Retrospective analysis of specimens sent for 16S rDNA investigation

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Use of 16S rDNA in Clinical Microbiology

Based on comparative sequencing of the unique 16S ribosomal DNA genes

The use of 16S rDNA sequencing has played a huge role in

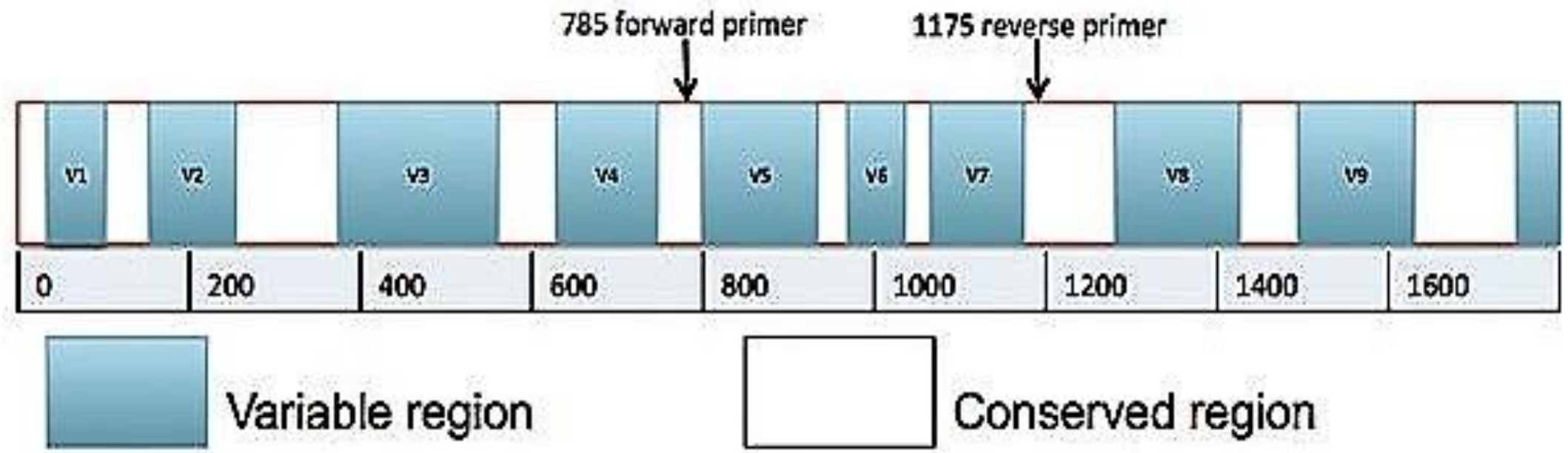
- discovery of novel bacteria
- reclassification of bacteria
- accurate identification of bacteria from clinical specimens

Use of 16S rDNA in Clinical Microbiology

16S rDNA in clinical microbiology has proven to be most useful in the identification of

- slow-growing bacteria
- fastidious bacteria
- uncultivable bacteria
- non-viable bacteria
- culture-negative infections

16S rRNA gene showing variable and conserved regions with primer location



Patel *et al*., 2017

Which specimens should be sent for 16S?

- There are no strict guidelines in SVUH regarding which specimens should be referred for 16S testing
- Each specimen is referred on a case by case basis
- The Colindale user manual provides information regarding specimen acceptance criteria but does not provide details on clinical indications for testing

SVUH – reasons that specimens are sent for 16S

- Culture negative
- From a normally sterile site
- Those in which one would expect a pathogen to be found e.g., liver abscess, empyema etc.
- Those from patients that are not clinically improving despite therapy
- Those from patients that have had prior antibiotics or those that are on immunosuppressive therapy e.g., steroids

Enrichment Culture & Extended Incubation

- In SVUH we use cooked meat anaerobic broth (LIP) for enrichment culture
  - Specimens of tissue, pus, joint fluids, devices etc.
  - Incubated in air at 35°C for 7 days* and subbed onto blood agar, CPSO and neomycin for 24 – 48 hours

- Extended incubation for anaerobes and fungi is carried out on samples of tissue, pus and devices
  - Extending incubation of anaerobic blood agar/neomycin and SDA (in 30°C) for 10 days

