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Genomic Investigation of Clinically Significant Coagulase-Negative Staphylococci

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Coagulase-negative staphylococci, whole-genome sequencing, blood culture, periprosthetic joint infection

Abstract

Introduction

Coagulase-negative staphylococci have been recognised both as emerging pathogens and contaminants of clinical samples. High resolution genomic investigation may provide insights into their clinical significance.

Aims

To review the literature regarding coagulase-negative staphylococcal infection and the utility of genomic methods to aid diagnosis and management and to identify promising areas for future research.

Methodology

We searched Google Scholar with the terms (*Staphylococcus*) AND (sequencing OR (infection)). We prioritised papers which addressed coagulase-negative staphylococci, genomic analysis, or infection.

Results

A number of studies have investigated specimen-related, phenotypic and genetic factors associated with colonisation, infection and virulence, but diagnosis remains problematic.

Conclusion

Genomic investigation provides insights into genetic diversity and natural history of colonisation and infection. Such information allows the development of new methodologies to identify and compare relatedness and predict antimicrobial resistance. Future clinical studies that employ suitable sampling frames coupled with the application of high-resolution whole genome sequencing may aid the development of more discriminatory diagnostic approaches to coagulase-staphylococcal infection.

Introduction

The term “Staphylococcus” was introduced by Sir Alexander Ogston to describe grape-like clusters of bacteria associated with post-surgical infection (1). Friedrich Rosenbach adopted the name when in 1884 he described “*Staphylococcus pyogenes aureus”* and *“Staphylococcus pyogenes albus”* (2). Both forms, differentiated by their golden and white

colony pigmentation, were considered by the early authors to be *bona fide* pathogens. In 1891, Welch reported the recovery of apparently non-pathogenic strains from the epidermis and from aseptic wounds, which he called "*Staphylococcus epidermidis albus*" (3). In 1940, Fairbrother noted that coagulase activity could be used to differentiate *S. aureus* from generally less pathogenic coagulase-negative staphylococci (4). Until the 1970s, the names "*S. albus"* and *S. epidermidis* were applied widely to coagulase-negative staphylococci (CoNS) (5). Novel staphylococci continue to be described (6,7) and at the time of writing the genus *Staphylococcus* comprises 55 validly published separate species plus an additional 12 separate subspecies, among which, *S. aureus* is preeminent as a pathogen of humans (8). In clinical practice the distinction between coagulase-positive (*S. aureus* and related species such as *S. intermedius*) and coagulase-negative (mostly low-virulence organisms, including *S. epidermidis*) staphylococci remains important.

Coagulase-negative staphylococci: colonisation and infection

In contrast with *S. aureus* which is associated with a wide range of community-acquired and nosocomial syndromes of infection, CoNS species are generally considered to be low-virulence organisms and less significant as causes of human disease. However, two CoNS species are associated with distinctive disease profiles. Unlike other staphylococci *Staphylococcus saprophyticus* possesses ion transport systems and adhesins which allow it to colonise the lower urinary tract (9) and this species is a common cause of urinary tract infection among young women (10). *Staphylococcus lugdunensis,* a commensal of inguinal and axillary skin, displays virulence mechanisms similar to *S. aureus* including an iron capture system, adhesins (fibronectin binding proteins) and biofilm formation. *S. lugdunensis* causes a spectrum of serious infections similar to *S. aureus* including endocarditis and osteomyelitis (11). However, clustered, regularly interspaced, short palindromic repeats (CRISPR) and restriction-modification systems limit its uptake of mobile genetic elements and associated virulence and antimicrobial resistance genes (12–14). Both species have been recently reviewed and will not be considered further (15,16). Other CoNS species are found extensively amongst humans, animals and the environment. Some are ubiquitous commensals of human skin and mucosal surfaces (17). As many as 18 different species have been cultured from the skin of healthy adults at densities of 10 to 10^3 colony-forming units per cm^2 (17,18). *Staphylococcus epidermidis* is the species most commonly identified in diagnostic microbiology accounting for over half of all CoNS

isolates across published series (Table 1), while *S. haemolyticus*, *S. hominis* and *S. capitis*, collectively account for over a quarter of isolates. Although usually identified in diagnostic microbiology as specimen contaminants, these species can also represent pathogens in certain patient groups. They have been termed 'accidental' pathogens (19) or 'Pathogens Associated with Medical Progress' (20) due to their ability to establish infection in the context of implanted medical devices. The infections they cause are typically indolent and complicate invasive hospital procedures such as the insertion of intravenous catheters and the implantation of biosynthetic materials. Coagulase-negative staphylococci are common causes of biomaterial infections including vascular grafts, prosthetic heart valves, cardiac pacemakers, central nervous system shunts, continuous ambulatory peritoneal dialysis and urinary catheters (5,16,18,21–24). Occasionally they cause native tissue infections including native-valve endocarditis, osteomyelitis, otitis media, ocular infections and surgical-site infections (10,22,25,26). Antibiotic resistance is widespread among CoNS species. Rates of methicillin resistance in clinical isolates can be as high as 80 % for *S. epidermidis* and other species (27–29) and in many healthcare settings are higher than for *S. aureus* (28,30,31). Resistance to other antimicrobials is increasing amongst clinical isolates of CoNS (28,32). As CoNS are common specimen contaminants, are important nosocomial pathogens and often resistant to many antibiotic classes, the interpretation of their significance in clinical specimens from normally sterile sites is a challenging problem which may require combined information from microbiological, histological and clinical investigations.

