

Susceptibility testing of anaerobic bacteria:

MIC distributions for
Bacteroides spp., *Prevotella* spp., *Fusobacterium
necrophorum*, *Clostridium perfringens*
and *Cutibacterium acnes*.

Supplementary material for the general consultation 4 October – 15
November, 2021.

2021 October

Background

- EUCAST has reviewed and revised breakpoints and developed a disk diffusion method for rapidly growing anaerobes on Fastidious Anaerobe Agar (FAA) for the following species:
 - *Bacteroides* spp.
 - *Prevotella* spp.
 - *Fusobacterium necrophorum*
 - *Clostridium perfringens*
 - *Cutibacterium acnes*

Reference MIC values performed with agar dilution at UKARU (Cardiff)

- UK Anaerobe Reference Unit in Cardiff performed Agar dilution according to CLSI M11 with anaerobic incubation at 35-37°C for 42-48 h, on two media in parallel:
 - Brucella Blood Agar (BBA)
 - Fastidious Anaerobe Agar (FAA)
- The following antimicrobial agents were tested at concentrations from 0.008-256 mg/L:
 - Benzylpenicillin
 - Piperacillin-tazobactam
 - Meropenem
 - Vancomycin
 - Clindamycin
 - Metronidazole

Bacteroides and *Prevotella* spp.

These were the *Bacteroides* and *Prevotella* available to us during the development of susceptibility testing of anaerobic bacteria; subsets consisting of 50 – 60 of each were used for different parts of the project.

Species	No	Newly re-named to
<i>Bacteroides fragilis</i>	112	
<i>Bacteroides thetaiotaomicron</i>	23	
<i>Bacteroides thetaiotaomicron/faecis</i>	1	
<i>Bacteroides ovatus</i>	9	
<i>Bacteroides dorei/vulgatus</i>	6	<i>Phocaeicola dorei/vulgatus</i>
<i>Bacteroides vulgatus/dorei</i>	1	<i>Phocaeicola vulgatus/dorei</i>
<i>Bacteroides vulgatus</i>	6	<i>Phocaeicola vulgatus</i>
<i>Bacteroides dorei</i>	1	<i>Phocaeicola dorei</i>
<i>Bacteroides caccae</i>	3	
<i>Parabacteroides distasonis</i>	2	
<i>Bacteroides cellulosilyticus</i>	1	
<i>Bacteroides faecis</i>	1	
<i>Bacteroides uniformis</i>	1	
<i>Bacteroides xylanisolvens</i>	1	
<i>Parabacteroides merdae</i>	1	

Species	No
<i>Prevotella bivia</i>	17
<i>Prevotella buccae</i>	11
<i>Prevotella denticola</i>	7
<i>Prevotella disiens</i>	3
<i>Prevotella heparinolytica</i>	2
<i>Prevotella melaninogenica</i>	2
<i>Prevotella nanceiensis</i>	2
<i>Prevotella bergensis</i>	1
<i>Prevotella corporis</i>	1
<i>Prevotella intermedia</i>	1
<i>Prevotella melanogenica</i>	1
<i>Prevotella nanciensis</i>	1
<i>Prevotella salivae</i>	1

The fact that some species have changed genus since "then" was brought up during the anaerobe workshop 20 September.

Isolates

- 263 isolates were selected and also tested with disk diffusion.

Species	Number
<i>Bacteroides</i> spp.	50
<i>Prevotella</i> spp.	50
<i>Fusobacterium necrophorum</i>	51
<i>Clostridium perfringens</i>	58
<i>Cutibacterium acnes</i>	54

Presentation of results

- Results for the 263 isolates are presented as:
 - MIC distributions comparing MIC values on BBA and FAA.
 - Available reference MIC distributions from the EUCAST website
 - Proposed EUCAST breakpoints are shown for each organism-agent combination
- Aggregating all five species, 45 degree analysis showing MICs on BBA vs. FAA per antimicrobial were performed.
 - Benzylpenicillin (all species except *Bacteriodes* spp.)
 - Piperacillin-tazobactam (all species)
 - Meropenem (all species)
 - Vancomycin (Gram-positive species only)
 - Clindamycin (all species)
 - Metronidazole (all species)

Disk diffusion methodology, QC criteria and reading guidance is published on the EUCAST website

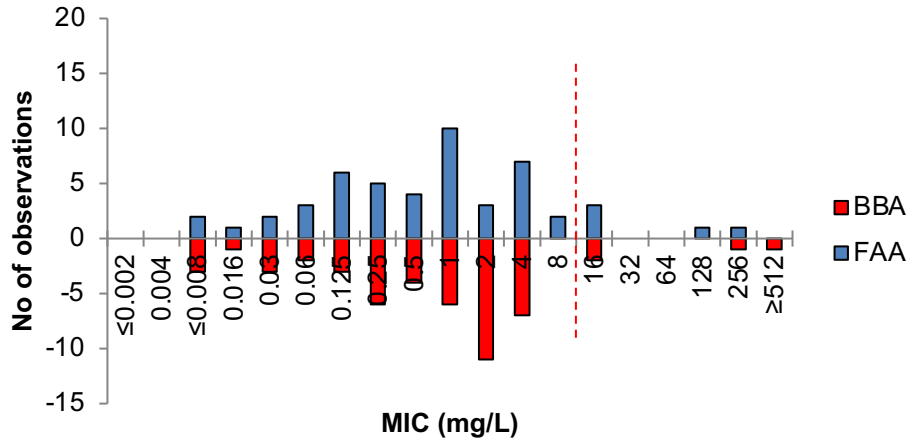
- Disk diffusion methodology on FAA and reference methodology agar dilution on FAA was already discussed and is published in CMI and manuals, QC criteria and reading guide now posted on EUCAST website: https://www.eucast.org/ast_of_bacteria/disk_diffusion_methodology/).
- Laboratory trial of disk diffusion methodology and QC criteria
 - Phase 1 completed and discussed in an online seminar September 2021.
 - Phase 2 until 15 November – the disk diffusion testing of "interesting" isolates collected individually in 16 laboratories. MIC will be determined as appropriate.

