

Case Report

Coagulase Negative Staphylococcal Isolates from Prosthetic Knee Infections Show Diverse Speciation and a High Rate of Antibiotic Resistance

Arthur R. Bartolozzi, Niaz Banaei, Indre Budvytiene, Robert Manasherob, Stuart B. Goodman, James I. Huddleston, William J. Maloney and Derek F. Amanatullah*

Department of Orthopaedic Surgery, Stanford Hospital and Clinics, 450 Broadway Street, C402, Redwood City CA 94063, USA

*Address for Correspondence: Derek F. Amanatullah, Stanford Hospital and Clinics, 450 Broadway Street, Redwood City, CA, USA, E-mail: dfa@stanford.edu

Received: 29 July 2019; Accepted: 05 November 2019; Published: 06 November 2019

Citation of this article: Bartolozzi, AR., Banaei, N., Budvytiene, I., Manasherob, R., Goodman, SB., Huddleston, JI., Maloney, WJ., Amanatullah, DF. (2019) Coagulase Negative Staphylococcal Isolates from Prosthetic Knee Infections Show Diverse Speciation and a High Rate of Antibiotic Resistance. *Int J Orth*, 2(2): 054-057.

Copyright: © 2019 Bartolozzi, AR, et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

ABSTRACT

The incidence of infection associated with primary total knee arthroplasty (TKA) is up to 2.5% with \$1.62 billion estimated in system costs. Adverse outcomes depend on bacterial species with methicillin resistant strains carrying the highest burden. Prior research has focused on *Staphylococcus aureus*. Our study identifies the species and resistance patterns of coagulase negative *Staphylococcus* species that infect knee prostheses. TKA infections positive for coagulase negative *Staphylococcal* species subsequently treated by four joint surgeons from 2015-2019 were included. Matrix-assisted laser desorption ionization (MALDI) time of flight mass spectrometry was used and scores greater than 2.00 were considered to identify unique species. There were 68 TKA infections treated during the study period and 29 (42%) cultured a coagulase negative *Staphylococcal* species. Of these, 16 (55%) were *S. epidermidis*, 6 (21%) were *S. lugdunensis*, 5 (17%) were *S. capitis*, 1 (3%) was *S. warneri* and 1 (3%) was *S. haemolyticus*. Further, 14 (48%) were positive for the *mecA* gene conferring resistance to methicillin. All species were identified by MALDI with an average score of 2.12 ± 0.13 . Coagulase negative *Staphylococcal* species from TKA infections showed a range of species which could suggest multiple etiologies for sustaining an infection. While the *mecA* gene was present more commonly than reported averages, it was present in strains with resistance to many antibiotics not just beta lactams.

Keywords: Prosthetic joint infection, Antibiotic resistance

Introduction

Prosthetic joint infection (PJI) is a devastatingly complication after total knee arthroplasty (TKA). The incidence of PJI after primary TKA is estimated up to 2.5% with an overall cost to the health system estimated at \$566 million in 2009 rising to \$1.62 billion by 2020 [1,2]. The specific subtypes implicated in PJI are extensively discussed in a review by Tande and Patel with *Staphylococcus* species accounting for 62% of all infections across an aggregated 2,435 joints [3]. Methicillin-resistant *Staphylococcus aureus* (MRSA) rates vary widely having been quoted from 23% to 61% [4-7]. The diversity and resistance patterns of infectious agents together with predisposing medical comorbidities create an adverse environment for patient outcomes

from shorter infection-free periods, more revision surgeries, longer hospital stays, and higher costs [8-11].

However, the rate of adverse outcomes depends on bacterial subtype. A recent study of 149 infections of hip prostheses demonstrated *Pseudomonas*, *Proteus*, and MRSA infections were each correlated with lower infection-free rates, additional surgeries, and longer hospital stays compared to PJI with other organisms [12]. These findings have been corroborated by other studies investigating multi-drug resistant (MDR) PJI with failure rates from 10% to 33% [5,13]. The rate of PJI with MDR strains are less prevalent than susceptible strains in nearly all studies with no consensus on whether the rates are rising [14,15]. To better quantify these differences, a more detailed molecular understanding is necessary.