Virulence of coagulase-negative staphylococci

A considerable body of research has established the wide-ranging mechanisms that allow *S. epidermidis* and other CoNS to colonise and cause disease. These have been reviewed elsewhere (19,33–35). Many are analogous to mechanisms described for *S. aureus* and include immune evasion through cellular internalisation and persistence, protection from host antimicrobial peptides, adhesion to host matrix proteins, biofilm formation and antimicrobial resistance.

Investigation of strain differences has successfully identified mechanisms of nosocomial adaptation. Biofilm formation is associated with nosocomial infection and hospital endemicity (19,22,35–38). Polysaccharide intercellular adhesin (PIA) encoded by the *icaADBC* operon is a key factor for formation of the cellular aggregations and protective

extracellular matrix that comprise the biofilm (37,39,40). The *icaADBC* operon is more frequently present in nosocomial than community isolates of *S. epidermidis* (29,36,41–46). The *icaR* regulatory gene is found upstream of *icaADBC*, and is one factor controlling its regulation allowing for phase-variability (47). The insertion sequence IS*256* plays an important role in the regulation of the *ica* gene locus expression and PIA formation (33,48). IS*256* has been found more frequently amongst nosocomial than community isolates (36,42–44,46). However, the *ica* locus does not always confer a PIA-producing phenotype (40,47,49,50) and biofilm formation is not completely reliant on PIA production (40,49,51). The accumulation-associated protein (*aap*) and Bap-homologous protein (*bhp*) genes present in some strains of *S. epidermidis* respectively encode proteins Aap and Bhp that are associated with PIA-independent biofilm formation (51–54). Both the *aap* and *bhp* genes have been detected with similar frequency amongst infecting, hospital carriage and community carriage isolates (36,42,44,45). However, Post *et al*. (55) reported that the presence of *bhp* was significantly associated with a failed treatment outcome for *S. epidermidis* orthopaedic device-related infections and may be the result of rapid primary attachment to abiotic surfaces during the early stages of biofilm formation. The Arginine Catabolic Mobile Element (ACME) is associated with the epidemic strain of *S. aureus* USA300 and encodes several putative virulence factors (56). However, in *S. epidermidis* it occurs at equal or higher rates in community strains compared with hospital-related strains (36,57). Moreover, Granslo *et al.* (56) found that the presence of the ACME was not associated with an increased inflammatory response in isolates of *S. epidermidis* obtained from neonate blood cultures (56). The formate-dehydrogenase (*fdh*) gene has been proposed as a genetic marker of commensalism due its higher prevalence in non-infecting isolates than clinical isolates of *S. epidermidis* (46). However, as *fdh* is infrequently detected even among community strains, its value as an indicator of commensalism appears at best to be limited (36).

Pan-genome analyses of *S. epidermidis* and *S. haemolyticus* show that their genomes comprise 20 % and 25 % respectively of accessory genes (46,58). This open genome has also been demonstrated at an inter-species level (59). The similarity of resistance genes harboured by staphylococci alludes to frequent horizontal gene transfers between species *in vivo* (59–63). The *mecA* gene which confers methicillin resistance in *S. aureus* and CoNS is thought to have a chromosomal origin in *Staphylococcus sciuri* (64). The staphylococcal chromosome cassette *SCCmec* is the mobile genetic element which contains the mobile

