of eukaryotic DNA in the sample would not problematic. The benefits of gaining higher DNA yields using this method, however, must be weighed against the potential distress caused to a child who is sampled in this manner. As such, it was decided to perform the final cohort of sampling using a MoBio Catch-All swab.

Chapter 5

16S amplicon sequencing

5.1 Introduction

Since the advent of next-generation sequencing, microbiome studies have become increasingly more affordable to conduct.

Illumina have a range of sequencing platforms; MiSeq and HiSeq are currently the most commonly used and differ primarily in their readlength and output[179]. MiSeq generates up to 300bp dual index reads, whereas the HiSeq generates up to 150bp reads. The HiSeq is designed for large-scale genome projects, outputting over 50Gb of data per day, compared to the Miseq which is designed for smaller scale sequencing, and can output 1.5Gb per day. As such, Illumina MiSeq is generally better suited to 16S amplicon sequencing due to the longer read length possible.

Illumina flow cells have oligonucleotides bound to the membrane which bind to Illumina adaptors, at both the 3' or 5' end[180]. This allows for sequencing of both DNA strands and identification of them using barcodes. There are two methods employed to enable dual index sequencing[181]. The first involves two rounds of PCR; in the first, the primers anneal to the region of interest with an overhang to link to the Illumina index and adaptor, while in the second primers anneal to linker and have an overhang of the Illumina index and adaptor. This has the limitations of requiring multiple PCR cycles, thus increasing the rate of PCR error. A second approach involves only one PCR cycle of forward and reverse primers with the Illumina pad-linker-primer combination already. The purpose of the pad in this case is to prevent hairpin formation. This has the advantage of limiting PCR cycles. The dual index approach can also produce longer reads by not completely overlapping the regions subject to PCR, thus extending

readlength up to 500bp.

Both platforms employ sequencing by synthesis, whereby reversible terminator dye nucleotides are incorporated into a genomic DNA clusters anchored to the flow cell (a chamber within the machine)[96]. First, genomic DNA (under 600bp long) with Illumina adaptors[96, 181] attached to each end of the DNA strand is placed in the flow cell where the DNA fragments anchor to the surface of the flow cell via the adaptors[96]. The DNA fragments then undergo isothermal PCR, forming individual clusters[96]. During each sequencing cycle, engineered DNA polymerase adds a fluorescently labelled nucleotide to anchored DNA fragments, after which a laser excites the fluorophore and a camera captures the image, after which the fluorophore is cleaved and the process start again[96]. Each sequencing run may involve hundreds of cycles.

Sequencing errors specific to Illumina chemistry include long homopolymer tracts and also GGC motifs in GC-rich zones[182]. Illumina requires heterogenous base composition within the libraries[182], as such it is often necessary to introduce higher genetic diversity through spiking the library with greater quantities of the control, PhiX[181]. The overall error rate of Illumina is under 0.4%[182].

Pyrosequencing was pioneered by Roche, culminating in the 454 GS-FLX (454 Life Sciences) and was arguably the first high-throughput sequencing technology available[95]. Initially a picotitre plate, each of the 1.7 millions wells with a bead that only binds one DNA fragment is introduced, followed by PCR creating many identical fragments on the same bead, and melting rendering the sample ready for sequencing. Pyrosequencing[95] is a sequencing by synthesis technology, whereby natural nucleotides are incorporated to a DNA strand with DNA polymerase, each time releasing a pyrophosphate group. An enzymatic cascade follows of pyrophosphate converting to ATP via sulphurylase followed by ATP use by luciferase to emit light which is then detected by a high resolution camera (using fibreoptic bundles attached to a charge coupled device). This sequencing technology gains on average, 400bp reads[95].

Pacific Biosciences released a sequencing platform, the PacBio RSII[183], which performs single molecule real-time (SMRT) sequencing through direct observation of DNA polymerisation incorporating fluorescently labelled nucleotides. Zero-mode waveguide

is employed, allowing direct observation of fluorescence at volumes orders of magnitude lower than confocal fluorescence microscopy[184]. It bypasses the need for PCR, although requires higher quantities of input DNA[182]. Unlike other technologies, the fluorophore is attached to the phosphate backbone of dNTPs, allowing uninterupted synthesis, and thus rendering it suitable for real-time observation[183]. This technology achieves readlengths several kilobases long, with some reads over 10,000kb[185]. In addition, this technology is able to observe methylation patterns which is desirable in an era where bacterial epigenomics is of growing interest[186]. The error rate is high, at 13% and distributed evenly through the reads[182], however this can be reduced to negligible quantities by performing more sequencing cycles to find consensus bases (known as circular consensus sequencing technology) or mapping with high quality short reads obtained from the same genome[187]. The aims of this chapter are to gain sequencing results from the V4 region of the 16S gene from DNA extracted from the second cohort of samples where forearm, axilla, nare, and oropharynx were sampled. In addition, a swab negative (DNA extracted from a swab with no sampling) and a PCR negative (DEPC water put through the library preparation) will be included in the sequencing run to assess contamination.

5.2 Results

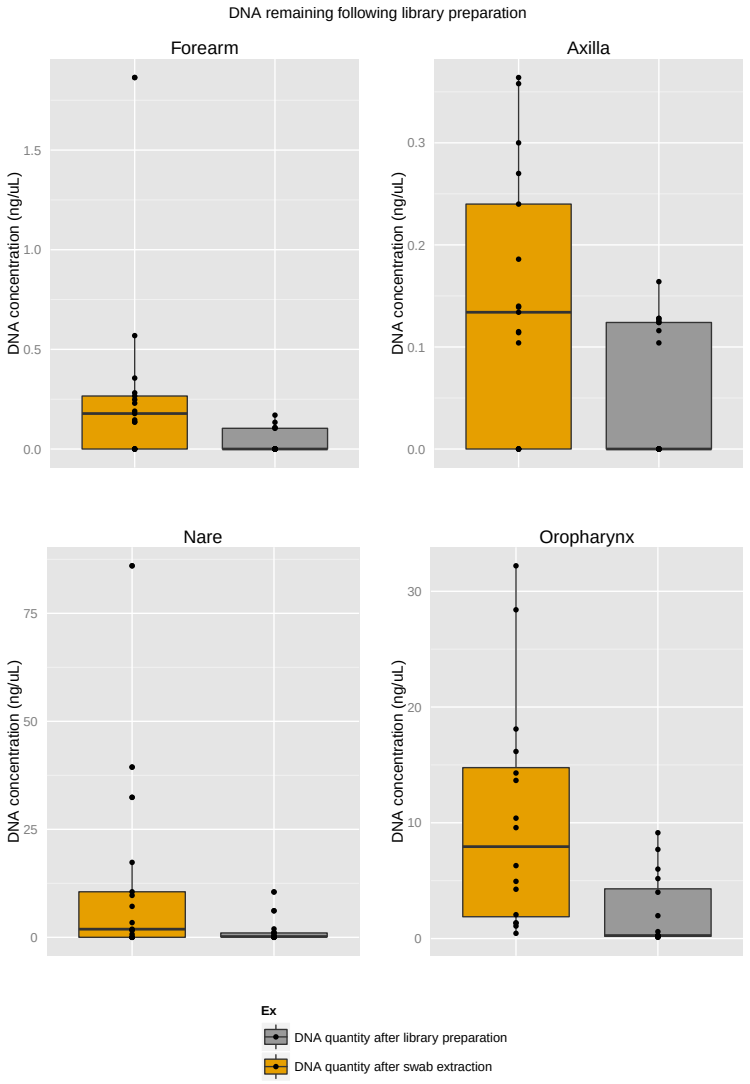


Figure 5.1: Loss in DNA quantity during library preparation for each sample type

Figure 5.1 demonstrates the quantity of DNA lost following library preparation. The DNA concentration of the DNA extraction product demonstrates the yields obtained using MoBio Ultraclean protocol finalised by optimisation. The pooling strategy for different sample types was derived from DNA concentration of the library for each sample type. Given the great differences between DNA quantity available from each sample type, a pooling strategy was applied whereby each sample type is input at approximately similar concentrations for sequencing. A full table of DNA concentrations obtained from extraction after concentration with SpeedVac, library DNA concentra-

tions obtained, and pooling strategy is given in the appendix.

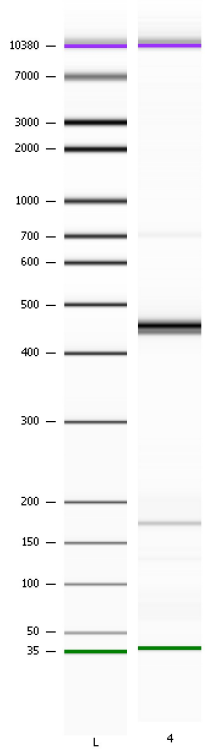


Figure 5.2: Quality check electrophoresis gel
This gel (lane 4) demonstrates bands of intensity, corresponding to concentration of DNA according to length of fragments (bp) in the library. The ladder (lane L) provides a reference.

Figure 5.2 demonstrates the electrophoresis gel on the entire pooled DNA sample from the Bioanalyzer as part of quality checks prior to sequencing. The majority of DNA was approximately 450bp length, matching the expected size of the V4 hypervariable region. There is a small band of approximately 150bp length which likely represents small fragments of primer-dimer introduced during the library preparation PCR cycles.

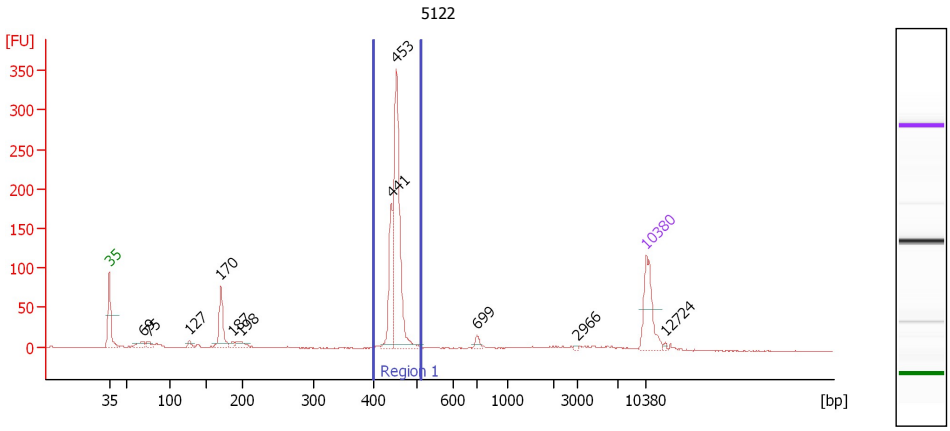


Figure 5.3: Quality check electropherogram
This quality check electropherogram demonstrates quantities of DNA according to size (bp) of fragments in the library.

Figure 5.3 shows the electropherogram from Bioanalyzer quality check prior to sequencing. This graph demonstrates the same result as seen in figure 5.2, that there is an expected peak of 453bp and a smaller peak of 441bp consistent with V4 hypervariable region and many smaller peaks of small fragments likely to be primer-dimer as a result of library preparation PCR.

Due to the primer-dimer, further clean-up of the sample was undertaken using Ampure beads (performed by the Centre for Genomic Research, University of Liverpool).

The pooled sample then passed quality checks and proceeded to sequencing on the Illumina MiSeq (performed by Centre for Genomic Research, University of Liverpool).

Following sequencing, some barcodes could not be detected due to low input quantities of DNA. The assembled reads are shown in table 5.4. It demonstrates the uneven results between samples from sequencing. Following quality control of those assembled reads, 42 of the original 70 samples including negative controls remained for analysis.

Sample	Site	Raw reads	R1/R2 read pairs	Assembled sequences of correct length
CF 16	Forearm	12	6	0
CF 16	Axilla	2	1	0
CF 16	Nare	71424	34057	298399
CF 16	Oropharynx	14102	7048	6894
CF 28	Forearm	2	1	0
CF 28	Axilla	54648	27269	26535
CF 28	Nare	5802	2898	2834
CF 28	Oropharynx	14572	6214	3924
CF 13	Forearm	72	36	0
CF 13	Axilla	2	1	0
CF 13	Nare	621588	305468	298399
CF 13	Oropharynx	484	237	149
Healthy 34	Forearm	212	106	15
Healthy 34	Axilla	14	7	0
Healthy 34	Nare	20502	10232	9756
Healthy 34	Oropharynx	1813572	906278	890771
Healthy 33	Forearm	830	116	17
Healthy 33	Axilla	24	12	0
Healthy 33	Nare	9436	4689	4632
Healthy 33	Oropharynx	4423460	2210459	2162777
Healthy 35	Forearm	2	1	0
Healthy 35	Axilla	2	1	0
Healthy 35	Nare	746964	373242	365893
Healthy 35	Oropharynx	414	206	144
CF 14	Forearm	2	1	0
CF 14	Axilla	2	1	0
CF 14	Nare	850	366	85
CH 14	Oropharynx	218	109	75
Healthy 31	Forearm	1076	537	528
Healthy 31	Axilla	31304	15638	15423
Healthy 31	Nare	198	99	0
Healthy 31	Oropharynx	2600486	1297388	1271799
CF 12	Forearm	30	15	0
CF 12	Axilla	346	172	1
CF 12	Nare	330262	164994	163070
CF 12	Oropharynx	206	102	71
CF 17	Forearm	4984	2490	2436
CF 17	Axilla	3236	1614	1596
CF 17	Nare	1128	547	533
CF 17	Oropharynx	28702	14342	13892
CF 18	Forearm	4	2	0
CF 18	Axilla	4	2	0
CF 18	Nare	488	242	231
CF 18	Oropharynx	62568	31254	30680
Healthy 30	Forearm	64	32	0
Healthy 30	Axilla	306	153	148
Healthy 30	Nare	112	56	0
Healthy 30	Oropharynx	199290	99435	97037
Equivocal 36	Forearm	456	228	224
Equivocal 36	Axilla	602	300	290
Equivocal 36	Nare	98	49	0
Equivocal 36	Oropharynx	4603630	2296522	0
Healthy 29	Forearm	14	7	0
Healthy 29	Axilla	2	1	0
Healthy 29	Nare	270406	135137	134398
Healthy 29	Oropharynx	2794	1396	1139
CF 11	Forearm	2	1	0
CF 11	Axilla	2	1	0
CF 11	Nare	554	171	100
CF 11	Oropharynx	120	60	0
CF 15	Forearm	90	45	0
CF 15	Axilla	26	13	0
CF 15	Nare	39178	19568	19411
CF 15	Oropharynx	1820972	910031	895682
Healthy 32	Forearm	210	105	2
Healthy 32	Axilla	4	2	0
Healthy 32	Nare	1246	673	664
Healthy 32	Oropharynx	2315788	1157375	1139970
None	None	20	10	895682
None	None	736	368	361

Figure 5.4: Reads resulting from samples.

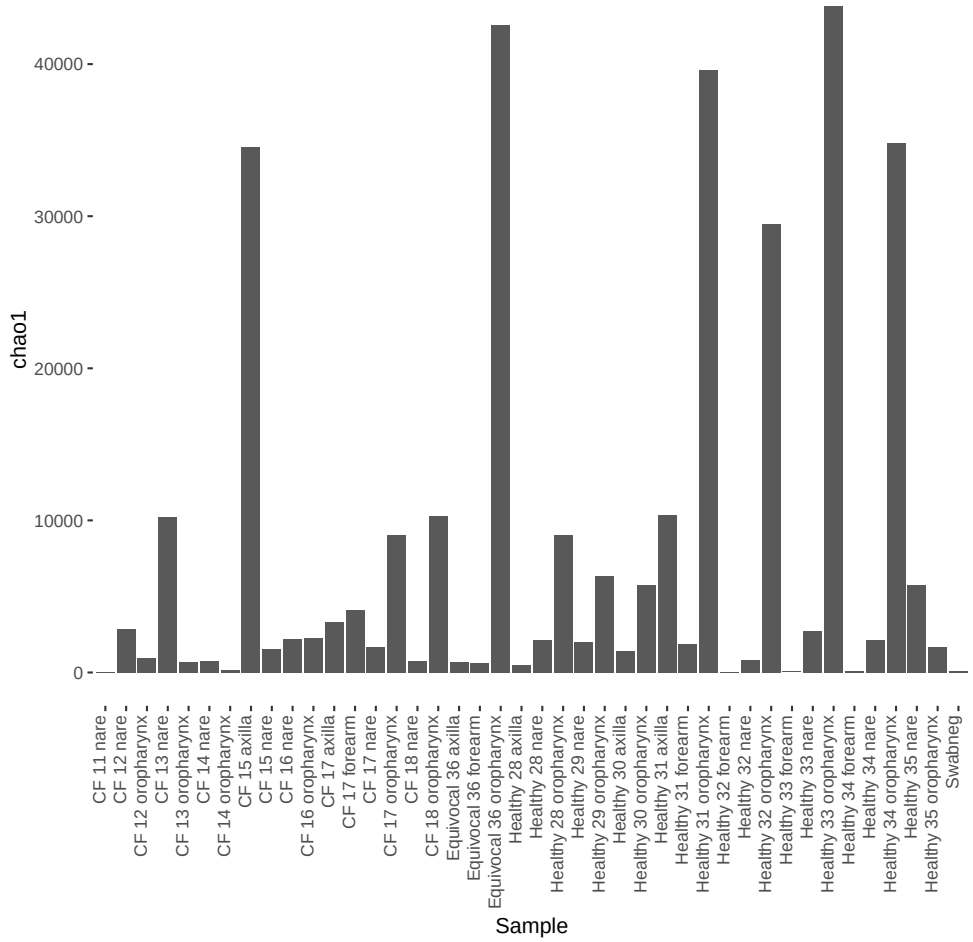


Figure 5.5: Alpha diversity of samples estimated on rare OTUs present.

Alpha diversity of samples, chao1, is shown in figure 5.5. High diversity appears to correspond with those samples with high biomass and read count as seen in figure 5.4.