Aim

To audit specimens sent for 16S rDNA detection between 2014 – 2017

To assess the impact these results had on clinical management

Materials and Methods

- The laboratory information system (APEX) was retrospectively examined for all specimens sent for 16S rDNA detection
- Patient demographics, culture results and pathogens detected were recorded
- Specimens were referred to the Bacterial Identification Service (BIDS) at the Antimicrobial Resistance and Healthcare Associated Infections (AMRHAI) Reference Unit in Colindale, London
- Detection was performed by real-time 16S rDNA PCR followed by comparative sequencing

Turn Around Time

- Turn Around Time for 16S rDNA in SVUH is <= 14 days
- Covers the period from specimen dispatch to authorisation of the final paper report
- Overall, 68% (N = 94) samples met the required TAT
- However, this changed to 86% (N = 118) when the time between dispatch and returned reports was analysed
  - Delays in entering reports?
  - Delays in second-checking reports?
  - Delays in authorisation?

Cost

Transport

- Biomnis: €45.36 + 23% VAT = €55.79
  - Transport Monday to Thursday
  - Weight less than 1.0 kg

Testing

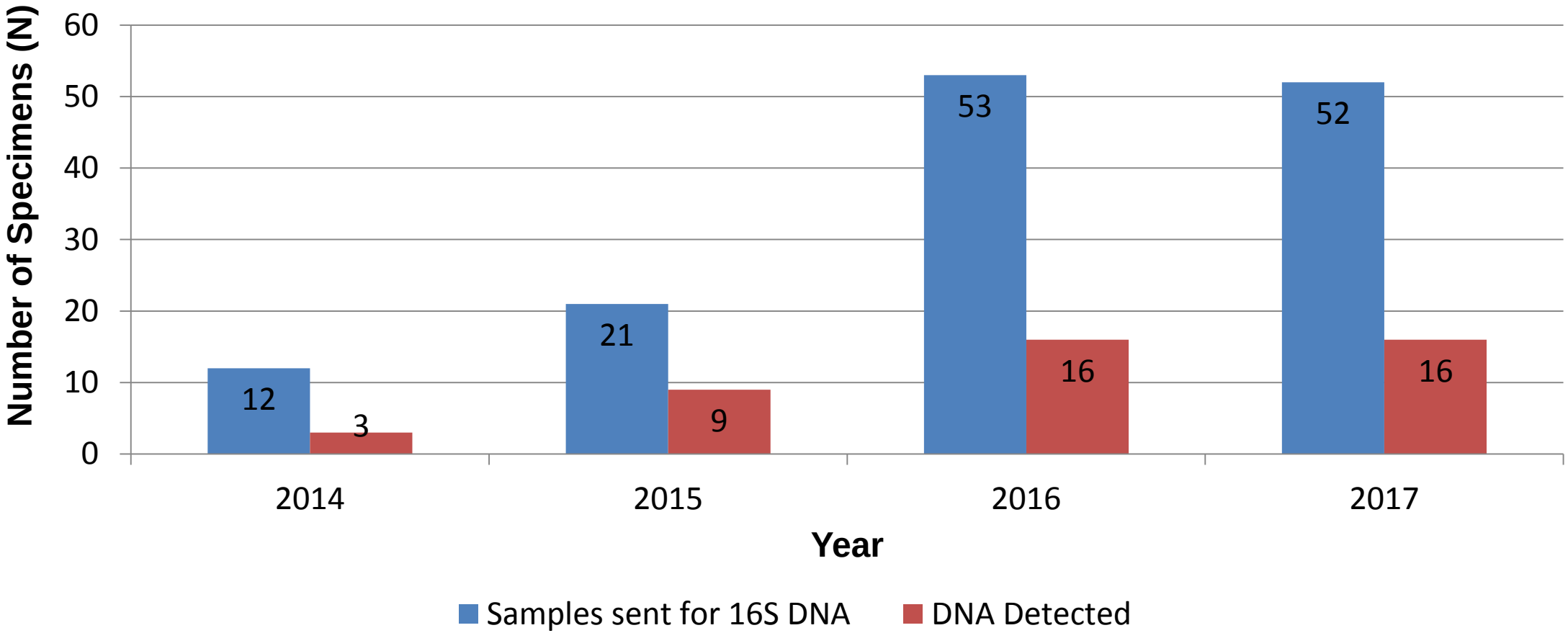
- Public Health England, Colindale, London: £273.00 (~€305.00)