172 *mec* gene complex amongst staphylococcal species. Whilst a number of *SCCmec* types
173 have been catalogued (65) high homology exists between *mecA* genes, suggestive of a
174 common origin. The *mecA* gene has been investigated as a potential marker of clinical
175 significance. The *mecA* homologues *mecB*, *mecC* and *mecD* have also been detected in
176 staphylococci by WGS (66–70). The *mecA* gene is strongly associated with nosocomiality in
177 CoNS with around 80 % of CoNS isolated from hospital sources carrying the gene (33).
178 However, *mecA* occurs frequently amongst both infecting and contaminating isolates
179 (36,42,46,57).
180 Linezolid is regarded as an important antibiotic in the treatment of methicillin-resistant
181 staphylococci since it can penetrate tissues and can be administered orally and
182 intravenously (71). Resistance to linezolid is still relatively rare amongst staphylococci. It
183 can be used to treat multidrug-resistant infections (72–74). Resistance to linezolid may
184 result from modification of the 23S rRNA part of the 50S subunit (73–76). A number of
185 point mutations have been identified in 23S rRNA genes which confer resistance to
186 linezolid in staphylococci (73,75,76) . The plasmid-borne *cfr* gene produces a ribosomal
187 RNA large subunit methyltransferase which methylates the adenine at position 2503 in 23S
188 rRNA and confers resistance to linezolid, chloramphenicol, lincosamides and
189 streptogramin$_A$ (73,76) . Mutations in the L3, L4, and L22 ribosomal proteins also confer
190 resistance to linezolid in staphylococci (73,75,76). Some strains of *S. epidermidis* harbour
191 all 3 mechanisms (23S rRNA mutation, *cfr*-mediated and ribosomal L-protein mutation) of
192 linezolid resistance (77,78). Linezolid resistance in *S. epidermidis* was first reported in 2004
193 in the USA (32) and has since been reported from Europe, China and Brazil in a number of
194 different sequence types (73,75,77–81).
195 The glycopeptides vancomycin and teicoplanin are important antibiotics in the treatment
196 of methicillin-resistant staphylococci infections. Transfer of the *vanA* gene from
197 enterococci to staphylococci has been demonstrated as a feasible mode of acquisition and
198 a public health concern since expression of the *vanA* gene changes the dipeptide terminus
199 from D-alanine-D-alanine to D-alanine-D-lactate to reduce the affinity of vancomycin a
200 thousand fold (82,83). Glycopeptide resistance in staphylococci may occur as
201 heteroresistance and reduced susceptibility (83,84). Vancomycin may promote biofilm
202 formation and cell wall thickening of *S. epidermidis* which, in turn, reduces the penetration
203 of vancomycin resulting in heteroresistance (85). Heteroresistance to vancomycin can also
204 result from mutations in the RNA polymerase B enconding gene *rpoB* which also confers

rifampicin resistance (77). Resistance to vancomycin and teicoplanin has been found in CoNS isolated from bloodstream infection (BSI) and orthopaedic device-related infection (ODRI) (77,84,86–89).

The plasmid-borne quaternary ammonium compound (*qac*)-resistance genes encode multidrug and multi-antiseptic efflux pumps (90). Different *qac* genes have been observed in *S. epidermidis, S. haemolyticus* and *S. hominis* (90,91). The presence of *qacA* has been shown to correlate with poor treatment outcome of orthopaedic device-related infections (55). Their presence may enhance persistence at surgical sites despite the use of antiseptics, thus facilitating accidental introduction onto an implanted prosthesis.

Coagulase-negative staphylococci as pathogens

Given the spectrum of disease associated with CoNS species two frequent situations in which determination of pathogenicity has significant clinical implications are in the diagnosis of BSI and periprosthetic-joint infection (PJI). In both these situations a CoNS identified in the laboratory may represent a true pathogen but is also frequently present as a result of sample contamination (92,93).

Blood stream infection

The great majority of CoNS cultured from blood samples represent sample contaminants (93,94). However, CoNS are common causes of BSI (95–99). Infections are often catheter-related (99,100). They may arise at the percutaneous entry site with subsequent migration along the extraluminal catheter surface (101,102). More rarely, they result from contamination of infusion fluid or haematogenous seeding from other foci (101). Determination of the significance of CoNS cultured in blood in a susceptible patient is challenging. Time-to-positivity of blood cultures has been assessed as a potential indicator of true infection but fundamentally just reflects bacterial density at the time of inoculation into the bottle and does not usefully differentiate culture contaminants from true bacteraemia (93,103–105). Clinical management involves catheter removal and antimicrobial therapy (101,102). Compared with negative blood culture results, false-positive results due to sample contamination are associated with a median increase in patient bed days of 8 to 12.5 and a median increase in total cost from $8731 to $13116 (106). When CoNS contaminate blood cultures, patients may receive unnecessary antibiotics. These are frequently parenteral agents such as glycopeptides (93).

Periprosthetic-joint infection

Implanted medical devices such as orthopaedic prostheses provide a nidus for bacterial growth and lack a vascular system that can deliver an immune response or sufficient antibiotics, making them vulnerable to infection by opportunistic pathogens (107). Biofilm producing CoNS strains infecting such devices are able to withstand clearance by host immune system and are relatively non-susceptible to antibiotics (40,108–110). Two studies investigating the clinical significance of biofilm formation however did not show biofilm production to be a reliable indicator of significance in CoNS isolated from blood cultures (111,112).

In clinical practice, periprosthetic-joint infections can be classified according to their temporal relationship with prosthesis insertion and the presumed mechanism of device infection. Early (≤3 months post-surgery) and delayed (3 – 24 months post-surgery) infections are usually associated with direct inoculation at the time of device insertion (113,114). Late (>24 months post-surgery) infections may result from haematogenous seeding (113,114) . Staphylococci, including *S. epidermidis*, are the most commonly cultured organisms from sampled prosthetic devices at all time points (110,115–119). The isolation of indistinguishable CoNS isolates from multiple periprosthetic tissue cultures (taken using separate sterile instruments to avoid cross contamination) is key for differentiating possible infections from likely contaminants (93,103,112). Determination of the indistinguishability of organisms relies on the typing method used being able to robustly determine whether two isolates are indeed the same strain. For the diagnosis of prosthetic joint infection at hip or knee revision arthroplasty, Atkins *et al* (120) demonstrated that detection of indistinguishable organisms from three or more operative samples was predictive of infection with a sensitivity of 65 % and a specificity of 99.6 %. These authors used morphotype, biochemical identification of the organism and an extended antibiogram to distinguish organisms. Isolates of the same phenotype however may represent genetically different strains (104,121). More recently, the Musculoskeletal Infection Society (https://www.msis-na.org/) updated their criteria for determining PJI with a score based on clinical, biochemical, histological and microbiological observations (122). Recognising that traditional phenotypic approaches to speciation are being replaced by molecular approaches the guidance suggests consideration of molecular diagnostic testing directly from samples.