Figure 5.6 shows those taxa which could be identified to species or genus level that comprise $> 1\%$ of any sample across all subjects and including the negative control swab. It is clear there is a great deal of overlap in taxa identified in CF subjects, healthy controls and the patient with equivocal diagnosis. *Acinetobacter* was found only in nare of subject 16, a CF patient who had not isolated this in clinic swabs from in the previous year. *H. influenzae* was found in nare of subject 14, a CF patient who had previously grown this pathogen from clinic swabs. *H. influenzae* was also found in the oropharynx of subject 16, a CF patient who had not had this pathogen isolated in the previous year and in this study was the only taxon found in this sample alongside other *Haemophilus* and *Veillonella dispar*. *H. influenzae* was also found in the nare of subject 35, a healthy control. Only two samples were found to contain *Pseudomonas*, the oropharynx of subject 18, a CF patient, and nare of subject 28, a healthy control. Strikingly, *Prevotella*, *Rothia*, *Streptococcus* and *Veillonella* species were found abundantly across CF subjects and healthy controls in all sites. The negative sample showed heavy *Rhodococcus* reads, which was not found in any other sample.

Figure 5.6: Heatmap of all samples showing relative abundance of all taxa reaching genus/species level which comprise $> 1\%$ in any sample.

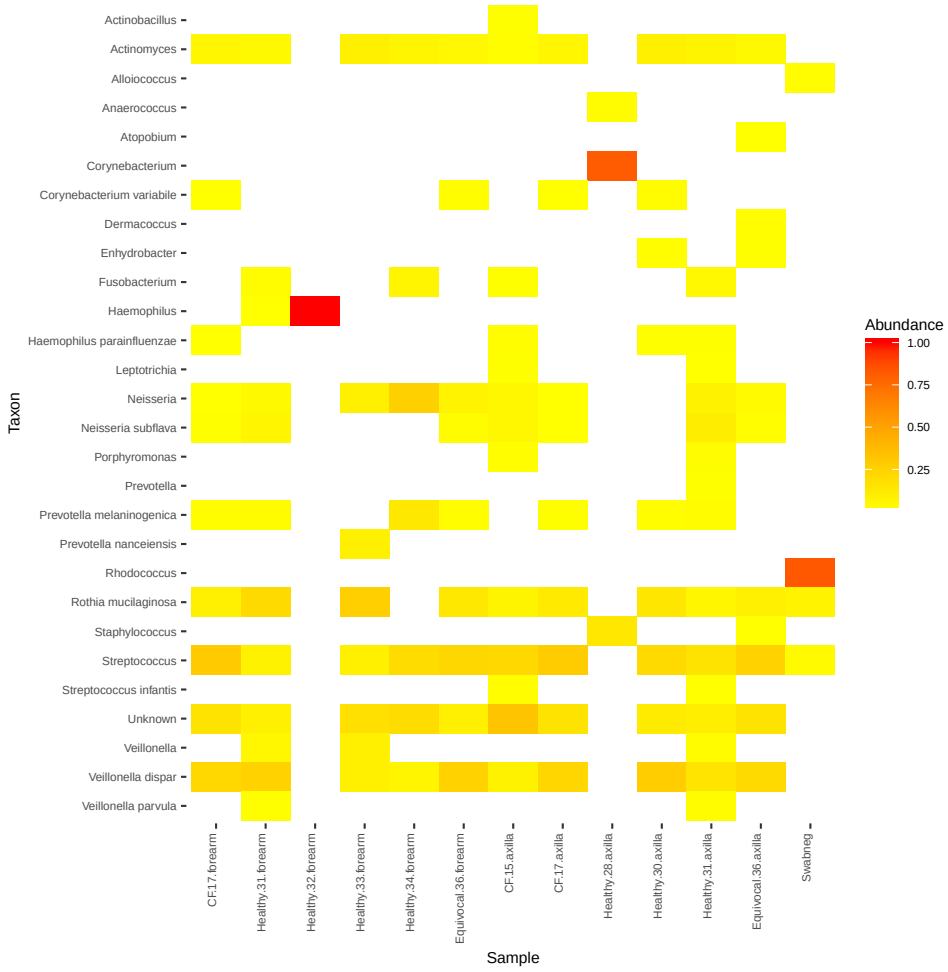


Figure 5.7: Heatmap of skin samples showing relative abundance of all taxa reaching genus/species level which comprise $> 1\%$ in any sample.

Figure 5.7 is a heatmap of all skin samples from CF patients and healthy controls showing taxa which comprise $> 1\%$ of any sample. It highlights the similarities between taxa found on forearm and axilla in both CF patients and healthy controls. Subject 32, a healthy control was found to have *Haemophilus* as the dominant taxon on the forearm in contrast to the other subjects where it is largely absent. The low abundance of *Staphylococcus* is also notable being found only in the axilla of two subjects.

Figure 5.8 shows a heatmap of taxa representing $> 1\%$ of any sample in nare and oropharynx of CF patients and healthy controls, of which there appears to be significant overlap. Predominant differences include *Staphylococcus*, *Alloicoccus*, *Corneyebacterium*, and *Moraxella* which appear to be largely present in CF and healthy subjects in the nare but not oropharynx. In addition, *Haemophilus* species seem to be found more frequently in the oropharynx compared to the nare of both CF and healthy subjects.

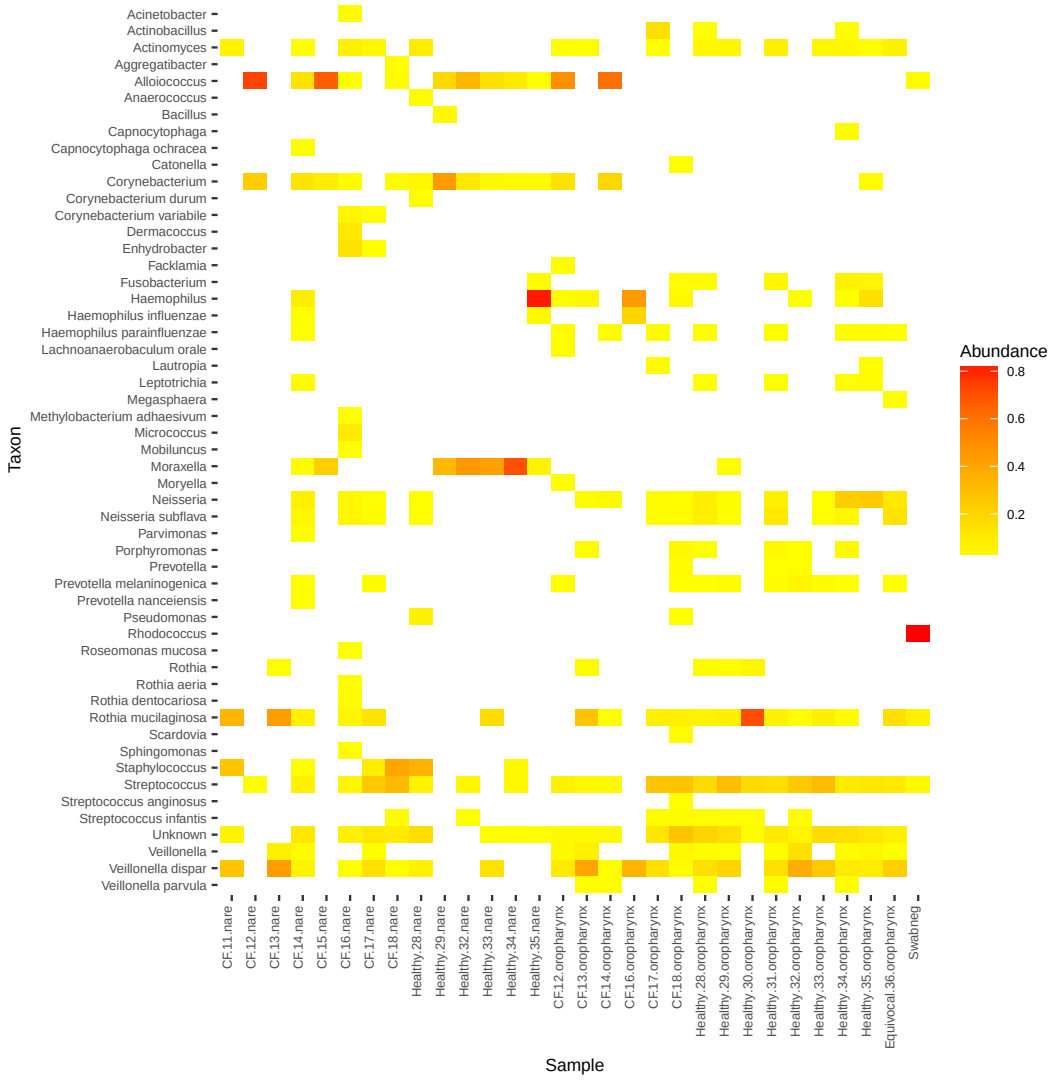
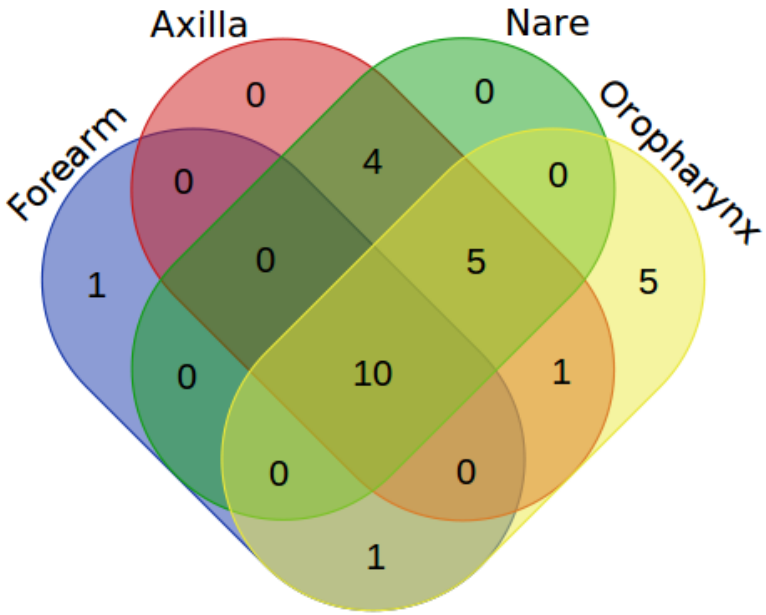


Figure 5.8: Heatmap of nare and oropharyngeal samples showing relative abundance of all taxa reaching genus/species level which comprise $> 1\%$ in any sample.

Sites	Sum	Taxa
Axilla, Forearm, Nare, Oropharynx	8	Streptococcus, Haemophilus parainfluenzae, Neisseria subflava, Rothia mucilaginosa, Actinomyces, Neisseria, Prevotella melaninogenica, Veillonella dispar
Axilla, Forearm, Nare	1	Corynebacterium variabile
Axilla, Nare, Oropharynx	3	Fusobacterium, Porphyromonas, Streptococcus infantis
Axilla, Nare	1	Leptotrichia
Axilla, Oropharynx	1	Actinobacillus
Nare, Oropharynx	4	Prevotella, Veillonella, Veillonella parvula, Corynebacterium
Nare	3	Anaerococcus, Staphylococcus, Enhydrobacter
Oropharynx	12	Facklamia, Lautropia, Rothia, Lachnoanaerobaculum orale, Streptococcus anginosus, Pseudomonas, Moryella, Scardovia, Haemophilus, Catonella, Alloiococcus, Haemophilus influenzae

Figure 5.9: Venn diagram showing overlap in each site from CF patients of taxa identified to genus/species level comprising $> 1\%$ of any sample.

A Venn diagram demonstrating overlap of taxa at genus/species level in CF patients which represent $> 1\%$ of any sample is shown in figure 5.9. There is significant overlap at all sites of eight taxa including *Streptococcus*, *Rothia mucilaginosa*, *Neisseria*, *Prevotella melaninogenica*, and *Veillonella dispar*. Of interest, other *Prevotella* and *Veillonella* species are found in both nare and oropharynx though this is not found with *Pseudomonas* or *Haemophilus* species which can be found primarily in the oropharynx.



Sites	Sum	Taxa
Axilla, Forearm, Nare, Oropharynx	10	Streptococcus, Veillonella parvula, Neisseria, Prevotella melaninogenica, Neisseria subflava, Fusobacterium, Veillonella dispar, Veillonella, Rothia mucilaginosa, Actinomyces
Axilla, Nare, Oropharynx	5	Prevotella, Haemophilus parainfluenzae, Corynebacterium, Leptotrichia, Porphyromonas
Forearm, Oropharynx	1	Haemophilus
Axilla, Nare	4	Anaerococcus, Staphylococcus, Enhydrobacter, Corynebacterium variabile
Axilla, Oropharynx	1	Streptococcus infantis
Forearm	1	Prevotella nanceiensis
Oropharynx	5	Moraxella, Capnocytophaga, Lautropia, Rothia, Actinobacillus

Figure 5.10: Venn diagram showing overlap in each site from healthy controls of taxa identified to genus/species level comprising $> 1\%$ of any sample.

Figure 5.10 shows overlap of taxa at genus/species level in healthy subjects which represent $> 1\%$ of any sample. In the healthy subset, *Veillonella* can be found in all sites, in addition to *Rothia*, *Neisseria* and *Streptococcus*as seen in the CF patients.

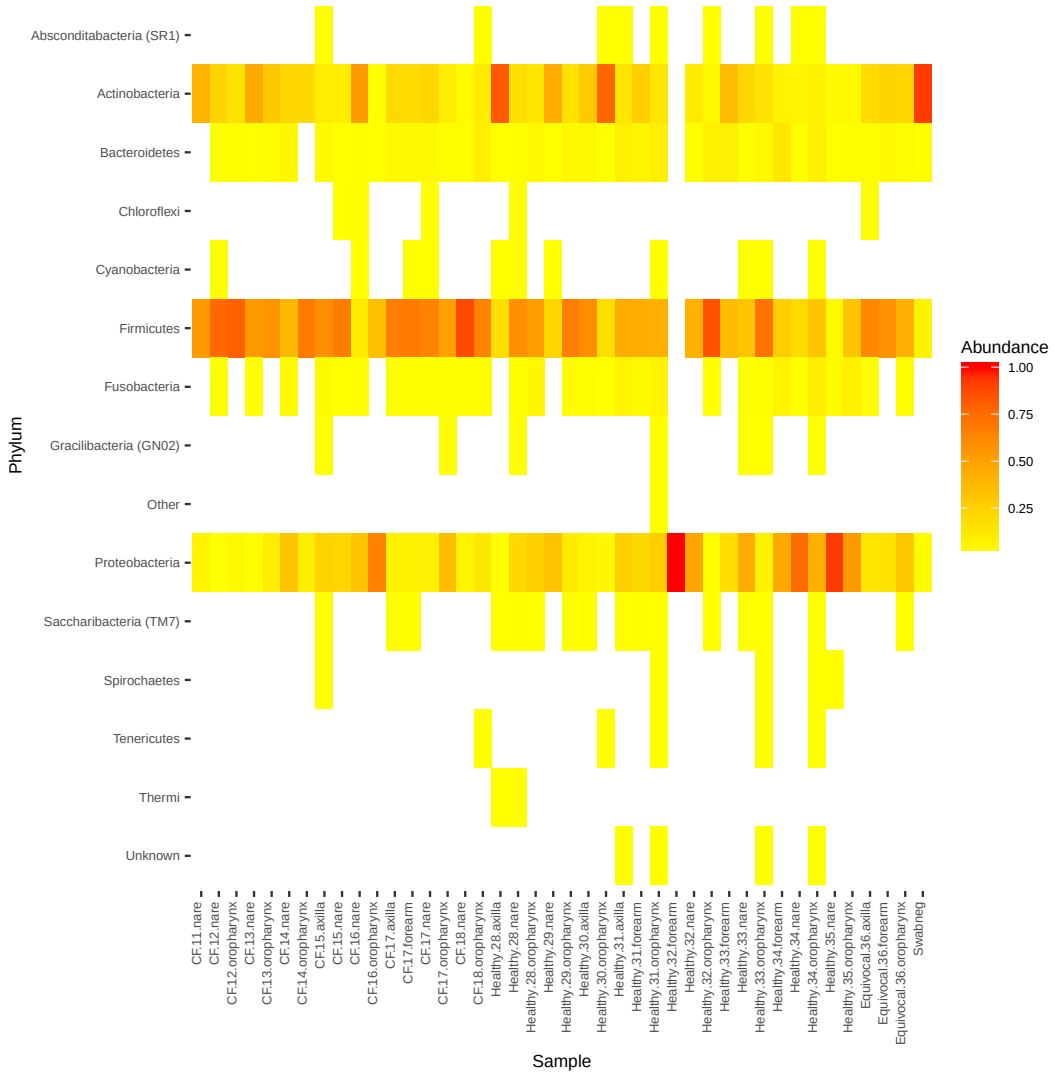


Figure 5.11: Heatmap showing relative abundance of phyla across samples.

The distribution of phyla for all subjects is seen in figure 5.11. Firmicutes appear to be in higher abundance in CF samples compared to healthy controls while Proteobacteria appear higher in abundance in healthy controls compared to CF patients. Actinobacteria appear to dominate the sample in the axilla and oropharynx of healthy subjects 28 and 30 respectively. The negative control is dominated by Actinobacteria due to the high read-count of *Rhodococcus* in the sample.

Heatmaps showing order, class and family taxa for all samples can be found in Appendix E.