Cost per test is ~€360.00

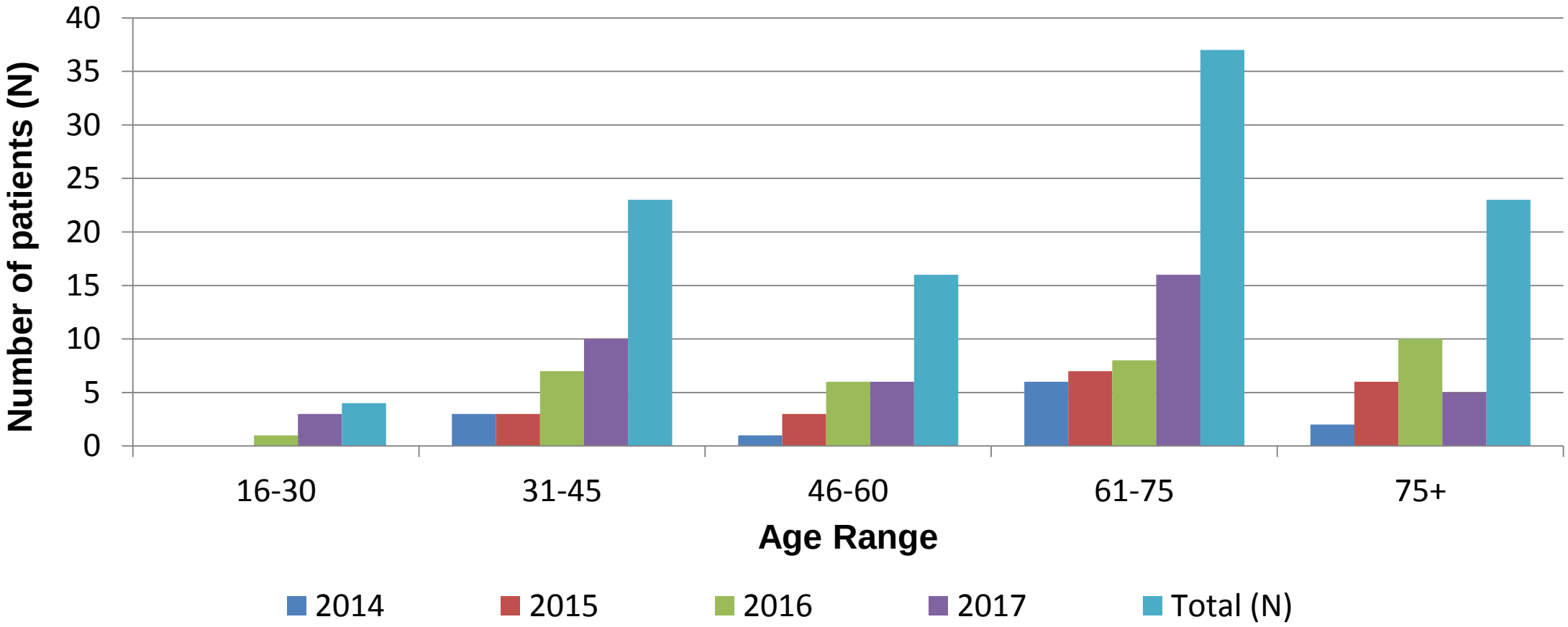
Results

- 138 specimens were referred for 16S rDNA from 103 patients
- 93.5% were sent when culture negative at 48 hours
- There was a predominance of specimens from females (54%)
- Most patients were aged 61 – 65 years
  - Large cohort of orthopaedic patients (33%)
- The most common specimens referred for testing were tissue (36%), joint fluid (18%), cerebrospinal fluid (10%) and pus (9%)
- Most patients had only one specimen sent (79%)