Phenotypic and genotypic identification of coagulase-negative staphylococci

Phenotypic methods used for species identification

In the 1970s, arrays based on phenotype (morphological, physiological, biochemical, antibiotic susceptibility and cell wall composition characteristics) were developed, which resulted in the discovery and description of new staphylococcal species (123–125). Subsequently, automated biochemical analysis systems such as VITEK 2 (BioMerieux S.A.) and Phoenix (Becton Dickinson Biosciences, Sparks, Md.) were developed to allow the routine determination of antibiotic susceptibilities and species identification of bacteria in diagnostic laboratories. The development of commercial platforms using Matrix-Assisted Laser Desorption/Ionisation Time-of-Flight Mass Spectrometry (MALDI-TOF MS) including the MALDI Biotyper system (Bruker Daltonique, Wissembourg, France) or the VITEK MS (bioMérieux, Marcy l'Etoile, France) has revolutionised diagnostic microbiology by providing a cheap, quick and accurate method for identifying bacteria (126–128).

Differentiation of strains using phenotypic methods

Phenotypic methods such as antibiotyping and morphotyping are often used in clinical laboratories as typing methods to distinguish between different strains of a bacterial species, including staphylococci (4,124,125,129). These methods provide potentially useful epidemiological information at relatively low additional cost. However, information derived from such methods may be misleading as phenotypic characteristics may be shared by genetically unrelated strains and antibiotic susceptibility may vary within a single genetic lineage. Genes that determine antimicrobial resistance or morphology may vary in their expression and obscure epidemiological links between related isolates (130). To avoid such pitfalls, guidance documents have been published regarding the validation and application of epidemiological typing systems (131,132).

Characterisation and identification using genotypic methods

16S rRNA gene sequencing has been successfully employed to identify staphylococci and investigate their phylogeny (133–136). As recently as 2015, this approach led to the description of the novel taxon *Staphylococcus petrasii* subspecies *pragensis* (137). Identification methods based on RNA polymerase B (*rpoB*), superoxide dismutase A (*sodA*) and elongation factor Tu (*tuf*) gene sequencing analysis have been proposed (23,138–141)

304 but have not replaced 16S rRNA gene sequencing (142). Genotypic methods are reliable
305 but their routine application in diagnostic settings has been impeded by cost and turn-
306 around-time.
307
308 *Differentiation of strains using genotypic methods*
309 Pulsed field gel electrophoresis (PFGE) employs restriction endonuclease enzymes to
310 cleave chromosomal DNA into fragments which are then subjected to gel electrophoresis
311 to separate them (143). The fragment size profiles are strain specific and so can be used as
312 a discriminatory typing method (143) . PFGE as a typing method for *S. epidermidis* was first
313 reported in 1992 (144,145). It has been used for hospital outbreak investigation and
314 comparison of isolates (104,146–148). Although highly discriminatory, PFGE may not allow
315 differentiation of some genetically distinct isolates (148). Furthermore, differences in PFGE
316 profile may be misleading if caused by plasmid loss (149), chromosomal rearrangements
317 (150,151) or changes in the insertion sequence IS*256*-specific hybridisation patterns (151).
318 When combined with staphylococcal chromosome cassette SCC*mec* cassette typing, PFGE
319 provides results similar to those obtained using multi-locus sequence typing (MLST) (152).
320
321 Multi-locus sequence typing exploits the sequence variation present in specific loci in a
322 number (six or seven) house-keeping genes (153). Different alleles are assigned a number
323 so that a sequence type based on the numerical profile of the six or seven loci can be
324 assigned (153). The pubMLST.org database is the internationally accepted database for
325 allele sequences. MLST has been used to investigate phylogeny and characterise clinically
326 important strains of *S. aureus, S. epidermidis*, *S. haemolyticus* and *S. hominis*
327 (65,118,120,121). The currently accepted scheme for *S. epidermidis* was developed by
328 Thomas *et al*. (154) (Table 2). Clonal complexes were defined as STs which differed by no
329 more than one allele from at least one other ST (154). The scheme was applied by Miragaia
330 *et al*. (155) to a collection of 217 clinical isolates, from which 74 different sequence types
331 (ST) were identified. A single clonal complex (CC2) accounted for 74 % of isolates;
332 comprised of 39 STs of which ST2 was the most frequently detected (67/217, 31 %) (155).
333 An eBURST analysis (which allows inference of the ancestral ST of a clonal complex)
334 suggested that ST2 was the founding sequence type of the largest clonal complex,
335 although a later investigation showed ST5 to be the ancestor of the largest clonal complex
336 (156,157). For *S. epidermidis* the predominant nosocomial strains ST2, ST5 and ST23 more
337 frequently harbour virulence-associated genetic elements both within clinical and hospital-