Matrix-assisted laser desorption ionization time of flight (MALDI-TOF) mass spectrometry provides increased specificity in organism detection through analysis of unique molecular signatures [16]. This has improved diagnostic sensitivity and led to new tools for species determinations even in approximately 60% of infections with normal erythrocyte sedimentation rate and C-reactive protein [17]. It has emerged as a cost effective, highly accurate alternative to polymerase chain reaction based strain identification and demonstrated accuracy above 93% to 99% in analysis of coagulase negative *Staphylococcal* species [18-23]. To our knowledge, there is no study of MALDI-TOF mass spectrometry molecular signatures for PJI in resistant bacterial strains. This study was designed to elucidate whether there were molecular commonalities between coagulase negative *Staphylococcal* species strains in the service of understanding the pathogenesis of PJI.

Methods

Following institutional review board approval, a retrospective chart review was undertaken for all primary periprosthetic knee infections treated by four knee arthroplasty surgeons (DFA, JIH, SBG, WJM) at a single institution from 2015-2019. All infections met Musculoskeletal Infection Society criteria initially with either a draining sinus tract or combination of minor criteria as delineated in Parvizi, et al. [24]. They also required confirmatory culture and sensitivity data from revision surgery (either synovial fluid or tissue) with ≥ 2 samples positive for the same organism to avoid the issue of contamination. Patients were included with coagulase negative *Staphylococcal* species grown in traditional fashion. Patients with chronic infections or culture negative infections were excluded.

MALDI-TOF mass spectrometry was performed on overnight grown bacterial cultures. Individual colonies were picked with the sterile toothpick from nonselective sheep blood agar plates and thinly smeared directly on polished steel target. Smeared target spots were overlaid with 1 μ l of a MALDI-TOF mass spectrometry matrix (portioned solution of α -cyano-4-hydroxycinnamic acid, Bruker Daltonics, Bremen, Germany with 50% acetonitrile, 47.5% mass spectrometry grade water and 2.5% trifluoroacetic acid, Sigma-Aldrich, Germany) and allowed to dry at room temperature. Mass spectra, ranging from 2,000 to 20,000 Da, were acquired using

Microflex LT Biotyper (Bruker Daltonics, Bremen, Germany) in a linear positive mode at laser frequency of 30 Hz. The rest of detection parameters were set by the manufacturer. Instrument calibration was performed before spectra acquisition using freshly made bacterial test standard (BTS; Bruker Daltonics, Bremen, Germany). Data was analyzed using the Bruker Biotyper 3.4 software and MBT Compass Library – RUO 7311 (Bruker Daltonics, Bremen, Germany). Species level identification was determined by using an in-house validated score criteria, where scores from 1.800 to 3.000 indicate species level identification, scores from 1.700 to 1.799 indicate genus level identification, scores < 1.699 indicate no reliable identification. Resistance was determined by diffusion disc method and the *mecA* testing was performed by nucleic acid amplification in the traditional fashion on the same isolates.

Categorical variables are expressed as number and percent while continuous variables are expressed as mean and standard deviation. Statistics were computed in STATA (StataCorp College Station, TX). Regressions were performed with statistical significance determined at $p < 0.05$. When relevant, 95% confidence intervals (CI) are reported. For binary variables, chi-square proportion testing or logistic regressions were used reporting odds ratios (OR) while for count variables, Poisson regression was used reporting incidence rate ratios (IRR).

Results

There were 68 TKA infections treated during the study period and 29 (42%) cultured coagulase negative *Staphylococcal* species. Of these, 16 (55%) were *S. epidermidis*, 6 (21%) were *S. lugdunensis*, 5 (17%) were *S. capitis*, 1 (3%) was *S. warneri* and 1 (3%) was *S. haemolyticus*. Further, 14 (48%) were positive for the *mecA* gene conferring resistance to methicillin and all of these were *S. epidermidis* (Table 1). All species were identified by MALDI-TOF mass spectrometry speciation with a mean score of 2.12 ± 0.13 .