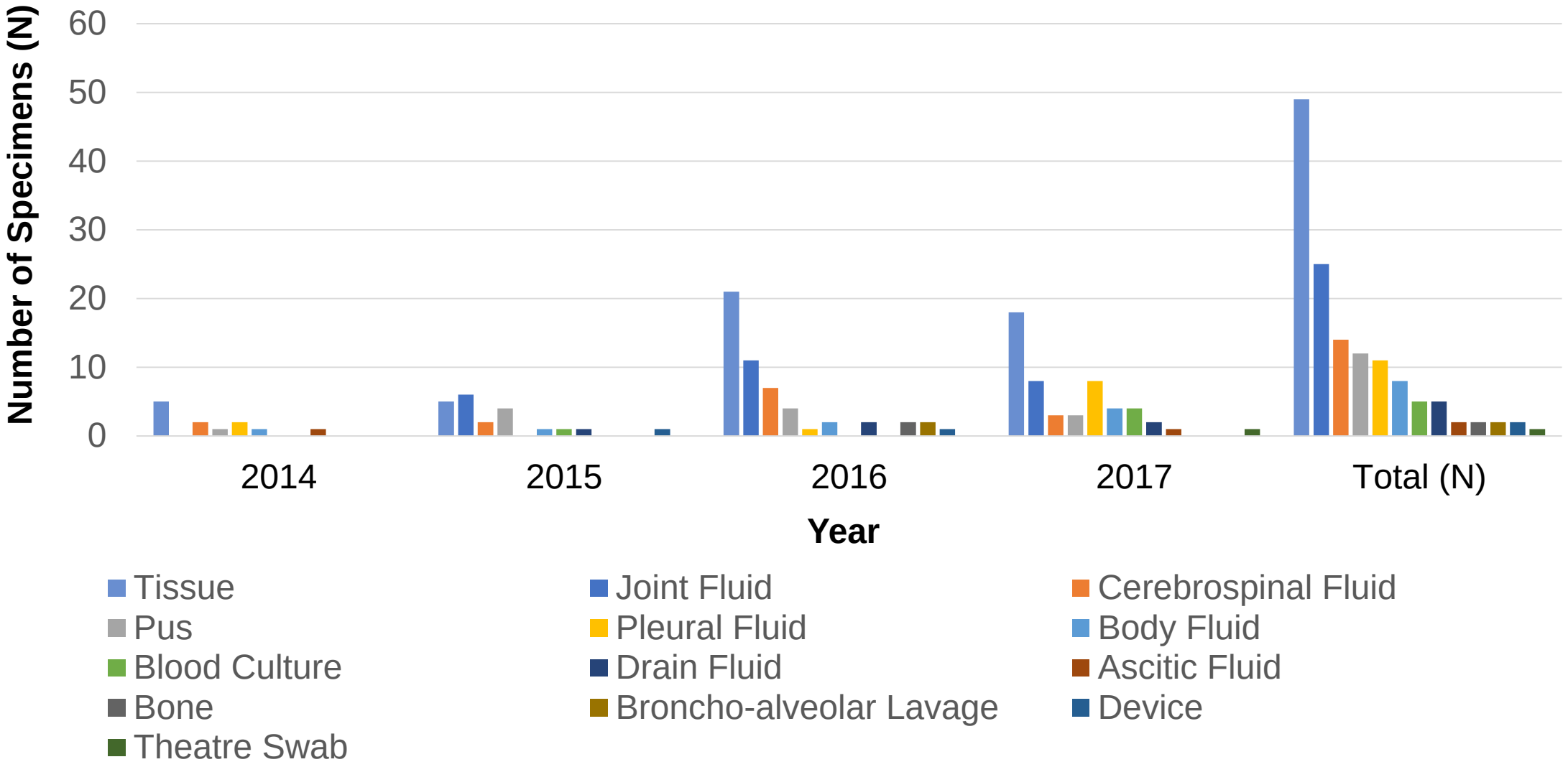
How many specimens are sent per year for 16S?