carriage isolates (27,50,155,158,159). ST-based analyses may be able to discriminate between community and nosocomial isolates but have been of limited value in discriminating between infecting and contaminating or carriage nosocomial isolates (36). However, since carriage often precedes infection (160) then identifying particular strains can be important. O'Connor *et al*. (161) employed a combination of PFGE and MLST to identify a single strain of ST2 *S. epidermidis* that was resistant to linezolid and was associated with an infection and carriage associated outbreak that was subsequently limited by enhanced infection control measures.

Multi-locus variable tandem repeat analyses (MLVA) and Multi-locus variable tandem repeat fingerprinting (MLVF) are similar methods based on the amplification and banding of several loci of repetitive regions in the genome. Although less laborious than PFGE, MLVF failed to resolve the population structure of a collection of *S. haemolyticus*. (162,163).

Genomic investigation of clinically significant coagulase-negative staphylococci

Whole-genome sequencing technologies have the potential to replace and improve upon traditional phenotypic and genotypic methods performed in diagnostic and public health laboratories. Short-read (a few hundred base pairs (bp)) sequencing such as Illumina technology involves the parallel sequencing of short fragments of genomic DNA which must then be reassembled into longer contigs. Whilst this method may yield higher error rates than capillary sequencing and struggle to resolve repetitive regions and plasmids it a high throughput method suitable for diagnostic and public health microbiology. Analysis of short-read genomic DNA has been applied widely to study transmission and predict antibiotic resistance of pathogens including *Mycobacterium tuberculosis, Neisseria gonorrhoeae* and *Staphylococcus aureus* (164–166) including sequencing direct from samples (167,168). Oxford Nanopore technology can produce long reads of around 10,000 bp and lends itself to metagenomic sequencing approaches and for the detection of bacterial and viral pathogens by directly sequencing clinical samples (169–171).

Many studies have focused on *S. aureus*, but at the time of writing there are more than 1866 deposits of *S. epidermidis* sequence read data derived from next-generation sequencing technologies in the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA) (172,173) providing a rich source of data for researchers to

survey for lineages or resistance or virulence determinants use as a comparator with their own isolates (Figure 1).

Research to establish the role of WGS in diagnostic and public health practice for *S. aureus* has focused on accurate pathogen identification, reliable prediction of antibiotic susceptibility, virulence profiling and assessment of transmission (166,174–177). Furthermore, for *S. epidermidis* it would be clinically valuable to develop methods that allow discrimination of infecting and contaminating isolates.

Pathogen identification

Whole-genome sequencing has the potential to replace MALDI-TOF MS and 16S rRNA gene sequencing as a tool to identify known and characterise novel staphylococci (69,178). Raw sequence data can be mapped to a reference genome with software such as SAMtools (179) and Stampy (180) or compared with a reference genome using the Basic Local Alignment Search Tool nucleotide (BLASTn) (181) from contigs assembled *de novo* assembled with such as Velvet (182). The Average Nucleotide Identity (ANI) method provides a plausible alternative to DNA-DNA hybridisation technique, the conventional standard method for measuring the genetic distance between bacteria (183,184). BLASTn and BLASTn-based ANI methods have been used to identify staphylococci from specimens routinely sent to clinical microbiology laboratories (185,186).

Velvet constructs de Brujin graphs from k-mers, short subsequences of DNA which overlap by all but one nucleotide (182). K-mer-based assembly has been used to identify bacterial pathogens by directly sequencing clinical samples (167,187). These methods offer the potential to aid in the diagnosis of culture-negative infection or might even replace culture-based microbiology.

Resistance detection

Various platforms that map resistance-associated genes curated in a number of different databases have been used to predict resistance in *Mycobacterium tuberculosis* and other pathogens, including *S. aureus* (164,175,188). Antibiotic Resistance Gene-ANNOTation (ARG-ANNOT), Comprehensive Antibiotic Resistance Database (CARD) and Resfinder employ a BLAST-based method to detect resistance genes in assembled genome sequences. Resfinder also allows genome assembly from short reads before analysis (181,189,190). Mykrobe software uses de Brujin graphs to detect resistance genes from

short reads of *S. aureus* (191). The Antimicrobial Resistance Identification By Assembly (ARIBA) also uses short-read data to detect resistance genes (192). Whilst there are several online sources of AMR elements none so far holds all known resistant elements for the staphylococci. For routine use such a resource would need to be accessible and contain a continually updated catalogue of resistance determinants. An ongoing role would exist for phenotypic investigation of novel determinants and epistatic effects on known determinants. Such work could be performed in reference laboratories (193).