5.3 Discussion

Illumina technology generates high-quality reads with a low error rate[182], and errors specific to the technology, such as difficulty with homopolymer tracts should not be of importance in amplicon sequencing. Compared to the Illumina HiSeq, the MiSeq produces longer read lengths (300bp compared to 150bp) but produces a lower data output. This renders it an obvious choice for small microbiome projects where a readlength capable of sequencing an entire hypervariable region is necessary. On average, 454-pyrosequencing generates 400bp reads, and this is still among the longest for next generation technologies today. It carries an average error rate of 1.07%, which are more common in homopolymer tracts, with longer reads there were an increased frequency of mismatch errors and ambiguous bases tending to occur at the end of the read, and a higher frequency of indels are found in particular spacial distributions of the sequencing plate[188]. This technology has largely been superseded by Illumina due to shorter but higher quality reads. Despite this, many early microbiome studies employed this technology. PacBio is able to characterise the full complexity of genomes, including repeating motifs, and as such is ideal for large scale genomics or to provide high quality reference genomes[183]. Despite criticism of its application in microbiome studies[189], likely due to the high error rates observed superficially, it has been used for this application with success[190]. With greater read lengths, a greater phylogenetic resolution can be obtained, assuming the error rate is reduced as discussed previously[190]. Cost and required input quantity of DNA is likely to make this technology prohibitory for most microbiome studies. Given the comparison of sequencing options, Illumina Miseq was clearly the appropriate choice for this study.

The 16S region comprises nine hypervariable regions, of which we chose hypervariable region 4 (V4) for amplicon sequencing. Each hypervariable region has a different pattern of sensitivity for particular genera. We chose V4 due to the length (approximately 450bp) rendering it suitable for sequencing on Illumina Miseq, and the lack of intragenomic heterogeneity found within it[88]. Increased intragenomic heterogeneity leads to an artificial increase in diversity within a sample, introducing greater error into comparative studies. Most skin microbiome studies have employed V1-V3 due to its

sensitivity to detect dominant skin commensals, such as firmicutes[191]. In designing a microbiome study, it is important to balance the practicality of downstream analysis resulting from choice of hypervariable region sequence and the sensitivity to detect the expected genera. Some studies have employed several hypervariable regions for this region, however cost and difficulty in analysing data resulting from this approach is a limiting factor.

Due to the low quantities of DNA yielded from skin swabs, and the necessary uneven pooling, there are limitations on the manner in which data from this study can be analysed. Uneven pooling of the DNA samples naturally leads to a greater read depth in particular samples over others due to the higher number of copies present in the sequencing flow cell. As such, a greater degree of variation may be observed in those with a greater read depth; α-diversity is a function of read depth[192]. Sample types with similar input DNA quantities can be compared to one another in terms of community ecology measures, however these measures cannot be compared between different sites. This prevents analysis of relationships between diversity of oropharyngeal microbiota and skin microbiota, which could have revealed whether the decline in diversity observed in the airways over time is linked to a systemic decrease in microbial diversity. It was important to include negative controls of both the swab itself and the PCR step in the library preparation due to the low biomass sample present; that in low DNA quantities, there may be a greater proportion of background noise from contamination. Encouragingly the PCR negative did not have enough reads to meet quality cut-off to be analysed and the swab negative showed predominantly *Rhodococcus*, a bacterial genus which inhibits soil and is frequently isolated from DNA extraction and PCR kits[176] probably related to their manufacture in low-oxygen environments which allow nitrogen-fixing bacteria to thrive.

This study found significant overlap of taxa between all sites in both CF patients and healthy controls. Notably, *Veillonella* and *Prevotella*, Gram-negative anaerobes recently postulated to have a role in CF infection[136, 193], were found in both nare and oropharynx of CF patients. This reinforces the theory that the upper respiratory tract may be a niche which offers pathogens relative protection from the immune response and

antimicrobial therapy compared to the lower respiratory tract, thus creating a reservoir of pathogens which can eventually seed back to the lower respiratory tract[112, 115]. Alpha diversity measures appeared to be highest in samples with a high read count limiting the conclusions that can be drawn from this measure. In further studies if even pooling could be achieved this would be a useful measure to determine early changes in population structure of skin and upper respiratory tract that may be implicated in disease progression in CF, as has previously been demonstrated in sputum samples from adults with CF[125].

At phylum level, it appears that CF patients tend to have a higher abundance of Firmicutes compared to healthy controls, while healthy controls have a higher abundance of Proteobacteria across all sample types. This appears to be contradictory to the assertion the CF lung microbiome has a dominant fraction of proteobacteria compared to healthy lung microbiome[123]. Previous studies only investigated adults with CF for phylum-level differences who may have more advanced lung disease and thus a more distinct microbial signature. In addition, this study only investigates samples from the upper respiratory tract and skin which may not be representative of samples from the lower respiratory tract. The high abundance of Firmicutes is reflected in the frequent identification of *Veillonella* and *Streptococcus* and infrequent identification of *Pseudomonas*. There are also some unculturable phyla present at low levels, Gracilibacteria(GN02) and Saccharibacteria (TM7) which did not resolve below order level so their significance cannot be determined. Thermi were also found which is likely *Thermus aquaticus* DNA contamination from PCR reagents.

Strikingly, *Prevotella* and *Veillonella*, anaerobic Gram-negative bacteria were found on the skin of CF patients and healthy controls in addition to the upper respiratory tract. The skin is an unexpected environment for even a low abundance of anaerobic bacteria. Since 16S microbiome studies may identify DNA from dead as well live bacteria, these reads could represent dead bacteria which have been transferred from other niches where these may be commensals. It is unlikely this is sample contamination as the swab negative control did not contain either of these genera. Other possibilities to consider are that all subjects had contact with the hospital environment and may have

acquired these genera from contact with other patients and the hospital environment. Finding *Prevotella* and *Veillonella* on the skin of CF patients and healthy subjects is an important finding that deserves further study to confirm the possibility of carriage on skin and in the upper airways given the role these anaerobes likely have as CF pathogens[136, 193].

Streptococcus was also found abundantly on all sites in CF patients and healthy controls, however this is a normal skin commensal[160] was also found in the negative control, limiting the significance of this finding. *Staphylococcus* was interestingly absent from most samples which would be expected to form a significant proportion of the skin microbiome[160]. This finding is also in conflict with the culture results obtained from the same patients. This is probably due to difficulty of extraction given these are Gram-positive bacteria, reinforcing difficulty of extracting DNA for studies of the skin microbiome[160]. This is study used beadbeating and lysozyme treatment to increase yield of DNA. Use of harsher protocols to increase yield carry the risk of shearing DNA decreasing its quality for sequencing[176] and use of more abrasive sampling techniques may have reduced recruitment given increased discomfort. This indicates that the skin microbiome presented in this study does not represent the full diversity of the skin microbiota present in these subjects.

Chapter 6

General Discussion

This study has demonstrated a culture-based core microbiota of *Staphylococcus* and *Micrococcus* universal to both CF patients and healthy controls across all sites. Pathogens have been found on the skin of both CF patients and healthy controls, however this is infrequent and not present in sequential samples, suggesting it is a transient microbiota, in agreement with the limited literature suggesting this[161]. In addition, issues of low biomass samples from the skin became apparent with extremely low DNA yields from swabs, which has been a feature recognised elsewhere[162]. This study has contributed to an optimised protocol, however has not succeeded in providing high enough yields that skin microbiota may be compared to the DNA yields of other sites to fulfill even pooling of a maximum sequencing capacity. The samples obtained from 18 CF patients recruited, 17 healthy subjects, and one subject with equivocal diagnosis has undergone amplicon sequencing of the V4 hypervariable region of 16S, and will likely generate interesting results of a preliminary study into the landscape of CF skin, however unfortunately it was not possible to present the data in this thesis. Subjects with equivocal diagnosis of CF have never been subject to a comprehensive microbiological investigation. Although only one subject was recruited in this feasibility study, it may provide important insights to their airway microbiome in this emerging diagnosis where there is a great deal of debate over best practices in monitoring colonisation in clinics[31].

Pathogens had been isolated at a variety of sites in each patients, and not always on the hands as might be expected. However, a similar pattern was seen in healthy subjects. This may imply a common route by which CF pathogens, such as *P. aeruginosa*, gain entry to the respiratory tract in children, leaving those who have CF prone to colonisation of the respiratory tract. This is in agreement with literature suggesting that transmission of pathogens occur with direct contact[153, 154, 170]. However, these studies only examined the hands of CF patients and recruited no healthy controls. In examining several sites, we demonstrate that *P. aeruginosa* may be present in any of these regions without being present on the hand. Although it is clear that over a period of months the colonisation is transient, the length of colonisation normally cannot be ascertained and these niches may play a role in harbouring pathogens prior to infection. The significance of an isolated finding of *Pseudomonas* in the axilla is unclear from this work but raises the possiblity that hand hygeine may not be adequate to prevent transmission of pathogens and raises the possiblity of wider hygeine interventions to control infection in CF. The possibility that community structure may render CF skin more prone to transient pathogens may become clearer after analysis of the microbiome data. Considering the high salt content of CF skin, it may be hypothesised that this provides a specific niche free of many genera contributing to healthy microbiota, and thus could provide a niche where an invasive species is more likely to thrive. Further work on this perspective would comprise long-term studies with sequential sampling of skin microbiota and strain typing of pathogens.

It is also not clear whether there are differences in characteristics of pathogens isolated from skin compared to those from the oropharynx in CF patients and healthy controls. This will form part of further work emanating from this study, particularly in characterising the strain type and phenotypic characteristics such as auxotrophy and virulence of *Pseudomonas* and *S. aureus* isolated in this study. Further understanding of the characteristics of pathogens in transient skin microbiota may lead to an understanding of how infection is established in CF.

The vast majority of microbiome studies in CF have concentrated on the airways, the site of chronic infection[194]. These have drawn conclusions focused on the community ecology of the CF lung, generating hypotheses for community interactions, and identification of novel pathogens which commonly evade culture-based techniques. Many key questions in these areas can be addressed by examining microbiomes outside of the lung.

Decreased α-diversity of the lung microbiome is thought to be have a central role in CF exacerbation and is associated with decreased lung function over time[127, 131], however the reasons behind this are unclear; decrease in diversity may relate to antibiotic therapy, high systemic levels of inflammatory cytokines, local inflammation in the lung parenchyma, or colonisation with an invasive species. In studying microbiomes outside of the CF lung, it would be possible to observe whether the decrease in microbial diversity is systemic or local to the lung, narrowing the possible reasons for the change in diversity observed. Furthermore, we observed it is possible to identify pathogens on the skin of CF patients, so it may be possible if studying sequential samples over time, to track the transmission of an invasive species into the lung microbiota and elucidate determinants of infection. Studies of this kind will likely inform the design of robust interventional studies in murine models or alternative systems for studying interventions in microbiomes, such as the cheese rind model[195].

This study demonstrated significant overlap in genera in each niche in both CF patients and healthy controls. There is mounting evidence the the upper respiratory tract is a niche where *P. aeruginosa* diversifies and evolves to then seed to the lower respiratory tract[108, 109]. Finding potential pathogens such as *Prevotella* and *Veillonella* in the nare and oropharynx may indicate they have adapted to niches in the upper respiratory tract for a similar end. It would be worthwhile exploring this further with whole-genome sequencing of these anaerobic bacterial populations in the nare, oropharynx, and lower respiratory tract to explore the possibility of the upper respiratory tract as a niche allowing for diversification and adaptation to the lower airways.

This study also demonstrated only *S. aureus* in some patients with CF but not healthy controls. It is known that carriage of *S. aureus* is higher in CF patients compared to healthy controls and that it is a predominant pathogen in childhood CF but that this can be reduced with antistaphylococcal antibiotics[49]. Another interpretation of this is that *S. aureus* carriage in all sites of this population was dramatically lower than reported in other studies of children such as Goerke *et al* who report 29%. This could indicate a weakness of this study in a lack of sensitivity for detecting *S. aureus* since this study lacked funding to use tube coagulase test or MALDI-TOF. The finding of *S. aureus* only in subjects with CF and no healthy controls should prompt further investigation in larger studies with more appropriate identification methods for *S. aureus*. Further studies should also evaluate the impact of *S. aureus* carriage on the progression of CF lung disease and the potential role for topical *S. aureus* decolinisation in this population.

Through lung microbiome studies, difficult to cultivate bacteria have emerged as potential pathogens when found to be a dominant genera in the CF lung, such as the Gram-negative anaerobic bacillus *Prevotella*[136]. *Prevotella* is a member of normal microbiota in the oral cavity and gut, however it is not clear whether this is a CF pathogen and how transmission from the environment occurs. It seems unlikely that anaerobic bacteria are transmitted via the skin, however finding of these genera on the skin in this population of CF patients and healthy controls should lead to further studies to confirm this. Performing comparative microbiome studies on oral and gut microbiota may further help elucidate the mechanism by which anaerobic bacteria inhabit the CF lung, and how much the contents of the gut, oral cavity, and skin can influence the composition of the CF lung.

In performing a feasibility study for the skin microbiome in cystic fibrosis, we have successfully characterised the difficulties in DNA extraction from swabs, and have isolated pathogens from different sites on the CF patient and healthy controls where further characterisation will determine any changes in behaviour of these pathogens specific to the niche they inhabit. Analysis of the microbiome data has revealed shared taxa between the skin, nare, and oropharynx to form a starting ground whereby larger

studies can examine the temporal relationships between the dynamics of this ecosystem. In addition, the skin microbiome of cystic fibrosis will be defined over both the axilla and forearm and hand. The strengths of this study are the rigorous attention to detail in optimisation of DNA extraction from a range of sample types to find an acceptable balance of yield between types, and in collecting culture isolates from each site for further analysis in the context of the microbiome data. Weaknesses may include sampling of entire forearm and hand which are arguably two very different niches, and as such cannot produce informative or reproducible community ecology metrics. In addition, the uneven pooling necessary to produce a library for sequencing forfeits potential aspects of analysis (particularly between different sample types within the same patient). Given the small patient sample and difficulties generating microbiome data from low biomass samples it was also impractical to stratify patients based on clinical data or hygeine data. In further studies, it would be useful to stratify based on these measures to explore any associations between clinical state or hygeine measures and pathogen acquisition, as well as any difference in community structure of the microbiome on the skin and in the respiratory tract.

A further limitation is the use of children attending surgical outpatient appointments as healthy controls. All these subjects had contact with the hospital environment and some had a history of medical conditions and surgical procedures.

Potential pathogens have been isolated from surfaces in hospital environments, including S. aureus, P. aeruginosa and multi-drug resistant organisms [196, 197]. Evidence of biofilm formation despite cleaning with chlorine solutions was also found in one study set in an intensive care unit [Hu2015]. To date, no studies have examined microbiota in the outpatient environment. Inpatient settings may have more prolonged exposure to patients with bacterial infections compared to outpatient settings so it could be speculated outpatient settings are less likely to harbour pathogenic microbiota. Conversely, the same healthcare professionals work in both inpatient and outpatient areas and may carry pathogens between these settings on clothing [198]. The control subjects in this study could have potentially acquired a hospital-associated microbiota from their surroundings. Common exposure to the hospital environment for both healthy controls

and children with CF could have resulted in observing similar microbiota, however this may also have helped in identifying a CF-specific microbiome independent of the hospital-associated microbiota.

Control subjects may have previously been exposure to the inpatient environment for surgical procedures and may have had exposure to broad-spectrum antibiotic therapy as prophylaxis to surgical wound infections. Jernberg et al. [199] studied a group of healthy adults exposed to 7 days of clindamycin, detecting changes in faecal microbiota, specifically decrease in Bacteroides diversity, lasting throughout follow-up of two years. Although the authors did not examine other microbial niches and did not include children, it suggests that broad-spectrum antibiotics can cause long-term changes in host microbiome and this may have impacted the observed microbiome in control subjects in this study. Previous use of broad-spectrum antibiotics may have long-term impacts on the control subject microbiome, possibly rendering it unrepresentative of the healthy microbiome.

Although there are limitations of the control group employed by this study, this group of healthy children remain the most appropriate to use within the scope of the protocol approved by the research ethics committee. Recruiting healthy children from the community would not have been practicable given the further ethical approvals needed and anticipated difficulties in recruitment.

Future work on this study should include full characterisation of bacterial and fungal isolates. Identification to species level should use an appropriate technology, such as MALDI-TOF as previously discussed, investigation of phenotypic characteristics, such as motility, auxotrophy, quorum sensing markers, and virulence. Initially, high-throughput methods using *Galleria* could be employed to assay virulence prior to selecting representative strains for testing in murine models. In addition, further characterisation of selected isolates could be undertaken using whole-genome and transcriptome sequencing to find candidate genes necessary for survival of pathogens on the skin. Coupled with probing more specific skin sites in CF subjects and healthy controls, this would provide a step forward in understanding the determinants of pathogen survival in this environment and could identify potential therapeutic targets. Furthermore, clinical trials may be undertaken to examine the effects of more rigorous hygeine routines on frequency of pathogen colonisation.

This has successfully provided a bacterial and fungal collection of isolates for further work with linked clinical data. In addition, it has provided 16S amplicon sequences of samples from skin and upper respiratory tract of CF patients and healthy controls, though unfortunately DNA yield from skin samples was inadequate despite extensive optimisation using a variety of extraction methods and swab types. Further studies should examine use of more abrasive sampling methods while considering issues of recruiting in the paediatric population where subject discomfort is a major problem to recruitment into observational studies. In addition, more robust DNA extraction protocols to target Firmicutes are necessary given the low numbers of *Staphylococcus* identified in the microbiome study is likely due to problems with extraction, especially in view of their frequent isolation in the culture study. This study has provided a starting point for a great deal of work on the subject of comparative microbiomes in cystic fibrosis which may help answer some of the pivotal questions surrounding the CF microbiome.