The patients had a mean age of 67.0 ± 7.9 years at the time of revision operation; 17 (59%) were male and 12 (41%) were female. Their mean body mass index was 34.0 ± 7.3 kg/m². The mean time from primary arthroplasty to infection identification was

Table 1: Antibiotic resistance data by species subtype following disc diffusion resistance testing. Per laboratory protocol, not all isolates were tested against all antibiotics.

Antibiotic	<i>S. epidermidis</i>	<i>S. lugdunensis</i>	<i>S. warneri</i>	<i>S. capitis</i>	<i>S. haemolyticus</i>
Penicillin	16 (100%)	3 (100%)	1 (100%)	4 (100%)	1 (100%)
Moxifloxacin	10 (77%)	--	--	--	--
Erythromycin	11 (69%)	3 (50%)	1 (100%)	1 (20%)	0 (0%)
Levofloxacin	12 (80%)	--	0 (0%)	--	--
Oxacillin	15 (94%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Clindamycin	8 (50%)	3 (50%)	0 (0%)	1 (20%)	0 (0%)
Trimethoprim-Sulfamethoxazole	10 (63%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Gentamicin	7 (44%)	1 (17%)	0 (0%)	0 (0%)	0 (0%)
Tetracycline	2 (13%)	--	--	--	--
Rifampin	1 (7%)	--	0 (0%)	--	--
Daptomycin	0 (0%)	--	--	--	--
Linezolid	0 (0%)	--	--	--	--
Vancomycin	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)

Table 2: Chi-squared testing of *mecA* positivity predicting resistance to other antibiotics. Penicillin, moxifloxacin, daptomycin, vancomycin, and linezolid were excluded because in our culture data they did not vary among CNS species.

Antibiotic	Chi-squared Statistic	p-value
Oxacillin	25.3	<0.001*
Trimethoprim-Sulfamethoxazole	10.6	0.001*
Gentamicin	3.2	0.075
Erythromycin	0.9	0.340
Clindamycin	0.8	0.362
Levofloxacin	0.8	0.383
Tetracycline	0.2	0.685
Rifampin	0.2	0.696

approximately 3.6 ± 4.7 years (range 0.1 – 18.8) and only 2 cases (7%) were documented within 90 days of the index procedure. Charlson comorbidity index (CCI) was calculated for each patient (mean 3.4 ± 2.0 , range 1-9).

MecA positivity correlated but did not significantly predict resistance to other antibiotic agents except trimethoprim-sulfamethoxazole and oxacillin (Table 2). Regarding demographic predictors, *mecA* positivity also showed a trend toward more complicated patients (increased CCI) but was not significant (OR 1.23, CI 0.82-1.83, $p=0.314$). Current or past smoking history was significantly correlated with less *mecA* positivity (OR 0.20, CI 0.05-0.94, $p=0.041$). Time to infection did not correlate with *mecA* positivity (OR 1.15, CI 0.95-1.39 $p=0.161$), however it did correlate significantly with *S. epidermidis* in so far as later presenting infections were more likely to be infection with *S. epidermidis* than any other isolate (IRR 2.68, CI 1.70-4.21, $p<0.001$).

Discussion

PJI continues to be a morbid complication of TKA. Of the infections we analyzed further, coagulase negative *Staphylococcal* species defined an important subgroup. Coagulase negative *Staphylococcal* species comprises a ubiquitous set of pathogens that demonstrate a range of clinical presentations from acute to chronic. In this series of TKA-PJI, all species were uniquely identified with MALDI species level specificity. *S. epidermidis* comprised most of the specific cultures. This is consistent with demonstrated epidemiologic incidence of indolent presentations [25]. *S. epidermidis* produces adhesive proteins specific for fibrinogen that mediate attachment to areas of scar formation facilitating localization to surgical wound beds. Further, it is known to produce polysaccharide intercellular adhesin when under hypoxic, osmotic, or antibiotic stress that in conjunction with implant hydrophobicity stimulates the production of biofilm [26]. A full review of biofilm and other pathogenic behavior of coagulase negative *Staphylococcal* species beyond the scope of this article, but is well summarized elsewhere [27].