Many of the genetic elements associated with resistance in *S. aureus* are located on mobile genetic elements or share homology with those observed in CoNS. Indeed there is evidence that some *S. aureus* resistance genes originated in CoNS (13,61,194–197). Modification of methods that allow resistance prediction in *S. aureus* for application to CoNS may therefore be a relatively simple process. In addition to detection of known resistance determinants, Fowler *et al.* (198) demonstrated a proof-of-principle that the detection of mutations in the dihydrofolate reductase (*dhfr*) gene in *S. aureus* can be used to predict the effect on the Dhfr enzyme. This in turn provides a measure of the reduction in the ability of trimethoprim to bind to the affected enzyme, thereby allowing prediction of minimum inhibitory concentration (MIC) (198).

Virulence profiling

In theory, the WGS methods developed for resistance gene detection can be applied to detect virulence-associated genes, although currently there is a lack of information regarding which genes are important as determinants of virulence in CoNS. Even for *S. aureus*, the importance of specific genetic markers which have been linked to specific disease phenotypes (e.g. Panton-Valentine leucocidin with skin and soft tissue infections, superantigen toxins with toxic shock syndrome, fibronectin binding protein polymoprhisms with cardiac device infections) remains unclear (177,199). Cataloguing confirmed and putative genetic factors associated with virulence could yield valuable information on the ability of particular strains and species of CoNS to persist on medical devices intra-corpus (34). Moreover, bacterial genome-wide association studies (GWAS) comparing isolates from cases with controls could be powered to detect genetic determinants of specific phenotypes. However, such studies are challenging because of the requirement to assemble very large sample sets involving well-defined clinical phenotypes. Moreover,

features of particular interest such as cell wall adhesins are often encoded in repetitive regions of the genome, which are poorly assembled from short-read sequencing data. Nevertheless, *S. epidermidis* GWAS are beginning to provide some insights. Meric *et al.* (200) applied GWAS to *S. epidermidis* to correlate k-mers with *in vitro* phenotypes associated with pathogenicity. The k-mers were present in 61 genes involved in biofilm formation, cell toxicity, Interleukin-8 response to infection in blood and methicillin resistance (200) . Such research will enhance our understanding of what differentiates infecting and commensal bacteria and could ultimately be applied in clinical practice to direct management decisions in individual patients. Wirth *et al*. (201) used a GWAS approach to investigate the emergence of *Staphylococcus capitis* NRCS-A clone as an agent of neonatal sepsis on neonatal intensive care units (ICUs). The success of the clone could be attributed to the acquisition of vancomycin resistance. At a national level, Stenmark *et al*. (202) used next-generation sequencing to investigate spread of the *S. capitis* NRCS-A clone in Sweden.

Assessment of transmission

Sequencing-based studies of *S. aureus* have advanced our understanding of endemic (166) and epidemic *S. aureus* transmission (176) and of outbreaks (176,203). Given the clonality of nosocomially adapted strains of *S. epidermidis, S. haemoloyticus and S. hominis* (27,58,73,78,204,205) and the poor resolution of conventional typing methods, WGS is likely to improve understanding of clonal spread, outbreaks and endemicity of CoNS. In one study 5/9 isolates of a single clonal linage of *S. epidermidis* were associated with neonatal sepsis (186). The nine isolates were highly related (diversity ranged from 0 to 18 SNPs between any two isolates) and all from patients with overlapping stay in the ward suggesting common ancestry of a virulent strain (186). At a larger scale WGS was used to resolve two distinct lineages of ST2 that would be indistinguishable by MLST, alongside a lineage of ST23 that have spread globally and show genotypic and phenotypic resistance to rifampicin, fusidic acid and aminoglycosides (85). Lazaris *et al*. (206) employed WGS to characterise an outbreak of linezolid resistant MRSE on an ICU. Although 13 isolates were indistinguishable by MLST and SCC*mec* typing, 12 isolates differed by 1 - 52 SNPs and were *cfr*-negative whilst one differed from them by 202 – 223 SNPs and harboured a novel *cfr*-positive plasmid (206) . This highlights the resolution afforded by WGS and demonstrates that both strain and plasmid surveillance can be performed using a single assay.

Discrimination of infection from sample contamination

Accidental introduction of organisms to an indwelling device such as a prosthetic joint or venous catheter is thought to account for many CoNS infections. As such infections are associated with nosocomial and commensal strains rather than with distinctive primary pathogens it is difficult to discriminate between infecting and contaminating isolates (36,42,207). Genome-wide linkage studies have substantially failed to identify simple genotype-phenotype relationships in staphylococcal infection. However, the study of sequential isolates has revealed the nature of within-host adaptations that accompany invasive *S. aureus* disease. Common mutations observed in invasive *S. aureus* undergoing within-host evolution (208) suggest that it may be possible to differentiate colonising and infecting staphylococci by detection of such mutations. Another diagnostic approach might be to measure the genetic relatedness of multiple isolates. In *S. aureus* infection it has been shown that a small subset of donor bacteria diversifies in the recipient host (209). The accumulation of point mutations over the course of time during chronic CoNS infection of orthopaedic devices would be expected to present a characteristic cloud of diversity that could be used to distinguish infection from sample contamination (Figure 2). Where multiple strains of a CNS species are inoculated into a surgical wound, discrimination down to the level of point mutations would allow isolates with different ancestors to be differentiated.