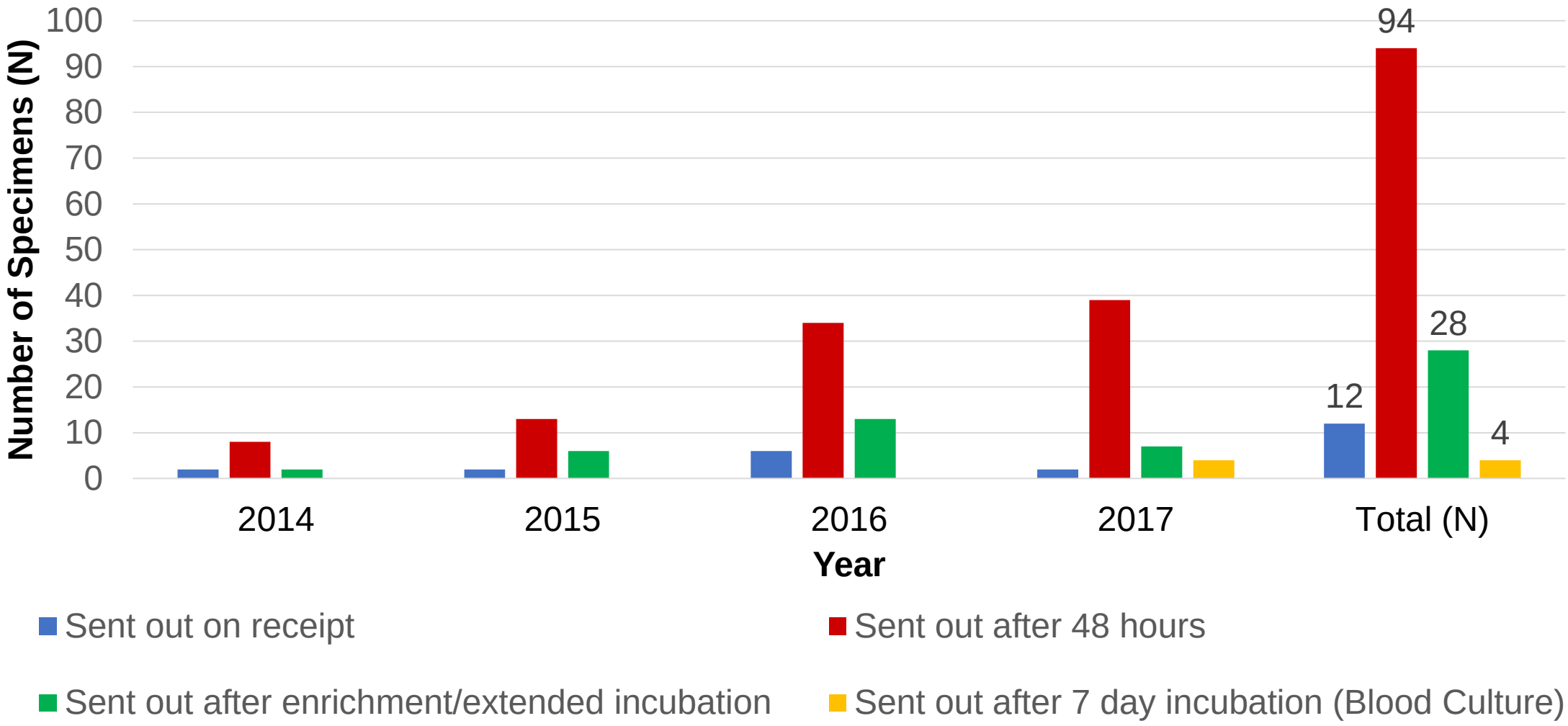
Age of patients with specimens referred for 16S



What specimens are sent for 16S?



When are specimens sent for 16S?