Appendix A

Research Ethics Committee approval

NHS
Health Research Authority
National Research Ethics Service
NRES Committee North West –Liverpool Central
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Telephone: 0161 625 7434

12 November 2013

Dr Kevin Southern
Reader and Honorary Consultant in Paediatric Respiratory Medicine
University of Liverpool
Alder Hey Children's Hospital NHS Trust
Eaton Road
Liverpool
L12 2AP

Dear Dr Southern

Study title: **A FEASIBILITY STUDY IN DETERMINING THE SKIN MICROBIOME OF CHILDREN WITH CYSTIC FIBROSIS COMPARED TO HEALTHY AGE-MATCHED CONTROLS**
REC reference: **13/NW/0740**
Protocol number: **UoL000992**
IRAS project ID: **130564**

Thank you for your email of 12 November 2013. I can confirm the REC has received the documents listed below and that these comply with the approval conditions detailed in our letter dated 11 November 2013

Documents received

The documents received were as follows:

Document	*Version*	*Date*
Participant Consent Form	2	11 November 2013
Participant Information Sheet: Parents/Carers - Control Group	1	11 November 2013
Participant Information Sheet: Children	3	11 November 2013

Approved documents

The final list of approved documentation for the study is therefore as follows:

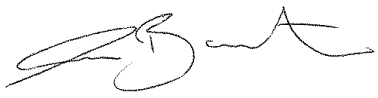
Document	*Version*	*Date*
Evidence of insurance or indemnity		05 August 2013
GP/Consultant Information Sheets		
Investigator CV	Southern	
Investigator CV	Forthergill	
Investigator CV	Pybus	

Letter from Sponsor		07 October 2013
Other: Date Collection Proforma CF	1	02 October 2013
Other: Date Collection Proforma Healthy	1	02 October 2013
Participant Consent Form: Assent	2	07 October 2013
Participant Consent Form	2	11 November 2013
Participant Information Sheet: Parents & Carers	1	02 October 2013
Participant Information Sheet: Parents/Carers - Control Group	1	11 November 2013
Participant Information Sheet: Children	3	11 November 2013
Protocol	2	04 October 2013
REC application	3.5	09 October 2013
Referees or other scientific critique report		03 October 2013

You should ensure that the sponsor has a copy of the final documentation for the study. It is the sponsor's responsibility to ensure that the documentation is made available to R&D offices at all participating sites.

13/NW/0740 **Please quote this number on all correspondence**

Yours sincerely



Anna Bannister
REC Assistant

E-mail: nrescommittee.northwest-liverpoolcentral@nhs.net

Copy to: *Mr Alex Astor,*
Ms Dot Lambert, Alder Hey Children's NHS Foundation Trust

Appendix B

Research and Development approval

Alder Hey Children's NHS
NHS Foundation Trust
Research & Development
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Dr Kevin Southern
Honorary Consultant in Paediatric Respiratory Medicine
Alder Hey Children's NHS Foundation Trust
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Liverpool
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14/01/2014

RE: A FEASIBILITY STUDY IN DETERMINING THE SKIN MICROBIOME OF CHILDREN WITH CYSTIC FIBROSIS COMPARED TO HEALTHY AGE-MATCHED CONTROLS
REC Ref: 13/NW/0740
R&D Ref: 13/76/RE

Dear Dr Southern

The protocol and supporting documentation for the above study have been submitted to the NIHR Co-ordinated System for Gaining NHS permission for governance checks to be undertaken in accordance with the requirements under the Research Governance Framework for Health and Social Care, and relevant legislation. I am pleased to confirm that following completion of these checks approval for the study to go ahead within the Alder Hey Children's NHS Foundation Trust is now granted.

This Trust is performance managed by the National Institute of Health Research (NIHR) in terms of the NIHR Higher Level objective of increasing the number of patients recruited to clinical trials. Our Trust investigators are supported by a number of data managers. You will be contacted by a member of that team who will advise you on the time and format in which data should be submitted. **R&D approval is conditional upon these data being submitted in a timely fashion each month**.

It will be the responsibility of the local Principal Investigator to comply with the responsibilities laid down, in the Research Governance Framework for Health and Social Care, by the Department of Health. Please see the enclosed leaflet for further information.

A full copy of the Research Governance Framework for Health and Social Care can also be obtained from the Department of Health website at www.doh.gov.uk or the R&D Office.

Yours sincerely

pp.

Dot Lambert
Research Business Unit Manager

RESEARCH GOVERNANCE FRAMEWORK FOR HEALTH AND SOCIAL CARE

RESPONSIBILITIES OF THE PRINCIPAL INVESTIGATOR

It is the principal investigator's responsibility to ensure that:

The dignity, rights, safety and well being of participants are given priority at all times by the research team.

The research is carried out in accordance with the research governance framework.

When a study involves participants under the care of a doctor, nurse or social worker for the condition to which the study relates, those care professionals are informed that their patients or users are being invited to participate and agree to retain overall responsibility for their care.

When the research involves user or carer or a child, looked after or receiving services under the auspices of the local authority, that the agency director or her deputy agrees to the person (and/or their carer) being invited to participate and is fully aware of the arrangements for dealing with any disclosure or other relevant information.

Unless participants or the relevant research ethics committee request otherwise participants' care professionals are given information specifically relevant to their care which arises in the research.

The study complies with all legal and ethical requirements.

A Material Transfer Agreement is in place with the receiving organisation for any samples sent outside of the Trust.

Each member of the research team is qualified by education, training and experience to discharge his/her role in the study.

Students and new researchers have adequate supervision, support and training.

The research follows the protocol approved by the research committee.

Any proposed changes or amendments to or deviations from the protocol are submitted for approval to the ethics committee, the research sponsor and any other appropriate body.

Procedures are in place to ensure collection of high quality, accurate data and the integrity and confidentiality of data during processing and storage.

Arrangements are made for the appropriate archiving of data when the research has finished.

The findings from the work are opened to critical review through the accepted scientific and professional channels.

Once established, findings from the work are disseminated promptly and fed back as appropriate to participants.

All data and documentation associated with the study are available for audit at the request of an auditing authority.

Appendix C

Media and buffers

Blood-Columbia was prepared from Columbia media (Oxoid) with the addition of 5% defibrinated horse blood (Fisher Scientific) following autoclaving and cooling of media to 60°C.

Carbohydrate fermentation broth (ASM microbe library) was prepared by adding 10g Proteose Peptone (Difco), 5g NaCl (Sigma), and 0.018g of phenol red (Sigma) to 800mL volume. pH was adjusted to 7.4 using Tris (Sigma). This base media is autoclaved, and then after cooling, a preparation of 10g carbohydrate (all produced by Sigma) dissolved in 200mL SDW is syringe filtered (Appleton Woods, 0.2μL pore diameter) into the broth.

Gelatin solid media (ASM microbe library) was prepared with 23g/L bacteriological agar (Sigma) and 8g/L gelatin. This media was then autoclaved.

Christensen's Urea solid media (ASM microbe library) was prepared with 1g/L peptone (Oxoid), 1g/L Dextrose (Sigma), 5g/L NaCl (Sigma), 2g/L monobasic potassium phosphate (Sigma), 20g/L urea (Sigma), 0.012g phenol red (Sigma). This media was then autoclaved.

Luria-Bertani (LB) Lennox broth was prepared with 10g Bacto-Tryptone (Difco), 5g Yeast Extract (Oxoid), and 10g NaCl (Sigma) in 1L volume.

LB glycerol was prepared as above with the Lennox version of LB, with the addition of 20% v/w of glycerol.

All other medias employed were produced from Oxoid, and manufacturer's instructions were followed regarding their preparation.

Phosphate-buffered saline (PBS) was prepared according to instructions from Sigma.

Tris-EDTA buffer was prepared using 10mM Tris in 1mM EDTA, adjusted to pH 8.0 with concentrated HCl

TE-Tween buffer was prepared by adding 1% Tween 20 to a preparation of Tris-EDTA.

All buffers were autoclaved prior to use.

Appendix D

Full culture and biochemical test results

Subject identifier is given as "ID". Cystic fibrosis status is given as "Y" for subjects with CF and "N" for subjects without CF. Aerophilic growth of the isolate is given as "Y" if initially cultured in aerobic conditions, "MA" is given if the isolate was originally isolated in microaerophilic conditions. Carbohydrate names indicate whether the isolate fermented to produce acid in the carbohydrate media provided. Pibwin score gives the probability associated with the assigned identification.

ID	Patient site	CF	Media	Aerophillic	Colony colour	Gram reaction	Morphology	Arrangement	Haemolysis	Catalase	Oxidase	Gelatin	Urea	MSA	Maltose	Fructose	Glucose	Lactose	Sucrose	Pibwin score	Pibwin ID
35	Axilla	N	NS	Y	Cream	+	Cocci	Chains	-	+	-	-	+	-	+	+	+	+	+	0.40327	Staphylococcus
35	Oropharynx	N	NS	MA	Yellow	+	Cocci	Chains	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
35	Oropharynx	N	NS	Y	Yellow	+	Cocci	Chains	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
35	Oropharynx	N	NS	MA	Yellow	+	Cocci	Chains	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
35	Axilla	N	St-spec	Y	Yellow	+	Cocci	Chains	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
35	Oropharynx	N	NS	MA	Yellow	+	Cocci	Chains	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
35	Oropharynx	N	NS	MA	Yellow	+	Cocci	Chains	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
35	Axilla	N	NS	Y	Yellow	+	Cocci	Chains	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
35	Oropharynx	N	NS	MA	Yellow	+	Cocci	Chains	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
35	Oropharynx	N	NS	MA	Yellow	+	Cocci	Chains	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
35	Axilla	N	St-spec	Y	Yellow	+	Cocci	Chains	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
35	Oropharynx	N	NS	MA	Yellow	+	Cocci	Chains	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
35	Axilla	N	St-spec	Y	Yellow	+	Cocci	Chains	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
35	Oropharynx	N	NS	MA	Yellow	+	Cocci	Chains	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
25	Oropharynx	N	NS	Y	Tan	+	Cocci	Chains	-	+	+	-	+	-	+	+	+	+	+	0.49045	Micrococcus varians
25	Forearm	N	NS	Y	Tan	+	Cocci	Chains	-	+	+	-	+	-	+	+	+	+	+	0.49045	Micrococcus varians
25	Forearm	N	NS	Y	Cream	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.40327	Staphylococcus
25	Nare	N	NS	Y	Yellow	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.59352	Staphylococcus
25	Forearm	N	NS	Y	Yellow	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.59352	Staphylococcus
25	Forearm	N	NS	Y	Yellow	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.59352	Staphylococcus
25	Axilla	N	NS	Y	Yellow	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.59352	Staphylococcus
25	Forearm	N	NS	Y	Yellow	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.59352	Staphylococcus
25	Nare	N	NS	Y	Yellow	+	Cocci	Chains	-	+	-	-	-	-	+	+	+	+	+	0.57697	Staphylococcus hominis
4	Axilla	Y	NS	Y	Cream	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.40327	Staphylococcus
4	Axilla	Y	NS	Y	Cream	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.40327	Staphylococcus
4	Axilla	Y	NS	Y	Cream	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.40327	Staphylococcus
4	Nare	Y	St-spec	Y	Cream	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.63592	Staphylococcus
4	ACF	Y	NS	Y	Cream	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.63592	Staphylococcus
4	Axilla	Y	NS	Y	Cream	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.63592	Staphylococcus

ID	Patient site	CF	Media	Aerophillic	Colony colour	Gram reaction	Morphology	Arrangement	Haemolysis	Catalase	Oxidase	Gelatin	Urea	MSA	Maltose	Fructose	Glucose	Lactose	Sucrose	Pibwin score	Pibwin ID
4 ACF		Y	NS	Y	Cream	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.63592	Staphylococcus
4 Axilla		Y	NS	Y	Yellow	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.96678	Staphylococcus
4 Axilla		Y	NS	Y	Cream	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.63592	Staphylococcus
4 Axilla		Y	NS	MA	Cream	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.63592	Staphylococcus
4 Axilla		Y	NS	Y	Cream	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.63592	Staphylococcus
4 ACF		Y	NS	Y	Yellow	+	Cocci	Clusters	β	+	-	+	+	+	+	+	+	+	+	0.85058	Staphylococcus aureus
4 ACF		Y	NS	Y	Yellow	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.9211	Staphylococcus aureus
4 Axilla		Y	NS	Y	Yellow	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.9211	Staphylococcus aureus
4 Oropharynx		Y	NS	Y	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
4 ACF		Y	NS	MA	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
4 Axilla		Y	NS	Y	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
4 ACF		Y	NS	Y	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
4 Axilla		Y	NS	Y	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
4 ACF		Y	St-spec	Y	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
4 ACF		Y	NS	MA	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
4 ACF		Y	NS	Y	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
4 Axilla		Y	NS	Y	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
4 ACF		Y	NS	Y	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
4 ACF		Y	NS	Y	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
4 Axilla		Y	NS	Y	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
4 Axilla		Y	NS	MA	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
4 Nare		Y	NS	Y	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
4 ACF		Y	NS	Y	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
4 Axilla		Y	NS	Y	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
4 ACF		Y	NS	MA	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
4 ACF		Y	NS	Y	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
4 Axilla		Y	NS	Y	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
4 ACF		Y	NS	MA	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis

ID	Patient site	CF	Media	Aerophilic	Colony colour	Gram reaction	Morphology	Arrangement	Haemolysis	Catalase	Oxidase	Gelatin	Urea	MSA	Maltose	Fructose	Glucose	Lactose	Sucrose	Pibwin score	Pibwin ID
4	Axilla	Y	NS	MA	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
4	ACF	Y	NS	Y	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
4	Axilla	Y	NS	Y	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
29	Forearm	N	NS	Y	Tan	+	Cocci	Clusters	-	+	+	-	+	-	+	+	+	+	+	0.49045	Micrococcus varians
29	Axilla	N	NS	Y	Cream	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.40327	Staphylococcus
29	Nare	N	NS	Y	Cream	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.40327	Staphylococcus
29	Forearm	N	NS	Y	Cream	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.40327	Staphylococcus
29	Axilla	N	NS	Y	Yellow	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.59352	Staphylococcus
29	Nare	N	NS	Y	Yellow	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.59352	Staphylococcus
29	Forearm	N	NS	Y	Yellow	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.59352	Staphylococcus
29	Axilla	N	NS	Y	Yellow	+	Cocci	Clusters	-	+	-	-	+	+	+	+	+	+	+	0.51899	Staphylococcus
29	Axilla	N	NS	Y	Yellow	+	Cocci	Clusters	α	+	-	-	+	+	+	+	+	+	+	0.51899	Staphylococcus
29	Nare	N	NS	Y	Yellow	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.51899	Staphylococcus
7	Nare	Y	NS	MA	Cream	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.40327	Staphylococcus
7	Axilla	Y	NS	MA	Cream	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.40327	Staphylococcus
7	ACF	Y	NS	MA	Cream	+	Cocci	Clusters	β	+	+	-	+	+	+	+	+	+	+	0.63592	Staphylococcus
7	Oropharynx	Y	NS	MA	Cream	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.63592	Staphylococcus
7	ACF	Y	NS	MA	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
7	ACF	Y	NS	MA	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
7	ACF	Y	NS	MA	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
7	Nare	Y	NS	Y	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
7	ACF	Y	NS	MA	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
7	Nare	Y	NS	Y	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
33	Forearm	N	NS	0	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
33	Forearm	N	NS	0	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
33	Forearm	N	NS	0	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
33	Axilla	N	NS	0	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
33	Nare	N	NS	0	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
33	Oropharynx	N	NS	0	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
33	Axilla	N	NS	0	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
33	Oropharynx	N	NS	0	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
10	(Forearm	Y	NS	Y	Tan	+	Cocci	Clusters	-	+	+	-	+	-	+	+	+	+	+	0.49045	Micrococcus varians
10	(ACF	Y	NS	MA	Cream	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.40327	Staphylococcus
10	(ACF	Y	NS	MA	Cream	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.40327	Staphylococcus

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4	Axilla	Y	NS		MA	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
4	ACF	Y	NS		Y	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
4	Axilla	Y	NS		Y	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
29	Forearm	N	NS		Y	Tan	+	Cocci	Clusters	-	+	+	-	+	-	+	+	+	+	+	0.49045	Micrococcus varians
29	Axilla	N	NS		Y	Cream	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.40327	Staphylococcus
29	Nare	N	NS		Y	Cream	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.40327	Staphylococcus
29	Forearm	N	NS		Y	Cream	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.40327	Staphylococcus
29	Axilla	N	NS		Y	Yellow	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.59352	Staphylococcus
29	Nare	N	NS		Y	Yellow	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.59352	Staphylococcus
29	Forearm	N	NS		Y	Yellow	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.59352	Staphylococcus
29	Axilla	N	NS		Y	Yellow	+	Cocci	Clusters	-	+	-	-	+	+	+	+	+	+	+	0.51899	Staphylococcus
29	Axilla	N	NS		Y	Yellow	+	Cocci	Clusters	α	+	-	-	+	+	+	+	+	+	+	0.51899	Staphylococcus
29	Nare	N	NS		Y	Yellow	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.51899	Staphylococcus
7	Nare	Y	NS		MA	Cream	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.40327	Staphylococcus
7	Axilla	Y	NS		MA	Cream	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.40327	Staphylococcus
7	ACF	Y	NS		MA	Cream	+	Cocci	Clusters	β	+	+	-	+	+	+	+	+	+	+	0.63592	Staphylococcus
7	Oropharynx	Y	NS		MA	Cream	+	Cocci	Clusters	β	+	+	-	+	+	+	+	+	+	+	0.63592	Staphylococcus
7	ACF	Y	NS		MA	Yellow	+	Cocci	Clusters	-	+	+	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
7	ACF	Y	NS		MA	Yellow	+	Cocci	Clusters	-	+	+	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
7	ACF	Y	NS		MA	Yellow	+	Cocci	Clusters	-	+	+	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
7	Nare	Y	NS		Y	Yellow	+	Cocci	Clusters	-	+	+	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
7	ACF	Y	NS		MA	Yellow	+	Cocci	Clusters	-	+	+	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
7	Nare	Y	NS		Y	Yellow	+	Cocci	Clusters	-	+	+	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
33	Forearm	N	NS		0	Yellow	+	Cocci	Clusters	-	+	+	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
33	Forearm	N	NS		0	Yellow	+	Cocci	Clusters	-	+	+	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
33	Forearm	N	NS		0	Yellow	+	Cocci	Clusters	-	+	+	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
33	Axilla	N	NS		0	Yellow	+	Cocci	Clusters	-	+	+	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
33	Nare	N	NS		0	Yellow	+	Cocci	Clusters	-	+	+	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
33	Oropharynx	N	NS		0	Yellow	+	Cocci	Clusters	-	+	+	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
33	Axilla	N	NS		0	Yellow	+	Cocci	Clusters	-	+	+	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
33	Oropharynx	N	NS		0	Yellow	+	Cocci	Clusters	-	+	+	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
10	(Forearm	Y	NS		Y	Tan	+	Cocci	Clusters	-	+	+	-	+	-	+	+	+	+	+	0.49045	Micrococcus varians
10	(ACF	Y	NS		MA	Cream	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.40327	Staphylococcus
10	(ACF	Y	NS		MA	Cream	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.40327	Staphylococcus