Investigation of within-host adaptation

During the course of an infection, a pathogen may undergo within-host evolutionary adaptive change associated with changes in virulence and tolerance to the host immune system or antimicrobial therapy. Whole genome sequencing of sequential isolates of *S. epidermidis* obtained from a pacemaker-associated infective endocarditis allowed investigation of genetic changes associated with increased biofilm formation, reduced growth and resistance to rifampicin over time (210). Similar studies of *S. aureus* endocarditis isolates have shown an accumulation of point mutations that lead to increased resistance to vancomycin following exposure (211,212).

Limitations of whole-genome sequencing

The prospect of adopting WGS in routine diagnostic microbiology to investigate CoNS infection is a tantalising one but several limitations currently impede the widespread adoption of WGS. A primary issue is cost. The sequencing instrument, reagents and the necessary laboratory and computational infrastructure are currently more costly than the equipment and consumables required for conventional culture-based techniques. Furthermore, specialist expertise is required to perform and analyse WGS. Whole genome sequencing-associated costs generally necessitate the batching of samples resulting in increased turn-around times that are unsuitable for diagnostic service. Unlike conventional phenotypic methods used to detect antimicrobial resistance, WGS-based methods require prior knowledge of the genetic basis for resistance and may fail to detect a hitherto unknown resistance element. Phenotypic methods also allow the observation of MIC creep which may not be detected by genotyping. Another limitation is the current absence of a widely accepted international standard for the interpretation of WGS for diagnostic purposes. Nevertheless, all of these limitations may eventually be overcome.

Conclusion

A significant body of research literature already exists as a result of genome-based studies of CoNS. This will grow as sequencing becomes more widely available. Genomic investigation of bacteria can provide information about genetic diversity, of population structure locally and globally and can provide insights into the natural history of colonisation and infection. Such information can be used to develop methods to identify and compare the relatedness of isolates, detect and investigate outbreaks, study transmission and predict antimicrobial resistance. These approaches have been applied to *S. aureus* (133,140–143,171,176–178) and the potential exists for their application to CoNS. WGS may eventually replace culture-dependent methods (135,179,180).

Coagulase-negative staphylococci are important causes of nosocomial infection. Although less virulent than *S. aureus,* nosocomial CoNS are often multi-resistant, necessitating the use of second line antibiotics. Infections are often associated with indwelling medical devices. Difficulties in discriminating between infecting and contaminating isolates can lead to unnecessary antibiotic therapy and device removal with impacts on patient care and antibiotic resistance. Genome sequencing lends itself to the detection of *post hoc* signals of infection since it can identify subtle changes in the genome, such as point

mutations acquired over time following inoculation or adaptive changes caused by environmental pressures (208,211–213).

Conflicts of Interest

The author(s) declare that there are no conflicts of interest

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1233 Figures and Tables

Country	USA	USA	Denmark	Japan	Brazil	Greece	Spain	France	India	Italy	India	Germany	Turkey	India	S. Korea	USA	
Study	Gill *et al.*, 1983 (214)	Kleeman *et al.*, 1993 (215)	Jarlov *et al.*, 1996 (216)	Kawamura *et al.*, 1998 (217)	del Alamo *et al.*, 1999 (218)	Petinaki *et al.*, 2001 (219)	Cuevas *et al.*, 2004 (220)	Sivadon *et al.*, 2005 (115)	Singhal *et al.*, 2006 (221)	Arciola *et al.*, 2006 (222)	Chaudhury *et al.*, 2006 (223)	Gatermann *et al.*, 2007 (224)	Koksal *et al.*, 2009 (225)	Jain *et al.*, 2011 (226)	Shin *et al.*, 2011 (23)	Roach *et al.*, 2015 (186)	
Specimen	CS	CS	BSI	CS	BSI	CS	CS	BJI	CS	PJI	CS	CRBI, BSI	BSI	CRBI	CAPD Peritonitis	CS	
No. of Isolates	**678**	**499**	**499**	**944**	**239**	**450**	**369**	**212**	**83**	**601**	**167**	**494**	**200**	**98**	**51**	**208**	**Mean%**
S. epidermidis	75	65	58	41	50	50	56	71	34	68	13	67	44	24	67	86	**54.3**
S. haemolyticus	7	13	9	15	10	15	5	2	13	7	72	12	12	37	12	11	**15.8**
S. hominis	5	7	12	5	12	11	18	4	2	8	6	9	9	3			**6.9**
S. capitis	2	4		5		4	3	6	10	5	1	1	8	12	4		**4.1**
S. warneri	3	4	2	3		4	4	7	1	5	1	2	4		8	2	**3.1**
S. lugdunensis		3		2		2		3	13	1	1	3	9				**2.3**
S. xylosus						4			6			1	5	13			**1.8**