Unlike *S. epidermidis* which can be considered a contaminant (though not in the setting of clinically meaningful PJI), *S. lugdunensis* and *S. warneri* are always considered pathogenic. [28]. *S. lugdunensis* is classically penicillin-sensitive but in our cohort all strains were penicillin-resistant [28]. Recently, it was shown that *S. lugdunensis* PJI leads to increased clinical symptoms than either *S. epidermidis* or *S. aureus* [28]. This speaks to varying acuity of chronic infection

exacerbations as well as the pathogenicity of *S. lugdunensis* in particular. *S. warneri* is isolated more rarely and is known to engage in biofilm production [29].

The results for *mecA* gene positivity in 48% of the cohort is consistent with if not slightly higher than other studies. *MecA* positivity was also correlated with resistance to many antibiotics not just cell wall synthesis inhibitors. In our cohort, 88% of *S. epidermidis* infections were resistant to gentamicin (of those resistant all were positive for *mecA*). Aminoglycoside resistance correlates with a “not cured” clinical outcome for coagulase negative *Staphylococcal* species-PJI – from testing at 2 year follow up, patients with “not cured” clinical status had ongoing treatment for PJI of any type medical or surgical [30]. To this end, it is interesting that all *mecA* positive strains were *S. epidermidis* and that smoking was both protective against *mecA* positive infection and associated with an earlier time to infection. It is well known that smoking increases the risk of PJI, but our data suggests smoking increases susceptibility to less virulent organisms at an earlier time point.

The varying species of coagulase negative *Staphylococcal* species in our isolates suggest multiple organism strains have the capability to generate clinical infection predicated on biofilm formation and MDR status. However, many infections are considered to be polymicrobial [31]. Questions therefore remain concerning the specific molecular factors that permit PJI, how the infectious agent(s) change over time, and what specific treatments should be employed to target those mechanisms [32]. This will be particularly relevant as new data regarding mechanisms like quorum sensing emerge with targeted quorum inhibitors and bacteriophage therapy [33].

In addition to the limited number of patients included, this study was limited by MALDI-TOF requiring species that result in a positive culture. This study also relied on traditional methods for determining antibiotic resistance and *mecA* gene positivity.

Conclusion

MALDI-TOF identified a range of coagulase negative *Staphylococcal* species that had wide and varied antibiotic resistance pattern. Interestingly, a history of smoking was protective against *mecA* positivity.

Acknowledgements

The authors have no financial relationships relevant to the content of this research study.

Author Contribution Statement

ARB harvested data, ran analysis, and drafted the manuscript. NB contributed to study design and bacterial analysis. IB ran the MALDI experiment. SBG, JIH, and WJM provided patient cases SBG, JIH, WJM, RM, and DFA reviewed the manuscript. DFA was the senior design author RM and DFA coordinated the project's completion.

References

- Kurtz, SM., Lau, E., Watson, H., Schmier, JK., Parvizi, J. (2012) Economic Burden of Periprosthetic Joint Infection in the United States. *J Arthroplasty*, 27(8): 61–65.
- Voigt, J., Mosier, M., Darouiche, R. (2015) Systematic review and meta-analysis of randomized controlled trials of antibiotics and antiseptics for preventing infection in people receiving primary total hip and knee prostheses. *Antimicrob Agents Chemother*, 59(11): 6696–6707.