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10 (	Nare	Y	NS	Y	Tan	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	-	0.55924	Staphylococcus
10 (	Forearm	Y	NS	Y	Yellow	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.59352	Staphylococcus
10 (	Axilla	Y	NS	MA	Cream	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.63592	Staphylococcus
10 (	ACF	Y	NS	MA	Cream	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.63592	Staphylococcus
10 (	Axilla	Y	NS	MA	Yellow	+	Cocci	Clusters	β	+	-	+	+	+	+	+	+	+	+	0.85058	Staphylococcus aureus
10 (	Axilla	Y	NS	MA	Yellow	+	Cocci	Clusters	β	+	-	+	+	+	+	+	+	+	+	0.85058	Staphylococcus aureus
10 (	Axilla	Y	NS	MA	Yellow	+	Cocci	Clusters	β	+	-	+	+	+	+	+	+	+	+	0.85058	Staphylococcus aureus
10 (	Axilla	Y	NS	MA	Yellow	+	Cocci	Clusters	β	+	-	+	+	+	+	+	+	+	+	0.85058	Staphylococcus aureus
10 (	Nare	Y	NS	Y	Yellow	+	Cocci	Clusters	-	+	-	-	-	-	+	+	+	+	+	0.77495	Staphylococcus hominis
10 (	Axilla	Y	NS	Y	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
10 (	Nare	Y	NS	Y	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
10 (	Oropharynx	Y	NS	Y	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
10 (	Axilla	Y	NS	Y	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
10 (	Oropharynx	Y	NS	Y	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
10 (	Axilla	Y	NS	Y	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
10 (	Oropharynx	Y	NS	Y	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
10 (	Axilla	Y	NS	Y	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
10 (	Oropharynx	Y	NS	Y	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
10 (	Axilla	Y	NS	Y	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
10 (	ACF	Y	NS	Y	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
10 (	Oropharynx	Y	NS	Y	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
10 (	ACF	Y	NS	Y	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
10 (	ACF	Y	NS	Y	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
10 (	Oropharynx	Y	NS	Y	Yellow	+	Cocci	Chains	α	-	-	-	-	-	+	+	+	+	-	0.95639	Streptococcus group O
11 (	Forearm	Y	NS	Y	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	-	+	+	+	-	0.70019	Micrococcus luteus 1
11 (	Forearm	Y	NS	Y	Tan	+	Cocci	Clusters	-	+	+	-	+	-	+	+	+	+	-	0.84912	Micrococcus varians
11 (	Nare	Y	Ps-spec	Y	Cream	-	Rods	-	-	+	+	+	-	-	-	-	-	-	-	0.38171	Pseudomonas
11 (	Axilla	Y	NS	Y	Cream	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.40327	Staphylococcus
11 (	Nare	Y	NS	Y	Cream	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.40327	Staphylococcus
11 (	Axilla	Y	NS	Y	Yellow	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.59352	Staphylococcus
11 (	Oropharynx	Y	NS	Y	Yellow	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.51899	Staphylococcus
11 (	Nare	Y	NS	Y	Yellow	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.9092	Staphylococcus aureus
11 (	Axilla	Y	NS	Y	Yellow	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.9092	Staphylococcus aureus
12 (	Oropharynx	Y	NS	Y	Yellow	+	Cocci	Clusters	-	+	-	-	-	-	-	-	-	-	-	0.99911	Micrococcus luteus 1

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12 (	ACF	Y	NS		Y	Cream	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.40327	Staphylococcus
12 (	Axilla	Y	NS		Y	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
12 (	Oropharynx	Y	NS		Y	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
12 (	Nare	Y	NS		Y	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
18	Forearm	Y	NS		Y	Tan	+	Cocci	Clusters	-	+	+	-	+	-	+	+	+	+	+	0.49045	Micrococcus varians
18	Forearm	Y	Ps-spec		Y	Cream	-	Rods	-	-	+	+	+	-	-	-	-	-	-	-	0.38171	Pseudomonas
18	Forearm	Y	NS		Y	Yellow	+	Cocci	Chains	β	-	-	-	+	+	+	+	+	+	+	0.58882	Staphylococcus
18	Axilla	Y	NS		Y	Cream	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.40327	Staphylococcus
18	Axilla	Y	NS		Y	Cream	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.40327	Staphylococcus
18	Forearm	Y	NS		Y	Cream	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.40327	Staphylococcus
18	Nare	Y	NS		Y	Cream	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.40327	Staphylococcus
18	Nare	Y	NS		Y	Yellow	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.59352	Staphylococcus
18	Axilla	Y	NS		Y	Yellow	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.59352	Staphylococcus
18	Oropharynx	Y	NS		Y	Yellow	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.59352	Staphylococcus
18	Axilla	Y	NS		Y	Yellow	+	Cocci	Clusters	-	+	-	-	+	+	+	-	+	+	+	0.58046	Staphylococcus
18	Forearm	Y	NS		Y	Yellow	+	Cocci	Clusters	-	+	-	-	+	+	+	-	+	+	+	0.58046	Staphylococcus
18	Forearm	Y	NS		Y	Yellow	+	Cocci	Clusters	β	+	-	-	+	+	+	-	+	+	+	0.58046	Staphylococcus
9	Axilla	Y	NS		Y	Yellow	+	Cocci	Clusters	-	+	+	-	+	-	-	+	+	+	-	0.95034	Micrococcus luteus 1
9	Axilla	Y	NS		Y	Tan	+	Cocci	Clusters	-	+	+	-	+	-	+	+	+	+	+	0.49045	Micrococcus varians
9	ACF	Y	NS		Y	Yellow	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.59352	Staphylococcus
9	Axilla	Y	NS		Y	Cream	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.40327	Staphylococcus
9	ACF	Y	NS		Y	Cream	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.40327	Staphylococcus
9	Oropharynx	Y	NS		Y	Yellow	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.59352	Staphylococcus
9	Nare	Y	NS		Y	Yellow	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.59352	Staphylococcus
9	Axilla	Y	NS		Y	Yellow	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.59352	Staphylococcus
8	Forearm	Y	NS		Y	Yellow	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.59352	Staphylococcus
8	Forearm	Y	NS		Y	Yellow	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.59352	Staphylococcus
8	Axilla	Y	NS		Y	Yellow	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.59352	Staphylococcus
8	Axilla	Y	NS		Y	Yellow	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.59352	Staphylococcus
8	Forearm	Y	NS		Y	Yellow	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.59352	Staphylococcus
8	Nare	Y	NS		Y	Yellow	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.59352	Staphylococcus
8	Nare	Y	NS		Y	Yellow	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.51899	Staphylococcus
8	Axilla	Y	NS		Y	Yellow	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.51899	Staphylococcus
8	Forearm	Y	NS		Y	Yellow	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.51899	Staphylococcus

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8	Oropharynx	Y	NS	Y	Yellow	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.51899	Staphylococcus
8	Axilla	Y	NS	Y	Yellow	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.9092	Staphylococcus aureus
8	Forearm	Y	NS	Y	Yellow	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.9092	Staphylococcus aureus
1	Nare	Y	NS	Y	Cream	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.40327	Staphylococcus
1	ACF	Y	NS	MA	Cream	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.40327	Staphylococcus
1	Oropharynx	Y	St-spec	Y	Cream	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.40327	Staphylococcus
1	Axilla	Y	NS	Y	Cream	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.40327	Staphylococcus
1	ACF	Y	St-spec	Y	Cream	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.40327	Staphylococcus
1	Oropharynx	Y	NS	Y	Cream	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.40327	Staphylococcus
1	Nare	Y	St-spec	Y	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
1	ACF	Y	NS	Y	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
1	Nare	Y	NS	MA	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
1	Oropharynx	Y	NS	Y	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
16	Oropharynx	Y	NS	Y	Tan	+	Cocci	Clusters	-	+	+	-	+	-	+	+	+	+	+	0.49045	Micrococcus varians
16	Oropharynx	Y	Ps-spec	Y	Cream	-	Rods	-	-	+	+	+	-	-	-	-	-	-	-	0.38171	Pseudomonas
16	Forearm	Y	NS	Y	Cream	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.63592	Staphylococcus
16	Nare	Y	NS	Y	Cream	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.63592	Staphylococcus
16	Axilla	Y	NS	Y	Cream	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.63592	Staphylococcus
16	Oropharynx	Y	NS	Y	Yellow	+	Cocci	Clusters	α	+	-	-	+	+	+	+	+	+	+	0.51899	Staphylococcus
3	Oropharynx	Y	NS	MA	Cream	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.63592	Staphylococcus
3	Axilla	Y	St-spec	Y	Cream	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.63592	Staphylococcus
3	Axilla	Y	St-spec	Y	Cream	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.63592	Staphylococcus
3	Axilla	Y	NS	MA	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
3	Nare	Y	St-spec	Y	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
3	ACF	Y	NS	MA	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
12	(2) Oropharynx	Y	Ps-spec	Y	Cream	-	Rods	-	-	+	+	+	-	-	-	-	-	-	-	0.38171	Pseudomonas
12	(2) Nare	Y	Ps-spec	Y	Cream	-	Rods	-	-	+	+	+	-	-	-	-	-	-	-	0.38171	Pseudomonas
12	(2) Forearm	Y	Ps-spec	Y	Cream	-	Rods	-	-	+	+	+	-	-	-	-	-	-	-	0.40437	Pseudomonas stutzeri
12	(2) Forearm	Y	NS	MA	Cream	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.40327	Staphylococcus
12	(2) Forearm	Y	NS	Y	Cream	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.63592	Staphylococcus
12	(2) Nare	Y	NS	MA	Cream	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.63592	Staphylococcus
12	(2) Axilla	Y	NS	MA	Cream	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.63592	Staphylococcus
12	(2) Oropharynx	Y	NS	Y	Cream	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.63592	Staphylococcus
12	(2) Nare	Y	NS	MA	Cream	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.63592	Staphylococcus

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30	ACF	N	NS	Y	Cream	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.63592	Staphylococcus
30	ACF	N	NS	Y	Cream	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.63592	Staphylococcus
30	ACF	N	NS	MA	Yellow	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.96678	Staphylococcus
30	Oropharynx	N	NS	Y	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
30	ACF	N	NS	Y	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
30	Axilla	N	NS	Y	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
30	Nare	N	NS	Y	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
10	Forearm	Y	NS	Y	Cream	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.40327	Staphylococcus
10	Forearm	Y	NS	MA	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
10	Nare	Y	NS	Y	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
10	Nare	Y	NS	Y	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
10	Oropharynx	Y	NS	MA	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
5	Oropharynx	Y	NS	Y	Cream	+	Cocci	Clusters	-	+	-	-	+	+	+	+	+	+	+	0.40327	Staphylococcus
5	Oropharynx	Y	NS	Y	Cream	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.63592	Staphylococcus
5	Oropharynx	Y	NS	Y	Yellow	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.96678	Staphylococcus
5	Oropharynx	Y	NS	Y	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
5	Nare	Y	NS	Y	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
5	Nare	Y	NS	MA	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
5	Oropharynx	Y	NS	Y	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
5	Nare	Y	NS	MA	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
5	Oropharynx	Y	NS	Y	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
5	Nare	Y	NS	MA	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
5	ACF	Y	NS	Y	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
5	Oropharynx	Y	NS	Y	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
5	Nare	Y	NS	MA	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
27	Nare	N	NS	Y	Cream	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.40327	Staphylococcus
27	Oropharynx	N	NS	Y	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
27	Nare	N	St-spec	Y	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
27	Axilla	N	St-spec	Y	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
27	Oropharynx	N	NS	Y	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
27	Oropharynx	N	NS	Y	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
27	Forearm	N	NS	Y	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
27	Oropharynx	N	NS	Y	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
2	Oropharynx	Y	St-spec	Y	Yellow	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.96678	Staphylococcus

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2 Nare	Nare	Y	St-spec	Y	Cream	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.63592	Staphylococcus
2 ACF	ACF	Y	NS	MA	Cream	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.63592	Staphylococcus
2 Axilla	Axilla	N	NS	Y	Cream	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.40327	Staphylococcus
2 ACF	ACF	Y	NS	Y	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
2 ACF	ACF	Y	NS	Y	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
2 ACF	ACF	Y	NS	Y	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
2 Nare	Nare	Y	St-spec	Y	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
2 ACF	ACF	Y	NS	MA	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
2 ACF	ACF	Y	NS	MA	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
2 Oropharynx	Oropharynx	Y	NS	MA	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
19 Axilla	Axilla	N	NS	Y	White	+	Cocci	Clusters	-	+	-	-	+	-	+	-	+	-	-	0.94931	Micrococcus luteus 3
19 Axilla	Axilla	N	NS	Y	Cream	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.40327	Staphylococcus
19 Nare	Nare	N	NS	Y	Cream	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.40327	Staphylococcus
19 Oropharynx	Oropharynx	N	NS	Y	Cream	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.40327	Staphylococcus
19 Forearm	Forearm	N	NS	Y	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
13 ACF	ACF	N	NS	Y	Cream	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.40327	Staphylococcus
13 ACF	ACF	N	NS	Y	Cream	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.40327	Staphylococcus
13 ACF	ACF	N	NS	Y	Cream	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.40327	Staphylococcus
13 ACF	ACF	N	St-spec	Y	Cream	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.63592	Staphylococcus
13 ACF	ACF	N	St-spec	Y	Cream	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.63592	Staphylococcus
13 ACF	ACF	N	NS	Y	Cream	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.63592	Staphylococcus
13 Axilla	Axilla	N	St-spec	Y	Cream	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.63592	Staphylococcus
13 Axilla	Axilla	N	St-spec	Y	Yellow	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.96678	Staphylococcus
13 Axilla	Axilla	N	St-spec	Y	Yellow	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.96678	Staphylococcus
13 ACF	ACF	N	NS	Y	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
13 Nare	Nare	N	NS	MA	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
13 ACF	ACF	N	NS	Y	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
13 Oropharynx	Oropharynx	N	NS	MA	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
13 ACF	ACF	N	NS	MA	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
13 Axilla	Axilla	N	St-spec	Y	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
13 ACF	ACF	N	NS	MA	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
13 Axilla	Axilla	N	NS	Y	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
13 Axilla	Axilla	N	St-spec	Y	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis

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13 Axilla	N	St-spec	Y	Yellow	+	Cocci	Clusters	-	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
13 Nare	N	NS	MA	Yellow	+	Cocci	Clusters	-	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
13 ACF	N	NS	Y	Tan	+	Cocci	Chains	α	-	-	-	-	+	-	+	+	+	-	+	0.66332	Streptococcus 'clinical'
24 Forearm	N	NS	MA	Yellow	+	Cocci	Clusters	-	+	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
24 Forearm	N	NS	Y	Yellow	+	Cocci	Clusters	-	+	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
24 Oropharynx	N	NS	MA	Yellow	+	Cocci	Clusters	-	+	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
24 Nare	N	NS	MA	Yellow	+	Cocci	Clusters	-	+	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
24 Nare	N	NS	Y	Yellow	+	Cocci	Clusters	-	+	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
24 Axilla	N	St-spec	Y	Yellow	+	Cocci	Clusters	-	+	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
11 (1 Nare	Y	NS	Y	Cream	+	Cocci	Clusters	-	+	+	-	-	+	-	+	+	+	+	+	0.40327	Staphylococcus
11 (1 Axilla	Y	NS	Y	Cream	+	Cocci	Clusters	-	+	+	-	-	+	-	+	+	+	+	+	0.40327	Staphylococcus
11 (1 Axilla	Y	NS	Y	Cream	+	Cocci	Clusters	-	+	+	-	-	+	-	+	+	+	+	+	0.40327	Staphylococcus
11 (1 ACF	Y	St-spec	Y	Cream	+	Cocci	Clusters	-	+	+	-	-	+	-	+	+	+	+	+	0.40327	Staphylococcus
11 (1 Axilla	Y	St-spec	Y	Cream	+	Cocci	Clusters	-	+	+	-	-	+	-	+	+	+	+	+	0.40327	Staphylococcus
11 (1 ACF	Y	NS	Y	Cream	+	Cocci	Clusters	-	+	+	-	-	+	-	+	+	+	+	+	0.40327	Staphylococcus
11 (1 ACF	Y	NS	Y	Cream	+	Cocci	Clusters	-	+	+	-	-	+	-	+	+	+	+	+	0.40327	Staphylococcus
11 (1 Axilla	Y	St-spec	Y	Cream	+	Cocci	Clusters	-	+	+	-	-	+	-	+	+	+	+	+	0.40327	Staphylococcus
11 (1 ACF	Y	NS	Y	Cream	+	Cocci	Clusters	-	+	+	-	-	+	-	+	+	+	+	+	0.40327	Staphylococcus
11 (1 Oropharynx	Y	NS	MA	Cream	+	Cocci	Clusters	β	+	+	-	-	+	+	+	+	+	+	+	0.63592	Staphylococcus
11 (1 Nare	Y	NS	MA	Cream	+	Cocci	Clusters	β	+	+	-	-	+	+	+	+	+	+	+	0.63592	Staphylococcus
11 (1 Oropharynx	Y	NS	MA	Cream	+	Cocci	Clusters	β	+	+	-	-	+	+	+	+	+	+	+	0.63592	Staphylococcus
11 (1 Oropharynx	Y	NS	MA	Cream	+	Cocci	Clusters	β	+	+	-	-	+	+	+	+	+	+	+	0.63592	Staphylococcus
11 (1 Axilla	Y	NS	MA	Yellow	+	Cocci	Clusters	-	+	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
11 (1 Axilla	Y	NS	MA	Yellow	+	Cocci	Clusters	-	+	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
11 (1 Nare	Y	St-spec	Y	Yellow	+	Cocci	Clusters	-	+	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
11 (1 Axilla	Y	NS	Y	Yellow	+	Cocci	Clusters	-	+	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
11 (1 Axilla	Y	NS	MA	Yellow	+	Cocci	Clusters	-	+	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
11 (1 ACF	Y	NS	MA	Yellow	+	Cocci	Clusters	-	+	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
11 (1 ACF	Y	NS	MA	Yellow	+	Cocci	Clusters	-	+	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
17 Forearm	Y	St-spec	Y	Yellow	+	Cocci	Clusters	-	+	+	-	-	+	-	-	-	-	-	+	0.98329	Micrococcus luteus 1
17 Forearm	Y	St-spec	Y	Cream	+	Cocci	Clusters	-	+	+	-	-	+	-	+	+	+	-	-	0.80458	Micrococcus varians
17 Nare	Y	St-spec	Y	Cream	+	Cocci	Clusters	-	+	+	-	-	+	-	+	+	+	+	+	0.40327	Staphylococcus
17 Nare	Y	St-spec	Y	Cream	+	Cocci	Clusters	-	+	+	-	-	+	-	+	+	+	+	+	0.40327	Staphylococcus