S. caprae				14				2		1		1			6		**1.5**
S. saprophyticus	1	1	1	5		6	5						3			1	**1.4**
S. simulans	1	2		6		2	1	1			2	1	2	1			**1.2**
S. cohnii		1	1		6	1				3		1		8			**1.3**
S. schleiferi									12			1					**0.8**
S. pasteuri								2							2		**0.3**
S. auricularis													1				**0.1**
Not determined	6		17	4	22	1	8	2	9	2	4	1	3	2	1		**5.1**

Table 1. Percentage of CoNS species isolated from clinical samples 1983 – 2015 (Adapted from Piette & Verschraegen G [23] & Becker *et al.* [34])
CS = Clinical specimen, BSI = Bloodstream infection, BJI = Bone & joint infection, PJI = Prosthetic joint infection, CRBI = Catheter-related bloodstream infection, CAPD = Continuous ambulatory peritoneal dialysis

Species	Loci						
S. aureus	*arcC*	*aroE*	*glpF*	*gmk*	*pta*	*tpi*	*yqiL*
S. epidermidis	*arcC*	*aroE*	*gtr*	*mutS*	*pyrR*	*tpiA*	*yqiL*
S. haemolyticus	*arcC*	*SH_1200*	*hemH*	*leuB*	*SH1431*	*cfxE*	*Ribose_ABC*
S. hominis	*arcC*	*glpK*	*gtr*	*pta*	*tpiA*	*tuf*	

Table 2. Loci employed by https://pubmlst.org/ for multi-locus sequence typing of four major staphylococcal human pathogens

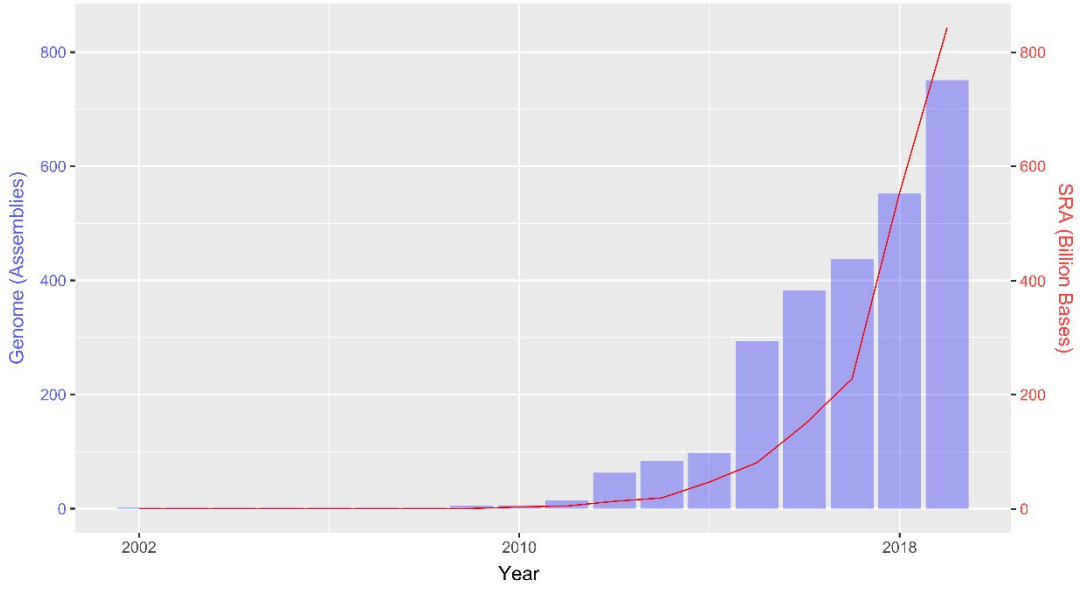

Figure 1. Number of *Staphylococcus epidermidis* assembled genomes released in the NCBI Genome database (blue) and bases released in the NCBI Sequence Read Archive (red) between 2002 and 2019

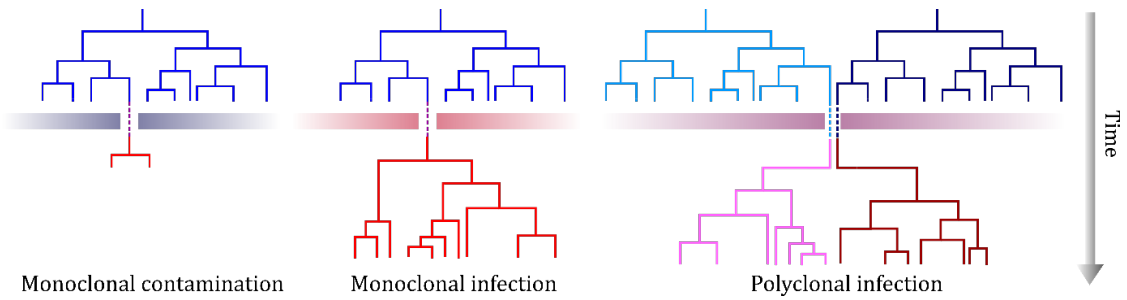

Figure 2. Working hypothesis of the difference in diversity between monoclonal contamination, monoclonal infection and polyclonal infection. The thick horizontal bars represent the inoculation event either during implantation (for infection) or specimen explantation/processing (for contamination). Adapted from Young *et al*. (208).