3. Tande, AJ., Patel, R. (2014) Prosthetic Joint Infection. *Clin Microbiol Rev*, 27(2): 302-345.
4. Murgier, J., Laffosse, J-M., Cailliez, J., Cavaignac, E., Murgier, P., Bayle-Iniguez, X., et al. (2016) Is the prognosis the same for periprosthetic joint infections due to *Staphylococcus aureus* versus coagulase-negative staphylococci? A retrospective study of 101 patients with 2-year minimum follow-up. *Arch Orthop Trauma Surg*, 136(10): 1357-1361.
5. Vasso, M., Schiavone, Panni, A., De, Martino, I., Gasparini, G. (2016) Prosthetic knee infection by resistant bacteria: the worst-case scenario. *Knee Surg Sports Traumatol Arthrosc*, 24(10): 3140-3146.
6. Parry, MC., Duncan, CP. (2014) The challenge of methicillin resistant staphylococcal infection after total hip replacement: overlooked or overstated? *Bone Joint J*, 96-B(11 Supple A): 60-65.
7. Parvizi, J., Ghanem, E., Azzam, K., Davis, E., Jaberi, F., Hozack, W. (2008) Periprosthetic infection: are current treatment strategies adequate? *Acta Orthop Belg*, 74(6): 793-800.
8. Tande, AJ., Palraj, BR., Osmon, DR., Berbari, EF., Baddour, LM., Lohse, CM., et al. (2016) Clinical Presentation, Risk Factors, and Outcomes of Hematogenous Prosthetic Joint Infection in Patients with *Staphylococcus aureus* Bacteremia. *Am J Med*, 129(2): 221.e11-20.
9. Gundtoft, PH., Pedersen, AB., Varnum, C., Overgaard, S. (2017) Increased Mortality After Prosthetic Joint Infection in Primary THA. *Clin Orthop Relat Res*, 475(11): 2623-2631.
10. Kapadia, BH., Berg, RA., Daley, JA., Fritz, J., Bhawe, A., Mont, MA. (2016) Periprosthetic joint infection. *Lancet*, 387(10016): 386-394.
11. Mahmoud, SS., Sukeik, M., Alazzawi, S., Shaath, M., Sabri, O. (2016) Salvage Procedures for Management of Prosthetic Joint Infection After Hip and Knee Replacements. *Open Orthop J*, 10: 600-614.
12. Cunningham, DJ., Kavolus, JJ., Bolognesi, MP., Wellman, SS., Seyler, TM. (2017) Specific Infectious Organisms Associated With Poor Outcomes in Treatment for Hip Periprosthetic Infection. *J Arthroplasty*, 32(6): 1984-1990.e5.
13. Murgier, J., Laffosse, JM., Cailliez, J., Cavaignac, E., Murgier, P., Bayle-Iniguez, X., et al. (2016) Is the prognosis the same for periprosthetic joint infections due to *Staphylococcus aureus* versus coagulase-negative staphylococci? A retrospective study of 101 patients with 2-year minimum follow-up. *Arch Orthop Trauma Surg*, 136(10): 1357-1361.
14. Mühlhofer, HML., Deiss, L., Mayer-Kuckuk, P., Pohlig, F., Harrasser, N., Lenze, U., et al. (2017) Increased Resistance of Skin Flora to Antimicrobial Prophylaxis in Patients Undergoing Hip Revision Arthroplasty. *In Vivo*, 31(4): 673-676.
15. Gundtoft, PH. (2017) Prosthetic Joint Infection following Total Hip Arthroplasty - Incidence, Mortality and Validation of the Diagnosis in the Danish Hip Arthroplasty Register. *Dan Med J*, 64(9).
16. Peel, TN., Cole, NC., Dylla, BL., Patel, R. (2015) Matrix-assisted laser desorption ionization time of flight mass spectrometry and diagnostic testing for prosthetic joint infection in the clinical microbiology laboratory. *Diagn Microbiol Infect Dis*, 81(3): 163-168.
17. Marmor, S., Bauer, T., Desplaces, N., Heym, B., Roux, AL., Sol, O., et al. (2016) Multiplex Antibody Detection for Noninvasive Genus-Level Diagnosis of Prosthetic Joint Infection. *J Clin Microbiol*, 54(4): 1065-1073.
18. Tran, A., Alby, K., Kerr, A., Jones, M., Gilligan, PH. (2015) Cost Savings Realized by Implementation of Routine Microbiological Identification by Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry. *J Clin Microbiol*, 53(8): 2473-2479.