ID	Patient site	CF	Media	Aerophilic	Colony colour	Gram reaction	Morphology	Arrangement	Haemolysis	Catalase	Oxidase	Gelatin	Urea	MSA	Maltose	Fructose	Glucose	Lactose	Sucrose	Pibwin score	Pibwin ID
17 Axilla	Y	NS	Y	Cream	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.40327	Staphylococcus
17 Forearm	Y	St-spec	Y	Cream	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.40327	Staphylococcus
17 Axilla	Y	NS	Y	Cream	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.40327	Staphylococcus
17 Forearm	Y	NS	MA	Cream	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.40327	Staphylococcus
17 Oropharynx	Y	St-spec	Y	Cream	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.40327	Staphylococcus
20 ACF	N	NS	MA	Cream	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.63592	Staphylococcus
20 Nare	N	NS	MA	Cream	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.63592	Staphylococcus
20 Oropharynx	N	NS	MA	Cream	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.63592	Staphylococcus
20 Oropharynx	N	NS	MA	Cream	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.63592	Staphylococcus
20 Axilla	N	NS	MA	Cream	+	Cocci	Clusters	-	+	-	-	+	+	+	+	+	-	+	0.45649	Staphylococcus warneri
36 Forearm	N	NS	Y	Cream	+	Cocci	Clusters	-	+	-	-	+	+	-	+	+	+	+	0.40327	Staphylococcus
36 Oropharynx	N	NS	MA	Cream	+	Cocci	Clusters	-	+	-	-	+	+	-	+	+	+	+	0.40327	Staphylococcus
36 Nare	N	NS	MA	Yellow	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.96678	Staphylococcus
36 Nare	N	NS	Y	Yellow	+	Cocci	Clusters	-	+	-	-	+	+	-	+	+	+	+	0.80567	Staphylococcus hominis
36 Oropharynx	N	NS	Y	Yellow	+	Cocci	Clusters	-	+	-	-	+	+	-	+	+	+	+	0.80567	Staphylococcus hominis
36 Axilla	N	NS	Y	Yellow	+	Cocci	Clusters	-	+	-	-	+	+	-	+	+	+	+	0.80567	Staphylococcus hominis
36 Oropharynx	N	NS	MA	Cream	+	Cocci	Clusters	-	+	-	-	+	+	-	+	+	+	+	0.40327	Staphylococcus
36 Axilla	N	NS	MA	Yellow	+	Cocci	Chains	β	+	-	-	+	+	+	+	+	+	+	0.96678	Staphylococcus
36 Nare	N	St-spec	Y	Yellow	+	Cocci	Chains	-	+	-	-	+	+	-	+	+	+	+	0.80567	Staphylococcus hominis
36 Nare	N	St-spec	Y	Yellow	+	Cocci	Chains	-	+	-	-	+	+	-	+	+	+	+	0.80567	Staphylococcus hominis
36 ACF	N	NS	MA	Yellow	+	Cocci	Clusters	-	+	-	-	+	+	-	+	+	+	+	0.80567	Staphylococcus hominis
23 Nare	N	NS	Y	Cream	+	Cocci	Clusters	-	+	-	-	+	+	-	+	+	-	-	0.80458	Micrococcus varians
23 Nare	N	NS	Y	Cream	+	Cocci	Clusters	-	+	-	-	+	+	-	+	+	+	+	0.40327	Staphylococcus
23 Axilla	N	NS	Y	White	+	Cocci	Clusters	-	+	-	-	+	+	-	+	+	+	+	0.40327	Staphylococcus
23 Axilla	N	NS	MA	Cream	+	Cocci	Clusters	-	+	-	-	+	+	-	+	+	+	+	0.40327	Staphylococcus
23 Nare	N	NS	Y	Cream	+	Cocci	Clusters	-	+	-	-	+	+	-	+	+	+	+	0.40327	Staphylococcus
23 ACF	N	St-spec	Y	Cream	+	Cocci	Clusters	-	+	-	-	+	+	-	+	+	+	+	0.40327	Staphylococcus
23 Axilla	N	NS	Y	Cream	+	Cocci	Clusters	-	+	-	-	+	+	-	+	+	+	+	0.40327	Staphylococcus
23 ACF	N	St-spec	Y	Cream	+	Cocci	Clusters	-	+	-	-	+	+	-	+	+	+	+	0.40327	Staphylococcus
23 Nare	N	NS	Y	Cream	+	Cocci	Clusters	-	+	-	-	+	+	-	+	+	+	+	0.40327	Staphylococcus
23 Axilla	N	NS	Y	Cream	+	Cocci	Clusters	-	+	-	-	+	+	-	+	+	+	+	0.40327	Staphylococcus
23 Axilla	N	NS	Y	Yellow	+	Cocci	Clusters	β	+	+	-	-	+	+	+	+	+	+	0.51899	Staphylococcus
23 Axilla	N	NS	MA	Cream	+	Cocci	Clusters	-	+	+	-	-	+	+	+	+	+	+	0.63592	Staphylococcus
23 ACF	N	NS	MA	Cream	+	Cocci	Clusters	β	+	+	-	-	+	+	+	+	+	+	0.63592	Staphylococcus

ID	Patient site	CF	Media	Aerophillic	Colony colour	Gram reaction	Morphology	Arrangement	Haemolysis	Catalase	Oxidase	Gelatin	Urea	MSA	Maltose	Fructose	Glucose	Lactose	Sucrose	Pibwin score	Pibwin ID
23	Oropharynx	N	NS	Y	Cream	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.63592	Staphylococcus
23	Nare	N	NS	Y	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
23	Nare	N	NS	Y	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
23	Nare	N	NS	Y	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
23	Axilla	N	NS	Y	White	+	Cocci	Chains	α	-	-	-	S	-	+	+	+	+	+	0.82138	Streptococcus
23	Nare	N	NS	Y	White	+	Cocci	Chains	α	-	-	-	+	-	+	+	+	+	+	0.82138	Streptococcus
23	Nare	N	NS	Y	Tan	+	Cocci	Chains	β	-	-	-	+	-	+	+	+	-	+	0.66332	Streptococcus 'clinical'
21	Forearm	N	NS	Y	Cream	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.40327	Staphylococcus
21	Oropharynx	N	NS	MA	Cream	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.40327	Staphylococcus
21	Nare	N	NS	Y	Cream	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.40327	Staphylococcus
21	Nare	N	NS	Y	Cream	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.40327	Staphylococcus
21	Axilla	N	St-spec	Y	Cream	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.40327	Staphylococcus
21	Axilla	N	NS	Y	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
21	Nare	N	NS	Y	Cream	+	Cocci	Clusters	-	+	-	-	+	+	+	+	+	-	+	0.45649	Staphylococcus warneri
31	Axilla	N	St-spec	Y	Cream	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.40327	Staphylococcus
31	Oropharynx	N	NS	MA	Cream	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.40327	Staphylococcus
31	ACF	N	NS	Y	Yellow	+	Cocci	Clusters	α	+	-	-	+	+	+	-	+	+	+	0.58046	Staphylococcus
31	Axilla	N	NS	MA	Cream	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.63592	Staphylococcus
31	Nare	N	NS	MA	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
31	ACF	N	NS	MA	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
31	ACF	N	NS	MA	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
31	Axilla	N	St-spec	Y	White	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	-	+	0.8953	Staphylococcus sp BP
22	Forearm	N	NS	Y	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	-	0.81419	Micrococcus varians
22	Forearm	N	NS	Y	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	-	0.81419	Micrococcus varians
22	Oropharynx	N	NS	MA	Cream	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.40327	Staphylococcus
22	Axilla	N	St-spec	Y	Cream	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.40327	Staphylococcus
22	Axilla	N	St-spec	Y	Cream	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.40327	Staphylococcus
22	Oropharynx	N	NS	MA	Cream	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.40327	Staphylococcus
22	Nare	N	NS	MA	Cream	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.40327	Staphylococcus
28	Nare	N	NS	Y	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	-	+	0.68308	Micrococcus sp
28	ACF	N	NS	Y	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	-	0.81419	Micrococcus varians
28	Axilla	N	NS	Y	Tan	+	Cocci	Clusters	-	+	+	-	+	-	+	+	+	+	+	0.49045	Micrococcus varians
28	Axilla	N	NS	Y	Tan	+	Cocci	Clusters	-	+	+	-	+	-	+	+	+	+	+	0.49045	Micrococcus varians
28	Axilla	Y	NS	MA	Cream	-	Rods	-	-	+	+	+	-	-	-	-	-	-	-	0.38171	Pseudomonas

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28 Axilla	Y	NS		MA	Cream	-	Rods	-	-	+	+	+	-	-	-	-	-	-	-	0.38171	Pseudomonas
28 Nare	Y	NS		MA	Cream	-	Rods	-	-	+	+	+	-	-	-	-	-	-	-	0.38171	Pseudomonas
28 ACF	Y	NS		MA	Cream	-	Rods	-	-	+	+	+	-	-	-	-	-	-	-	0.38171	Pseudomonas
28 ACF	N	NS		MA	Cream	-	Rods	-	-	+	+	+	-	-	-	-	-	-	-	0.38171	Pseudomonas
28 Nare	N	NS		MA	Cream	-	Rods	-	-	+	+	+	-	-	-	-	-	-	-	0.38171	Pseudomonas
28 ACF	N	NS		Y	Cream	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.40327	Staphylococcus
28 Nare	N	NS		Y	Cream	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.40327	Staphylococcus
28 ACF	N	NS		Y	Yellow	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.59352	Staphylococcus
28 Axilla	N	NS		Y	Yellow	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.59352	Staphylococcus
28 ACF	N	NS		Y	Yellow	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.59352	Staphylococcus
28 Axilla	N	NS		Y	Yellow	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.51899	Staphylococcus
28 ACF	N	NS		Y	Yellow	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.51899	Staphylococcus
28 Nare	N	NS		Y	Cream	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.63592	Staphylococcus
28 ACF	N	NS		MA	Cream	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.34449	Staphylococcus
28 Nare	N	NS		MA	Cream	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.63592	Staphylococcus
28 Axilla	N	NS		MA	Cream	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.63592	Staphylococcus
28 Axilla	N	NS		MA	Cream	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	-	+	0.61608	Staphylococcus
28 Nare	N	NS		MA	Cream	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.63592	Staphylococcus
28 Axilla	N	NS		MA	Cream	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.63592	Staphylococcus
28 Axilla	N	NS		MA	Cream	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.63592	Staphylococcus
28 Axilla	N	NS		MA	Cream	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.63592	Staphylococcus
28 ACF	N	NS		MA	Cream	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.63592	Staphylococcus
28 Nare	N	NS		MA	Cream	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.63592	Staphylococcus
28 Axilla	N	NS		MA	Cream	+	Cocci	Clusters	β	+	-	-	+	+	+	-	+	+	+	0.34188	Staphylococcus
28 Oropharynx	N	NS		MA	Cream	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.63592	Staphylococcus
28 Nare	N	NS		MA	Cream	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.63592	Staphylococcus
28 Nare	N	NS		MA	Yellow	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.96678	Staphylococcus
28 Nare	N	NS		Y	Yellow	+	Cocci	Clusters	-	+	-	-	-	-	+	+	+	+	+	0.77495	Staphylococcus hominis
28 Nare	N	NS		Y	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
28 Axilla	N	NS		MA	Cream	+	Cocci	Clusters	-	+	-	-	+	+	+	+	+	-	+	0.45649	Staphylococcus warneri
28 Nare	N	NS		MA	Cream	+	Cocci	Clusters	-	+	-	-	+	+	+	+	+	-	+	0.45649	Staphylococcus warneri
32 ACF	N	NS		Y	Yellow	+	Cocci	Clusters	-	+	-	-	-	-	+	+	+	-	+	0.68308	Micrococcus sp
32 ACF	N	NS		Y	Yellow	+	Cocci	Clusters	-	+	-	-	-	-	+	+	+	-	+	0.68308	Micrococcus sp
32 ACF	N	NS		Y	Yellow	+	Cocci	Clusters	-	+	-	-	-	-	+	+	+	-	+	0.68308	Micrococcus sp

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32	Oropharynx	N	NS	Y	Tan	+	Cocci	Clusters	-	+	+	-	+	-	+	+	+	+	+	0.49045	Micrococcus varians
32	Nare	N	NS	Y	Tan	+	Cocci	Clusters	-	+	+	-	+	-	+	+	+	+	+	0.49045	Micrococcus varians
32	ACF	N	NS	Y	Yellow	+	Cocci	Clusters	-	+	-	-	-	-	-	+	+	+	+	0.77243	Micrococcus varians
32	ACF	N	NS	Y	Yellow	+	Cocci	Clusters	-	+	-	-	-	-	-	+	+	+	+	0.77243	Micrococcus varians
32	Nare	Y	NS	MA	Cream	-	Rods	-	-	+	+	+	-	-	-	-	-	-	-	0.38171	Pseudomonas
32	Axilla	Y	NS	MA	Cream	-	Rods	-	-	+	+	+	-	-	-	-	-	-	-	0.38171	Pseudomonas
32	Oropharynx	Y	NS	MA	Cream	-	Rods	-	-	+	+	+	-	-	-	-	-	-	-	0.38171	Pseudomonas
32	Axilla	Y	NS	MA	Cream	-	Rods	-	-	+	+	+	-	-	-	-	-	+	-	0.40437	Pseudomonas stutzeri
32	Oropharynx	N	NS	Y	Cream	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.40327	Staphylococcus
32	ACF	N	NS	Y	Cream	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.40327	Staphylococcus
32	ACF	N	NS	Y	Cream	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.40327	Staphylococcus
32	ACF	N	NS	Y	Cream	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.40327	Staphylococcus
32	ACF	N	NS	Y	Cream	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.40327	Staphylococcus
32	ACF	N	St-spec	Y	Cream	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.40327	Staphylococcus
32	Oropharynx	N	NS	MA	Cream	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.40327	Staphylococcus
32	ACF	N	NS	Y	Cream	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.40327	Staphylococcus
32	ACF	N	NS	MA	Cream	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.40327	Staphylococcus
32	ACF	N	NS	Y	Cream	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.40327	Staphylococcus
32	ACF	N	St-spec	Y	Cream	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.40327	Staphylococcus
32	ACF	N	NS	Y	Cream	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.40327	Staphylococcus
32	ACF	N	St-spec	Y	Cream	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.40327	Staphylococcus
32	ACF	N	St-spec	Y	Cream	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.40327	Staphylococcus
32	ACF	N	NS	Y	Cream	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.40327	Staphylococcus
32	ACF	N	St-spec	Y	Cream	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.40327	Staphylococcus
32	ACF	N	NS	Y	Cream	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.40327	Staphylococcus
32	ACF	N	St-spec	Y	Cream	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.40327	Staphylococcus
32	ACF	N	NS	Y	Cream	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.40327	Staphylococcus
32	Axilla	N	St-spec	Y	Cream	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.40327	Staphylococcus
32	ACF	N	St-spec	Y	Cream	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.40327	Staphylococcus
32	ACF	N	St-spec	Y	Cream	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.40327	Staphylococcus
32	Oropharynx	N	NS	MA	Cream	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.40327	Staphylococcus
32	Oropharynx	N	NS	MA	Cream	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.40327	Staphylococcus
32	ACF	N	St-spec	Y	Cream	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.40327	Staphylococcus
32	ACF	N	St-spec	Y	Cream	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.40327	Staphylococcus