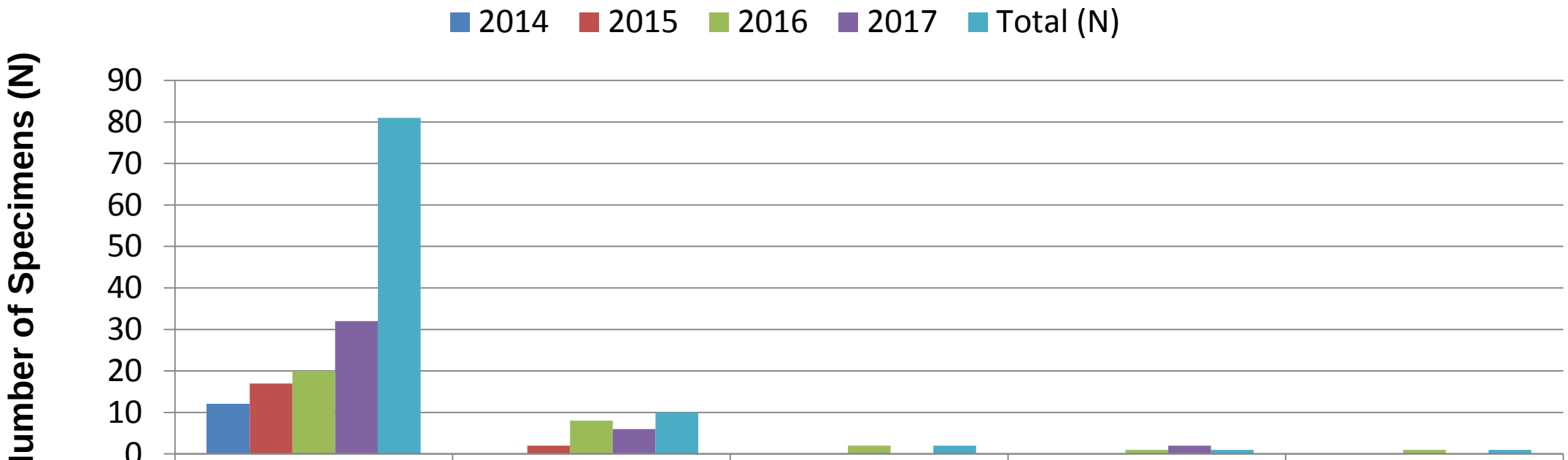
Specimens that were sent out for on receipt (1)

Specimen	Clinical Details	Culture Result	16S Result	Altered Management
Joint Fluid	WCC = 2610/cmm, 96% PMNs	*Staphylococcus epidermidis & Staphylococcus warneri* on 14-day enrichment	Not Detected	
Tissue	RT Finger; Seal bite	*Staphylococcus aureus*	*Staphylococcus* species	
CSF	Squamous cell carcinoma; Immunosuppressed	Negative	Not Detected	
CSF	Lymphocytic CSF	Negative	Not Detected	
Drain Fluid	Liver abscess	Negative	Not Detected	
Drain Fluid	Liver abscess	Negative	*Fusobacterium nucleatum*	No

Specimens that were sent out for on receipt (2)

Specimen	Clinical Details	Culture Result	16S Result	Altered Management
Pleural Fluid	Empyema	*Streptococcus intermedius*	*Streptococcus* species	No
Joint Fluid	WCC = >10,000/cmm, 87% PMNs; Hx STI	Negative	Not Detected	
Joint Fluid	WCC = >10,000/cmm, 93% PMNs; Hx STI	Negative	*Neisseria gonorrhoeae*	Yes
Drain Fluid	Prev OLT; Immunosuppressed	Negative	Not Detected	
Pleural Fluid	Empyema	Negative	*Streptococcus* species	No
CSF	Autoimmune systemic vasculitis	Negative	Not Detected	

Number of specimens per patient sent for 16S rDNA detection between 2014 – 2017 (N = 103)



	1 Sample	2 Samples	3 Samples	4 Samples	4+ Samples
2014	12				
2015	17	2			
2016	20	8	2	1	1
2017	32	6		2	
Total (N)	81	10	2	1	1

Specimens referred for 16S from 2014 – 2017

	DNA Detected	DNA NOT Detected
Culture Positive	18	16
Culture Negative	26	78

DNA Detected but Culture Negative (N = 26)

- Twenty-six specimens that had bacterial DNA detected were culture negative.
  - 8 Tissues
  - 4 Joint Fluids
  - 4 Pleural Fluids
  - 4 Pus Samples
  - 2 Body Fluids
  - 2 Drain Fluids
  - 1 Ascitic Fluid
  - 1 Bone Sample

Bacteria Detected by 16S rDNA	Specimen	Clinically Significant	Changed Management
Abiotrophia defectiva	Joint Fluid (Right Knee)	Yes	No
Enterobacteriaceae (Closest genera to *Escherichia coli* and *Shigella* species)	Tissue (Lung)	Yes	No
Finegoldia magna	Pleural Fluid	Data not available	Data not available
Fusobacterium nucleatum	Drain Fluid (Chest/Pigtail)	Yes	No
Fusobacterium species (99% homology to *Fusobacterium nucleatum* & *Fusobacterium naviforme*)	Pus (Liver Abscess)	Data not available	Data not available
Fusobacterium species	Pleural Fluid	Data not available	Data not available
Fusobacterium species	Tissue (Lung)	Data not available	Data not available
Moraxella osloensis	Bone (Right Proximal Humerus)	Yes	Yes