19. Loonen, AJM., Jansz, AR., Bergland, JNB., Valkenburg, M., Wolffs, PFG., van den Brule, AJC. (2012) Comparative study using phenotypic, genotypic, and proteomics methods for identification of coagulase-negative staphylococci. *J Clin Microbiol*, 50(4): 1437-1439.
20. Dupont, C., Sivadon-Tardy, V., Bille, E., Dauphin, B., Beretti, JL., Alvarez, AS., et al. (2010) Identification of clinical coagulase-negative staphylococci, isolated in microbiology laboratories, by matrix-assisted laser desorption/ionization-time of flight mass spectrometry and two automated systems. *Clin Microbiol Infect*, 16(7): 998-1004.
21. Tevell, S., Hellmark, B., Nilsdotter-Augustinsson, Å., Söderquist, B. (2017) *Staphylococcus capitis* isolated from prosthetic joint infections. *Eur J Clin Microbiol Infect Dis*, 36(1): 115-122.
22. Li, A., Gow, N., Atkins, BL., Taylor, A., Peto, T., McNally, MA., et al. (2017) Metalware-associated orthopaedic infections caused by *Staphylococcus lugdunensis*: An emerging pathogen. *J Infect*, 75(4): 368-370.
23. Harris, LG., El-Bouri, K., Johnston, S., Rees, E., Frommelt, L., Siemssen, N., et al. (2010) Rapid identification of staphylococci from prosthetic joint infections using MALDI-TOF mass-spectrometry. *Int J Artif Organs*, 33(9): 568-574.
24. Parvizi, J., Tan, TL., Goswami, K., Higuera, C., Della Valle, C., Chen, AF., et al. (2018) The 2018 Definition of Periprosthetic Hip and Knee Infection: An Evidence-Based and Validated Criteria. *J Arthroplasty*, 33(5): 1309-1314.e2.
25. Otto, M. (2009) *Staphylococcus epidermidis* - the "accidental" pathogen. *Nat Rev Microbiol*, 7(8): 555-567.
26. Le, KY., Park, MD., Otto, M. (2018) Immune Evasion Mechanisms of *Staphylococcus epidermidis* Biofilm Infection. *Front Microbiol*, 9: 359.
27. Becker, K., Heilmann, C., Peters, G. (2014) Coagulase-Negative Staphylococci. *Clin Microbiol Rev*, 27(4): 870-926.
28. Lourtet-Hascoët, J., Félicé, MP., Bicart-See, A., Bouige, A., Giordano, G., Bonnet, E. (2018) Species and antimicrobial susceptibility testing of coagulase-negative staphylococci in periprosthetic joint infections. *Epidemiol Infect*, 146(14): 1771-1776.
29. Bogut, A., Niedziadek, J., Koziol-Montewka, M., Strzelec-Nowak, D., Blacha, J., Mazurkiewicz, T., et al. (2014) Characterization of *Staphylococcus epidermidis* and *Staphylococcus warneri* small-colony variants associated with prosthetic-joint infections. *J Med Microbiol*, 63(Pt 2): 176-185.
30. Post, V., Harris, LG., Morgenstern, M., Mageiros, L., Hitchings, MD., Méric, G., et al. (2017) Comparative Genomics Study of *Staphylococcus epidermidis* Isolates from Orthopedic-Device-Related Infections Correlated with Patient Outcome. *J Clin Microbiol*, 55(10): 3089-3103.
31. Wimmer, MD., Friedrich, MJ., Randau, TM., Ploeger, MM., Schmolders, J., Strauss, AA., et al. (2016) Polymicrobial infections reduce the cure rate in prosthetic joint infections: outcome analysis with two-stage exchange and follow-up \geq two years. *Int Orthop*, 40(7): 1367-1373.
32. Swearingen, MC., DiBartola, AC., Dusane, D., Granger, J., Stoodley, P. (2016) 16S rRNA analysis provides evidence of biofilms on all components of three infected periprosthetic knees including permanent braided suture. *Pathog Dis*, 74(7).
33. Mooney, JA., Pridgen, EM., Manasherob, R., Suh, G., Blackwell, HE., Barron, AE., et al. (2018) Periprosthetic bacterial biofilm and quorum sensing. *J Orthop Res*, 36(9): 2331-2339.