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32 Oropharynx	N	NS		Y	Yellow	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.59352	Staphylococcus
32 Nare	N	NS		Y	Yellow	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.59352	Staphylococcus
32 Nare	N	NS		Y	Yellow	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.59352	Staphylococcus
32 Oropharynx	N	NS		Y	Yellow	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.51899	Staphylococcus
32 ACF	N	NS		Y	Cream	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.63592	Staphylococcus
32 ACF	N	St-spec		Y	Cream	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.63592	Staphylococcus
32 Oropharynx	N	NS		MA	Cream	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.63592	Staphylococcus
32 ACF	N	NS		Y	Cream	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.63592	Staphylococcus
32 ACF	N	St-spec		Y	Cream	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.63592	Staphylococcus
32 Nare	N	NS		MA	Tan	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.96678	Staphylococcus
32 Nare	N	NS		MA	Cream	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.63592	Staphylococcus
32 Axilla	N	NS		MA	Tan	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.96678	Staphylococcus
32 ACF	N	NS		MA	Cream	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.63592	Staphylococcus
32 Nare	N	NS		MA	Cream	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.63592	Staphylococcus
32 Oropharynx	N	NS		MA	Cream	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.63592	Staphylococcus
32 Axilla	N	NS		MA	Cream	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.63592	Staphylococcus
32 Oropharynx	N	NS		MA	Cream	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.63592	Staphylococcus
32 Axilla	N	NS		MA	Cream	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.63592	Staphylococcus
32 Axilla	N	NS		MA	Cream	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.63592	Staphylococcus
32 Oropharynx	N	NS		MA	Cream	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.63592	Staphylococcus
32 Axilla	N	NS		MA	Cream	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.63592	Staphylococcus
32 Axilla	N	NS		MA	Cream	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.63592	Staphylococcus
32 ACF	N	NS		Y	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
32 Axilla	N	St-spec		Y	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
32 Nare	N	NS		Y	White	+	Cocci	Clusters	-	+	-	-	+	-	+	+	-	-	+	0.8953	Staphylococcus sp. BP
6 Oropharynx	Y	NS		MA	Cream	-	Rods	-	-	+	-	-	+	+	-	H2S	+	-	+	0.99913	Acinetobacter
6 Oropharynx	Y	NS		MA	Cream	+	Cocci	Clusters	-	+	-	-	+	+	-	-	+	-	-	0.69027	Arthrobacter sp.
6 Oropharynx	Y	NS		MA	Cream	+	Cocci	Clusters	-	+	+	-	+	+	+	-	+	-	+	0.77237	Micrococcus luteus 3
6 Oropharynx	Y	NS		MA	Cream	+	Cocci	Clusters	-	+	-	-	+	+	+	-	+	-	+	0.38343	Micrococcus sp
6 Oropharynx	Y	NS		Y	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	-	+	+	+	+	0.77243	Micrococcus varians
6 ACF	Y	NS		Y	Tan	+	Cocci	Clusters	-	+	+	-	+	-	+	+	+	+	+	0.49045	Micrococcus varians
6 Oropharynx	Y	NS		Y	Orange	+	Cocci	Clusters	-	+	+	-	+	-	+	+	+	+	-	0.84812	Micrococcus varians
6 Nare	Y	NS		Y	Tan	+	Cocci	Clusters	-	+	+	-	+	-	+	+	+	+	+	0.49045	Micrococcus varians

ID	Patient site	CF	Media	Aerophillic	Colony colour	Gram reaction	Morphology	Arrangement	Haemolysis	Catalase	Oxidase	Gelatin	Urea	MSA	Maltose	Fructose	Glucose	Lactose	Sucrose	Pibwin score	
6	Oropharynx	Y	NS	Y	Tan	+	Cocci	Clusters	-	+	+	-	+	-	+	+	+	+	+	0.49045	Micrococcus varians
6	Oropharynx	Y	NS	Y	Tan	+	Cocci	Clusters	-	+	+	-	+	-	+	+	+	+	+	0.49045	Micrococcus varians
6	Nare	Y	NS	MA	Cream	-	Rods	-	-	+	+	+	-	-	-	-	-	-	-	0.38171	Pseudomonas
6	Nare	Y	NS	Y	Cream	-	Rods	-	-	+	+	+	-	-	-	-	-	-	-	0.38171	Pseudomonas
6	Sput	Y	Ps-spec	Y	Cream	-	Rods	-	-	+	+	+	-	-	-	-	-	-	-	0.38171	Pseudomonas
6	Oropharynx	Y	Ps-spec	Y	Cream	-	Rods	-	-	+	+	+	-	-	-	-	-	-	-	0.38171	Pseudomonas
6	Sput	Y	NS	Y	Cream	-	Rods	-	-	+	+	+	-	-	-	-	-	-	-	0.38171	Pseudomonas
6	Nare	Y	Ps-spec	Y	Cream	-	Rods	-	-	+	+	+	-	-	-	-	-	-	-	0.38171	Pseudomonas
6	Oropharynx	Y	NS	MA	Cream	-	Rods	-	-	+	+	+	-	-	-	-	-	-	-	0.40437	Pseudomonas stutzeri
6	ACF	Y	NS	Y	Cream	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.40327	Staphylococcus
6	Nare	Y	NS	Y	Cream	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.40327	Staphylococcus
6	ACF	Y	NS	Y	Cream	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.40327	Staphylococcus
6	ACF	Y	NS	Y	Cream	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.40327	Staphylococcus
6	ACF	Y	NS	Y	Cream	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.40327	Staphylococcus
6	Oropharynx	Y	NS	Y	Cream	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.40327	Staphylococcus
6	ACF	Y	NS	Y	Cream	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.40327	Staphylococcus
6	Axilla	Y	NS	Y	Cream	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.40327	Staphylococcus
6	ACF	Y	NS	Y	Cream	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.40327	Staphylococcus
6	Axilla	Y	NS	Y	Cream	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.40327	Staphylococcus
6	ACF	Y	NS	Y	Cream	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.40327	Staphylococcus
6	Axilla	Y	NS	MA	Cream	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.40327	Staphylococcus
6	ACF	Y	NS	MA	Cream	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.40327	Staphylococcus
6	Axilla	Y	NS	Y	Tan	+	Cocci	Clusters	β	+	-	-	+	+	+	-	+	+	+	0.58046	Staphylococcus
6	ACF	Y	NS	Y	Yellow	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.59352	Staphylococcus
6	ACF	Y	NS	Y	Yellow	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.59352	Staphylococcus
6	ACF	Y	NS	Y	Yellow	+	Cocci	Clusters	α	+	-	-	+	+	+	+	+	+	+	0.59352	Staphylococcus
6	Nare	Y	NS	Y	Yellow	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.59352	Staphylococcus
6	Oropharynx	Y	NS	Y	Yellow	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.59352	Staphylococcus
6	ACF	Y	NS	Y	Yellow	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.59352	Staphylococcus
6	ACF	Y	NS	Y	Yellow	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.59352	Staphylococcus
6	ACF	Y	NS	Y	Yellow	+	Cocci	Clusters	α	+	-	-	+	+	-	+	+	+	+	0.59352	Staphylococcus
6	Oropharynx	Y	NS	Y	Yellow	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.59352	Staphylococcus
6	Axilla	Y	NS	Y	Yellow	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.59352	Staphylococcus
6	Oropharynx	Y	NS	Y	Yellow	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.59352	Staphylococcus

ID	Patient site	CF	Media	Aerophilic	Colony colour	Gram reaction	Morphology	Arrangement	Haemolysis	Catalase	Oxidase	Gelatin	Urea	MSA	Maltose	Fructose	Glucose	Lactose	Sucrose	Pibwin score	Pibwin ID
6	ACF	Y	NS	Y	Yellow	+	Cocci	Clusters	α	+	-	-	+	+	+	-	+	+	+	0.58046	Staphylococcus
6	Oropharynx	Y	NS	Y	Yellow	+	Cocci	Clusters	α	+	-	-	+	+	+	-	+	+	+	0.58046	Staphylococcus
6	ACF	Y	NS	Y	Yellow	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.51899	Staphylococcus
6	ACF	Y	NS	Y	Yellow	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.51899	Staphylococcus
6	Nare	Y	NS	Y	Yellow	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.51899	Staphylococcus
6	ACF	Y	NS	Y	Yellow	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.51899	Staphylococcus
6	ACF	Y	NS	Y	Yellow	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.51899	Staphylococcus
6	Oropharynx	Y	NS	Y	Yellow	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.51899	Staphylococcus
6	ACF	Y	NS	Y	Yellow	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.51899	Staphylococcus
6	ACF	Y	NS	Y	Yellow	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.51899	Staphylococcus
6	Oropharynx	Y	NS	Y	Yellow	+	Cocci	Clusters	β	+	-	-	+	+	-	+	+	+	+	0.2173	Staphylococcus
6	Nare	Y	NS	MA	Cream	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.96678	Staphylococcus
6	Axilla	Y	NS	MA	Yellow	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.63592	Staphylococcus
6	Oropharynx	Y	NS	MA	Cream	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.63592	Staphylococcus
6	Nare	Y	NS	MA	Cream	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.63592	Staphylococcus
6	ACF	Y	NS	MA	Cream	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.63592	Staphylococcus
6	Nare	Y	NS	MA	Cream	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.63592	Staphylococcus
6	ACF	Y	NS	MA	Cream	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.63592	Staphylococcus
6	Nare	Y	NS	MA	Cream	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.63592	Staphylococcus
6	Oropharynx	Y	NS	MA	Cream	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.63592	Staphylococcus
6	ACF	Y	NS	MA	Cream	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.63592	Staphylococcus
6	Nare	Y	NS	MA	Cream	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.63592	Staphylococcus
6	Oropharynx	Y	NS	MA	Cream	+	Cocci	Clusters	α	+	-	-	+	+	+	+	+	+	+	0.63592	Staphylococcus
6	ACF	Y	NS	Y	Yellow	+	Cocci	Clusters	-	+	-	-	-	-	+	+	+	+	+	0.57697	Staphylococcus hominis
6	ACF	Y	NS	Y	Yellow	+	Cocci	Clusters	-	+	-	-	-	-	+	+	+	+	+	0.57697	Staphylococcus hominis
6	ACF	Y	NS	Y	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
6	ACF	Y	NS	Y	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
6	Oropharynx	Y	NS	Y	Tan	+	Cocci	Clusters	-	+	-	-	+	-	+	-	+	-	+	0.6028	Staphylococcus hominis
6	Oropharynx	Y	NS	MA	Cream	+	Cocci	Clusters	-	+	-	-	+	+	+	-	+	-	-	0.45649	Staphylococcus warneri
6	ACF	Y	NS	Y	White	+	Cocci	Chains	β	-	-	-	+	-	+	+	+	+	+	0.82138	Streptococcus
34	Axilla	N	NS	MA	Cream	-	Rods	Chains	-	+	-	-	+	+	+	-	+	+	+	0.99461	Acinetobacter
34	Nare	N	NS	Y	Yellow	+	Cocci	Clusters	-	+	-	-	-	+	+	+	+	-	+	0.68308	Micrococcus sp
34	Oropharynx	N	NS	Y	Tan	+	Cocci	Clusters	-	+	+	-	+	-	+	+	+	+	+	0.49045	Micrococcus varians

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34	Axilla	Y	NS	MA	Cream	-	Rods	-	-	+	+	+	-	-	-	-	-	-	-	0.38171	Pseudomonas
34	Oropharynx	N	NS	Y	Cream	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.40327	Staphylococcus
34	Axilla	N	NS	Y	Cream	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.40327	Staphylococcus
34	Axilla	N	NS	Y	Cream	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.40327	Staphylococcus
34	ACF	N	NS	Y	Yellow	+	Cocci	Clusters	-	+	-	-	-	-	+	+	+	+	+	0.59352	Staphylococcus
34	Staph	N	NS	Y	Cream	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.40327	Staphylococcus
34	Axilla	N	St-spec	Y	Cream	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.40327	Staphylococcus
34	Nare	N	NS	Y	Cream	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.40327	Staphylococcus
34	Nare	N	NS	Y	Cream	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.40327	Staphylococcus
34	Oropharynx	N	NS	Y	White	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.40327	Staphylococcus
34	Nare	N	NS	Y	Cream	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.40327	Staphylococcus
34	Nare	N	NS	Y	Cream	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.40327	Staphylococcus
34	ACF	N	NS	Y	Cream	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.40327	Staphylococcus
34	Nare	N	NS	Y	Cream	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.40327	Staphylococcus
34	ACF	N	NS	Y	Cream	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.40327	Staphylococcus
34	Nare	N	NS	Y	Cream	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.40327	Staphylococcus
34	ACF	N	NS	MA	Cream	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.40327	Staphylococcus
34	Nare	N	St-spec	Y	Cream	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.40327	Staphylococcus
34	ACF	N	NS	MA	Cream	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.40327	Staphylococcus
34	ACF	N	NS	MA	Cream	+	Cocci	Clusters	-	+	-	-	S	-	+	+	+	+	+	0.40327	Staphylococcus
34	Axilla	N	NS	MA	Cream	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.40327	Staphylococcus
34	Axilla	N	NS	Y	Tan	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.55924	Staphylococcus
34	Oropharynx	N	NS	Y	White	+	Cocci	Clusters	α	+	-	-	+	+	+	+	+	+	+	0.33319	Staphylococcus
34	Axilla	N	NS	Y	White	+	Cocci	Clusters	-	+	-	-	+	+	+	+	+	+	+	0.33319	Staphylococcus
34	Axilla	N	NS	Y	Yellow	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.51899	Staphylococcus
34	Axilla	N	NS	Y	Yellow	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.51899	Staphylococcus
34	ACF	N	St-spec	Y	Cream	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.63592	Staphylococcus
34	ACF	N	NS	Y	Cream	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.63592	Staphylococcus
34	ACF	N	St-spec	Y	Cream	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.63592	Staphylococcus
34	ACF	N	NS	Y	Cream	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.63592	Staphylococcus
34	ACF	N	St-spec	Y	Cream	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.63592	Staphylococcus
34	Nare	N	NS	Y	Cream	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.63592	Staphylococcus
34	ACF	N	NS	Y	White	+	Cocci	Clusters	β	+	-	-	S	+	+	+	+	+	+	0.59352	Staphylococcus
34	Axilla	N	NS	MA	Yellow	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.96678	Staphylococcus

ID	Patient site	CF	Media	Aerophillic	Colony colour	Gram reactior	Morphology	Arrangement	Haemolysis	Catalase	Oxidase	Gelatin	Urea	MSA	Maltose	Fructose	Glucose	Lactose	Sucrose	Pibwin score	Pibwin ID
34	ACF	N	NS	Y	White	+	Cocci	Clusters	β	+	-	-	S	+	+	+	+	+	+	0.59352	Staphylococcus
34	Nare	N	NS	MA	Cream	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.63592	Staphylococcus
34	Nare	N	St-spec	Y	Cream	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.63592	Staphylococcus
34	Axilla	N	NS	MA	Cream	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.63592	Staphylococcus
34	ACF	N	NS	MA	White	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.34449	Staphylococcus
34	ACF	N	NS	Y	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
34	ACF	N	NS	Y	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
34	Axilla	N	St-spec	Y	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
34	ACF	N	NS	Y	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
34	ACF	N	St-spec	Y	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
34	ACF	N	NS	Y	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
34	ACF	N	St-spec	Y	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
34	ACF	N	NS	Y	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
34	ACF	N	St-spec	Y	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
34	ACF	N	NS	Y	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
34	ACF	N	St-spec	Y	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
34	Axilla	N	NS	Y	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
34	Axilla	N	NS	Y	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
34	Nare	N	NS	Y	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
34	ACF	N	St-spec	Y	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
34	Axilla	N	St-spec	MA	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
34	ACF	N	St-spec	Y	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
34	Nare	N	NS	Y	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
34	Axilla	N	NS	MA	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
34	ACF	N	St-spec	Y	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
34	Nare	N	NS	Y	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
34	Axilla	N	NS	MA	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
34	ACF	N	St-spec	Y	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
34	Nare	N	NS	MA	Cream	+	Cocci	Clusters	-	+	-	-	+	+	+	+	+	-	+	0.45649	Staphylococcus warneri
26	Axilla	N	NS	Y	Tan	+	Cocci	Clusters	-	+	+	-	+	-	+	+	+	+	+	0.49045	Micrococcus varians
26	Axilla	N	NS	Y	Tan	+	Cocci	Clusters	-	+	+	-	+	-	+	+	+	+	+	0.49045	Micrococcus varians
26	Oropharynx	N	NS	MA	Tan	+	Cocci	Clusters	-	+	+	-	+	-	+	+	+	+	+	0.49045	Micrococcus varians
26	Oropharynx	N	NS	MA	Tan	+	Cocci	Clusters	-	+	+	-	+	-	+	+	+	+	+	0.49045	Micrococcus varians
26	Oropharynx	N	NS	Y	Cream	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.40327	Staphylococcus