Bacteria Detected by 16S rDNA	Specimen	Clinically Significant	Changed Management
Moraxella osloensis	Tissue (Right Knee)	No	No
Mycobacterium avium & *Staphylococcus* species	Pus (Left Shoulder)	Yes	No
Mycobacterium tuberculosis complex	Joint Fluid (Right Hip)	Yes	No
Mycoplasma species	Tissue (Right Index Finger)	Yes	Yes
Neisseria gonorrhoeae	Joint Fluid (Right Ankle)	Yes	Yes
Porphyromonas endodontonalis	Pleural Fluid		
Proprionibacterium acnes	Joint Fluid (Right Shoulder)	Yes	Yes
Staphylococcus epidermidis	Tissue (Knee Prosthesis)	Yes	No

Bacteria Detected by 16S rDNA	Specimen	Clinically Significant	Changed Management
Staphylococcus species (99% homology to *Staphylococcus aureus, Staphylococcus haemolyticus, Staphylococcus lugdunensis* and *Staphylococcus simiae*)	Pus	Yes	No
Stenotrophomonas maltophilia	Perigraft	Data not available	Data not available
Stenotrophomonas maltophilia	Tissue (Left Hip – Prosthesis)	Data not available	Data not available
Streptococcus agalactiae	Body Fluid (Right Thigh)	Data not available	Data not available
Streptococcus agalactiae	Drain Fluid (Pigtail)	Data not available	Data not available
Streptococcus pyogenes	Ascitic Fluid	Yes	Data not available
Streptococcus pyogenes	Body Fluid	Yes	Data not available
Streptococcus pyogenes	Joint Fluid (Right Knee)	Yes	No

Bacteria Detected by 16S rDNA	Specimen	Clinically Significant	Changed Management
Streptococcus species (99% homology to *Streptococcus anginosus* group)	Tissue (Chest)	Yes	Yes
Streptococcus species (99% homology to *Streptococcus pneumoniae, Streptococcus mitis, Streptococcus oralis* and *Streptococcus pseudopneumoniae*)	Pleural Fluid	Yes	No

Culture Positive and DNA Detected (N = 18)

- Eighteen specimens that grew bacteria and had DNA detected by 16S
- Of the eighteen specimens:
  - Eight were concordant between culture and molecular techniques
  - Six were discordant between techniques
  - Four were excluded as the organism was unidentifiable by culture techniques
- 5/6 of discordant specimens grew from enrichment culture
  - Prone to contamination
- The remaining specimen was a culture positive BAL

138 Specimens	Primary culture positive N = 9	PCR-positive (concordant) = 4
		PCR-negative = 0
		PCR-positive (discordant) = 1
		Unidentified culture = 4
	Enrichment culture only positive N = 25	PCR-positive (concordant) = 4
		PCR-negative = 16
		PCR-positive (discordant) = 5
	Culture negative N = 104	PCR-positive = 26
		PCR-negative = 78

Culture Positive but DNA NOT Detected (N = 16)

- Sixteen specimens that grew bacteria where no DNA was detected by 16S
- All grew from extended (N=1) or enrichment (N=15) incubation
- Eighteen organisms grew from the sixteen specimens:
  - Coagulase-negative staphylococci (N=15)
  - *Corynebacterium* spp. (N=1)
  - *Proprionibacterium acnes* (N=1)
  - *Staphylococcus aureus* (N=1)

Organisms that grew in primary, extended or enrichment cultures but were not detected by 16S rDNA PCR (N = 16)

Organism	Primary	Extended	Enrichment
Staphylococcus epidermidis			6
Staphylococcus capitis			2
Staphylococcus hominis			2
Staphylococcus auricularis			1
Staphylococcus pasteuri			1
Corynebacterium species			1
Staphylococcus aureus & Staphylococcus epidermidis			1
Proprionibacterium acnes		1	
Staphylococcus epidermidis & Staphylococcus warneri			1
Total		1	15