ID	Patient site	CF	Media	Aerophilic	Colony colour	Gram reaction	Morphology	Arrangement	Haemolysis	Catalase	Oxidase	Gelatin	Urea	MSA	Maltose	Fructose	Glucose	Lactose	Sucrose	Pibwin score	Pibwin ID
26 Axilla	N	NS		Y	Cream	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.40327	Staphylococcus
26 Axilla	N	NS		MA	Cream	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.40327	Staphylococcus
26 Nare	N	NS		MA	Cream	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.40327	Staphylococcus
26 Axilla	N	NS		MA	Cream	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.40327	Staphylococcus
26 Nare	N	NS		MA	Cream	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.40327	Staphylococcus
26 Axilla	N	NS		MA	Cream	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.40327	Staphylococcus
26 Axilla	N	NS		MA	Cream	+	Cocci	Clusters	-	+	-	-	S	-	+	+	+	+	+	0.40327	Staphylococcus
26 Axilla	N	NS		Y	Yellow	+	Cocci	Clusters	-	+	-	-	+	+	+	+	+	+	+	0.59352	Staphylococcus
26 Axilla	N	NS		Y	Yellow	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.59352	Staphylococcus
26 Axilla	N	NS		Y	Yellow	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.59352	Staphylococcus
26 Axilla	N	NS		Y	Yellow	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.51899	Staphylococcus
26 Axilla	N	NS		Y	Yellow	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.51899	Staphylococcus
26 Oropharynx	N	NS		MA	White	+	Cocci	Clusters	β	+	+	-	+	+	+	+	+	+	+	0.30094	Staphylococcus
26 Oropharynx	N	NS		MA	White	+	Cocci	Clusters	β	+	-	-	S	+	+	+	+	+	+	0.59352	Staphylococcus
26 Oropharynx	N	NS		Y	Tan	+	Cocci	Clusters	β	+	-	-	S	+	+	+	-	+	+	0.61608	Staphylococcus
26 Oropharynx	N	NS		Y	White	+	Cocci	Clusters	β	+	-	-	S	+	-	+	+	+	+	0.34188	Staphylococcus
26 Oropharynx	N	NS		Y	White	+	Cocci	Clusters	α	+	-	-	+	+	+	+	+	+	+	0.34449	Staphylococcus
26 Oropharynx	N	NS		Y	White	+	Cocci	Clusters	β	+	-	-	S	+	+	+	+	+	+	0.59352	Staphylococcus
26 Axilla	N	NS		MA	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
26 Axilla	N	NS		Y	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
26 Nare	N	NS		MA	Tan	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
26 ACF	N	NS		MA	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
26 Nare	N	NS		MA	White	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.4869	Staphylococcus hyicus
26 Axilla	N	NS		Y	White	+	Cocci	Clusters	-	+	-	-	-	-	-	+	+	+	+	0.44554	Staphylococcus sp BP V
26 Axilla	N	NS		Y	White	+	Cocci	Clusters	-	+	-	-	+	+	+	+	+	+	+	0.60538	Staphylococcus sp BP V
26 Axilla	N	NS		Y	White	+	Cocci	Clusters	α	+	-	-	+	+	-	+	+	+	+	0.52356	Staphylococcus sp BP V
26 Axilla	N	NS		Y	White	+	Cocci	Clusters	-	+	-	-	+	+	+	+	+	+	+	0.60538	Staphylococcus sp BP V
15 Axilla	N	NS		Y	Cream	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.40327	Staphylococcus
15 Nare	N	NS		MA	Cream	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.40327	Staphylococcus
15 ACF	N	NS		MA	Cream	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.40327	Staphylococcus
15 ACF	N	NS		Y	Cream	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.40327	Staphylococcus
15 ACF	N	NS		Y	Cream	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.40327	Staphylococcus
15 Nare	N	NS		Y	Cream	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.40327	Staphylococcus
15 Axilla	N	NS		Y	Cream	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.40327	Staphylococcus

ID	Patient site	CF	Media	Aerophillic	Colony colour	Gram reactior	Morphology	Arrangement	Haemolysis	Catalase	Oxidase	Gelatin	Urea	MSA	Maltose	Fructose	Glucose	Lactose	Sucrose	Pibwin score	Pibwin ID
15	Axilla	N	NS	Y	Cream	+	Cocci	Clusters	-	+	-	-	S	-	+	+	+	+	+	0.40327	Staphylococcus
15	Axilla	N	NS	Y	Yellow	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.51899	Staphylococcus
15	Axilla	N	NS	Y	Yellow	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.51899	Staphylococcus
15	Nare	N	NS	Y	Cream	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.63592	Staphylococcus
15	Axilla	N	NS	MA	Cream	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.63592	Staphylococcus
15	Axilla	N	NS	MA	Yellow	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.96678	Staphylococcus
15	Nare	N	NS	Y	White	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.34449	Staphylococcus
15	Nare	N	NS	Y	Yellow	+	Cocci	Clusters	β	+	-	-	+	+	+	+	+	+	+	0.9211	Staphylococcus aureus
15	Axilla	N	NS	Y	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
15	Oropharynx	N	NS	Y	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
15	Nare	N	NS	MA	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
15	Oropharynx	N	NS	Y	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
15	ACF	N	NS	MA	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
15	Nare	N	NS	Y	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
15	Axilla	N	St-spec	MA	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
15	Nare	N	NS	Y	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
15	Axilla	N	NS	MA	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
15	Nare	N	NS	Y	Tan	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
15	Nare	N	NS	Y	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
15	Nare	N	NS	Y	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
15	Nare	N	NS	Y	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
15	Nare	N	NS	Y	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
15	Nare	N	NS	Y	Yellow	+	Cocci	Clusters	-	+	-	-	+	-	+	+	+	+	+	0.80567	Staphylococcus hominis
15	Nare	N	NS	Y	Cream	+	Cocci	Clusters	-	+	-	-	-	-	+	+	+	+	+	0.28841	Staphylococcus hyicus
15	Nare	N	NS	Y	Cream	+	Cocci	Clusters	-	+	-	-	-	-	+	+	+	+	+	0.67235	Staphylococcus sp BP V
15	Axilla	N	NS	Y	Cream	+	Cocci	Clusters	-	+	-	-	-	-	+	+	+	+	+	0.67235	Staphylococcus sp BP V
15	Axilla	N	NS	Y	Cream	+	Cocci	Clusters	-	+	-	-	-	-	+	+	-	+	+	0.67235	Staphylococcus sp BP V
15	Nare	N	NS	MA	Cream	+	Cocci	Clusters	-	+	-	-	-	-	+	+	+	+	+	0.67235	Staphylococcus sp BP V
15	Nare	N	NS	Y	Cream	+	Cocci	Clusters	-	+	-	-	-	-	+	+	+	+	+	0.67235	Staphylococcus sp BP V
15	Axilla	N	NS	Y	Cream	+	Cocci	Clusters	-	+	-	-	-	-	+	+	+	+	+	0.67235	Staphylococcus sp BP V
15	Nare	N	NS	Y	Cream	+	Cocci	Clusters	-	+	-	-	-	-	+	+	+	+	+	0.67235	Staphylococcus sp BP V
15	Nare	N	NS	Y	Cream	+	Cocci	Clusters	-	+	-	-	-	-	+	+	+	+	+	0.67235	Staphylococcus sp BP V
15	Axilla	N	NS	Y	Cream	+	Cocci	Clusters	-	+	-	-	-	-	+	+	+	+	+	0.67235	Staphylococcus sp BP V
15	Axilla	N	NS	MA	Cream	+	Cocci	Clusters	-	+	-	-	+	+	+	+	+	-	+	0.45649	Staphylococcus warneri

ID	Patient site	CF	Media	Aerophilic	Colony colour	Gram reaction	Morphology	Arrangement	Haemolysis	Catalase	Oxidase	Gelatin	Urea	MSA	Maltose	Fructose	Glucose	Lactose	Sucrose	Pibwin score	Pibwin result
15 Nare		N	NS	MA	White	+	Cocci	Chains	α	-	-	-	+	-	+	+	+	+	+	0.82138	Streptococcus
15 Nare		N	NS	MA	White	+	Cocci	Chains	α	-	-	-	+	-	+	+	+	+	+	0.82138	Streptococcus

Appendix E

Order to family level OTU heatmaps

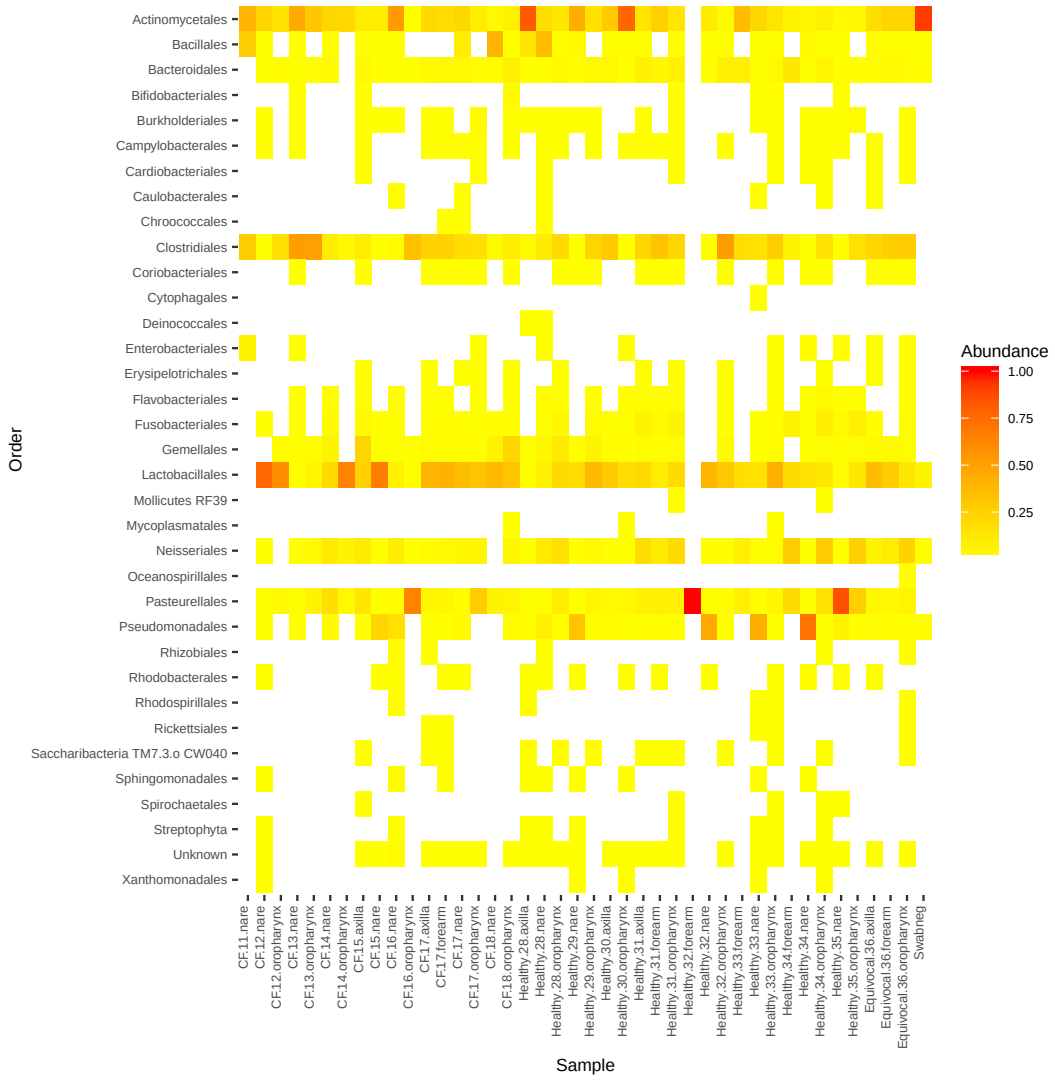

Figure E.1: Heatmap showing relative abundance of bacterial orders across samples.

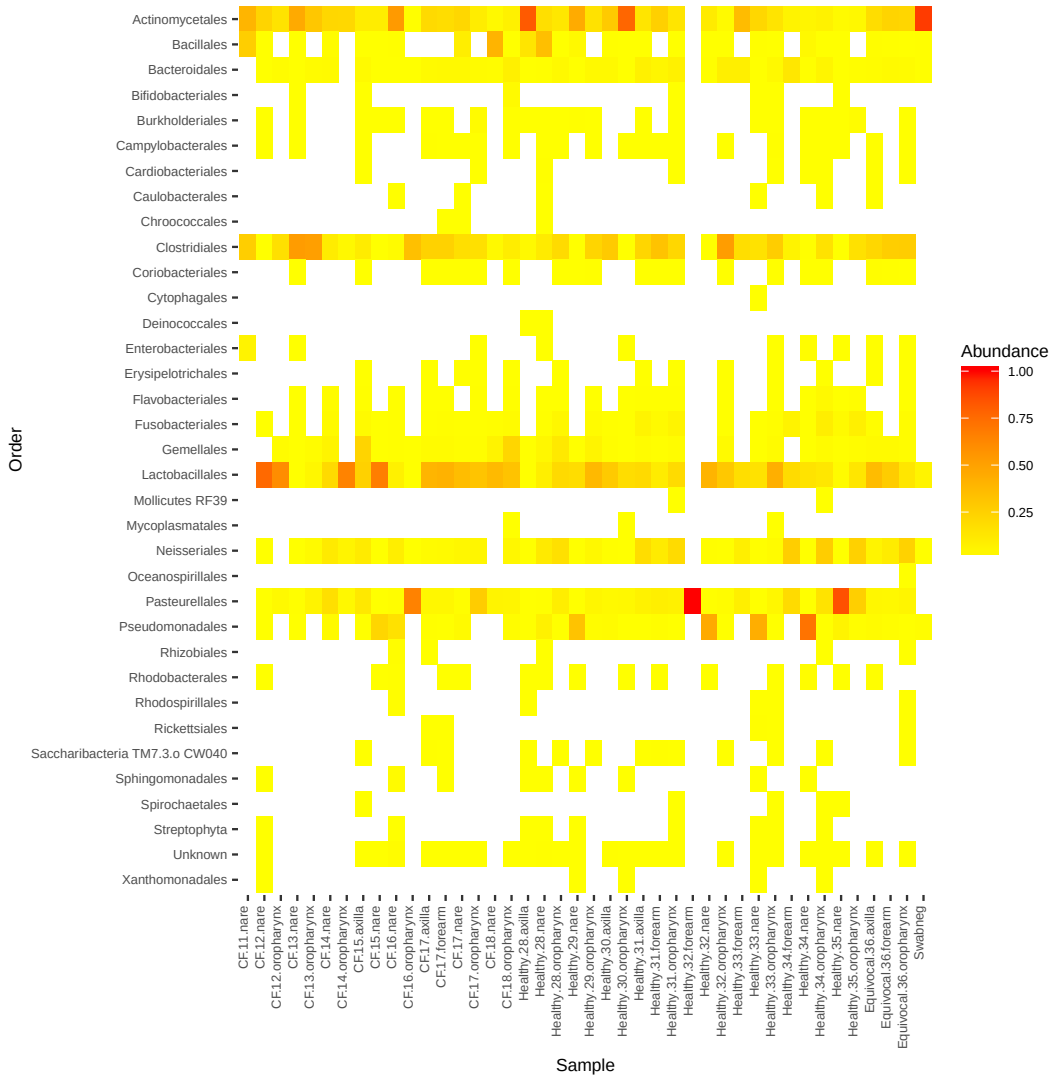

Figure E.2: Heatmap showing relative abundance of bacterial classes across samples.

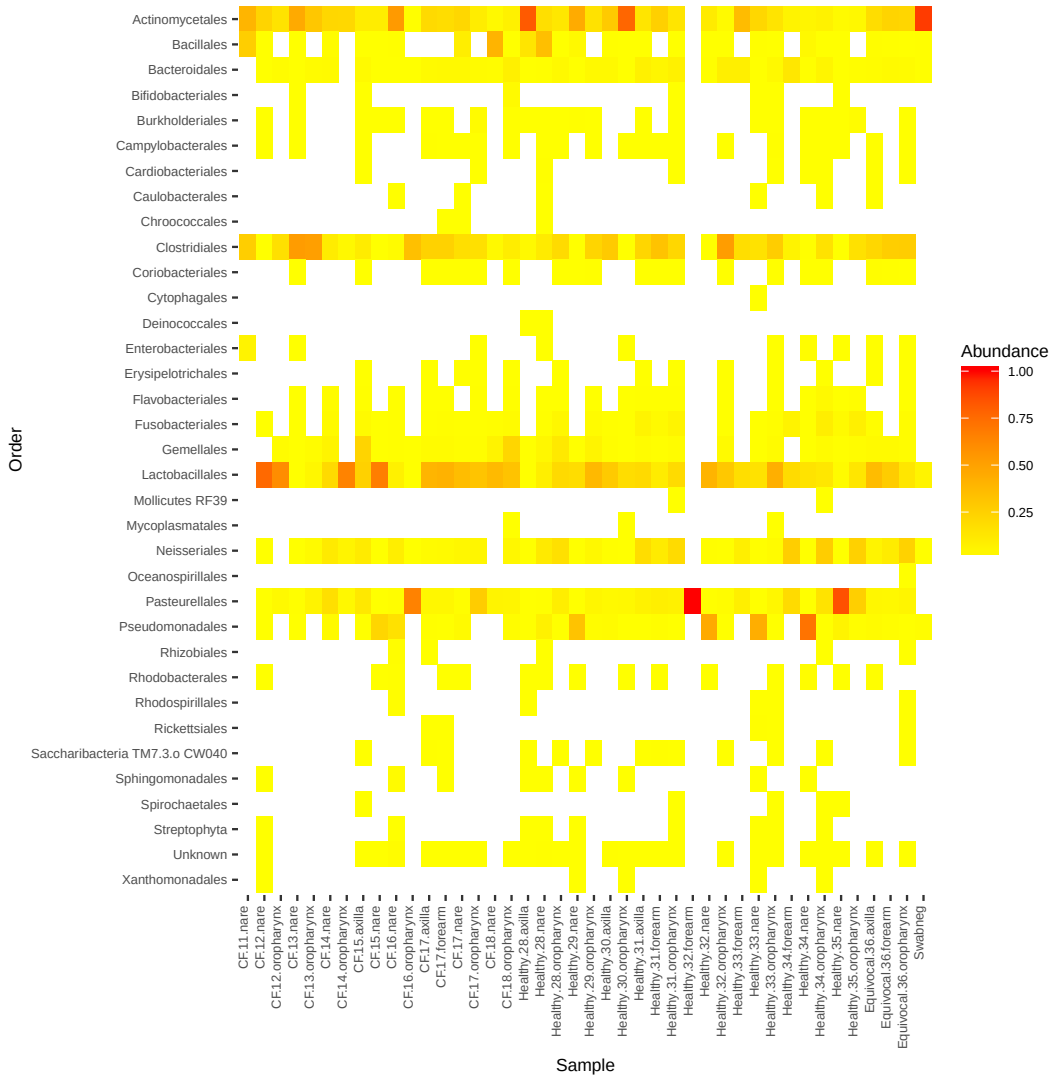

Figure E.3: Heatmap showing relative abundance of bacterial families across samples.

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