Role of 16S rDNA in the detection of anaerobes

- 15 anaerobes were identified from 138 specimens referred for 16S rDNA
  - We grew one – *Bacteroides fragilis* was isolated on enrichment culture from a laparoscopic pus sample

- Why?
  1. Some anaerobes that were identified will not readily grow within 48 hours on neomycin agar
  2. Some anaerobes were probably non-viable after delayed transport to the lab or the plates were not incubated quickly enough after being inoculated

Anaerobes identified by 16S rDNA from 2014-2017

Organism	Specimen	Culture	DNA
Actinomyces species	Pus – Lung	Failed to grow	DETECTED
Bacteroides fragilis	Pus – Laparoscopic Drainage	Grew from 7-day enrichment culture	DETECTED
Dialister microaerophilus	Pus – Finger	Failed to grow	DETECTED
Finegoldia magna	Pleural Fluid	Failed to grow	DETECTED
Finegoldia magna	Tissue – Left Thigh	Failed to grow	DETECTED
Fusobacterium nucleatum	Drain Fluid	Failed to grow	DETECTED
Fusobacterium nucleatum/naviforme	Liver Abscess	Failed to grow	DETECTED
Fusobacterium species	Pleural Fluid	Failed to grow	DETECTED

Anaerobes identified by 16S rDNA from 2014-2017

Organism	Specimen	Culture	DNA
Fusobacterium species	Pleural Fluid	Failed to grow	DETECTED
Fusobacterium species	Tissue – Lung	Failed to grow	DETECTED
Phocaeicola abscessus	Pus – Unknown	Failed to grow	DETECTED
Porphyromonas endodontalis	Pleural Fluid	Failed to grow	DETECTED
Prevotella histicola & Veillonella atypica	Bronchoalveolar Lavage	Failed to grow	DETECTED
Proprionibacterium acnes	Joint Fluid – Shoulder	Failed to grow	DETECTED
Proprionibacterium acnes	Tissue – Knee	Failed to grow	DETECTED

Some bacterial genera and species that that were difficult to differentiate using 16S (N =20)

Report	Comment
Enterobacteriaceae (N = 2)	Closest genera to *Escherichia coli* & *Shigella* species
Fusobacterium species (N = 3)	
Fusobacterium species	99% homology to *F. naviforme* and *F. nucleatum*
Klebsiella species	99% homology to *K. pneumoniae, K. quasipneumoniae* and *K. planticola*
Micrococcus species	99% homology to *M. luteus* & *M. yunnanensis*
Mycoplasma species	
Mycobacteria species	Proposed to be *M. tilburgii*
Mycobacterium tuberculosis complex	

Some bacterial genera and species that that were difficult to differentiate using 16S

Report	Comment
Staphylococcus species	99% homology to *S. arlettae, S. cohnii, S. nepalensis* and *S. saprophyticus*
Staphylococcus species	99% homology to *S. aureus, S. argentus* and *S. simiae*
Staphylococcus species	99% homology to *S. aureus, S. haemolyticus, S. lugdunensis* and *S. simiae*
Staphylococcus species	
Streptococcus species	*Streptococcus anginosus* Group
Streptococcus species	*Streptococcus anginosus* Group
Streptococcus species	*Streptococcus anginosus* Group
Streptococcus species	99% homology to *S. mitis, S. oralis, S. pneumoniae* and *S. pseudopneumoniae*
Streptomyces species	

Conclusions

- 16S rDNA is a valuable molecular identification tool but should be used in combination with bacterial culture
- 16S identified bacteria in 26/138 specimens which remained culture negative throughout incubation
- Some disadvantages with the technique so it is not suitable as a standalone test:
    1. Lacks sensitivity to identify some bacterial species due to high genetic similarity/inadequate sequences in Gene Bank.
    2. Does not provide antimicrobial sensitivity data.

Conclusions

- Enrichment cultures were frequently prone to contamination and should be interpreted with caution
- It is likely that more specimens need to be sent per patient to rule out or confirm infection and change patient management, especially in the case of orthopaedic patients

Acknowledgements

- Prof Kirsten Schaffer
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Questions?