Bacteroides spp.

Piperacillin-tazobactam

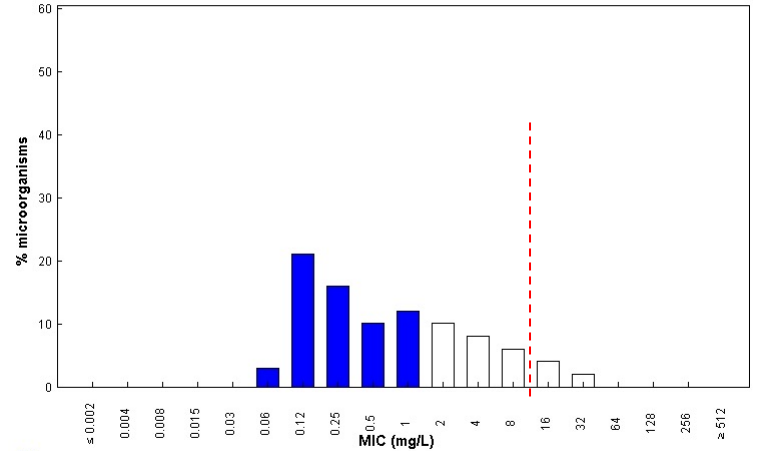
Proposed breakpoints: 8/8 mg/L

Piperacillin-tazobactam MIC FAA vs. BBA
Bacteroides spp., 50 isolates



Piperacillin-tazobactam / *Bacteroides fragilis*
International MIC Distribution - Reference Database 2020-09-18

MIC distributions include collated data from multiple sources, geographical areas and time periods and can never be used to infer rates of resistance



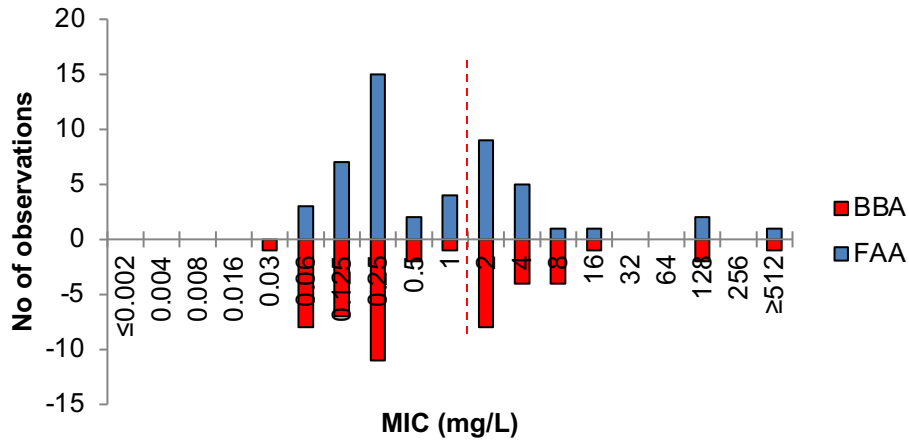
MIC
Epidemiological cut-off (ECOFF): 1 mg/L
Wildtype (WT) organisms: ≤ 1 mg/L

1908 observations (5 data sources)

Meropenem

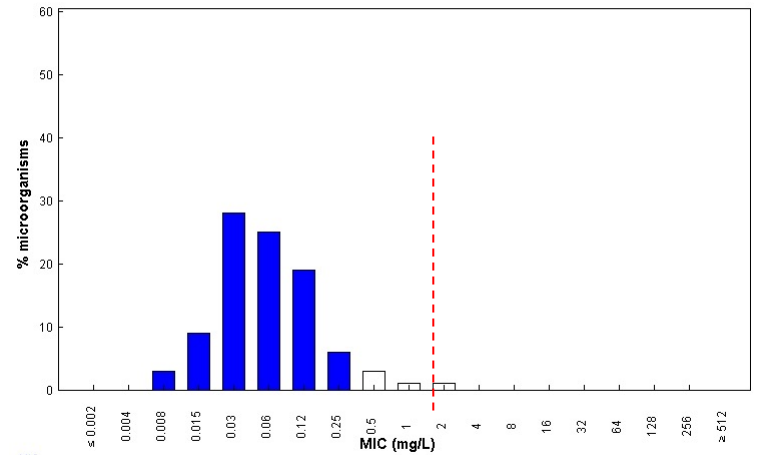
Proposed breakpoints: 1/1 mg/L

**Meropenem MIC FAA vs. BBA
Bacteroides spp., 50 isolates**



Meropenem / *Bacteroides fragilis*
International MIC Distribution - Reference Database 2020-09-18

MIC distributions include collated data from multiple sources, geographical areas and time periods and can never be used to infer rates of resistance

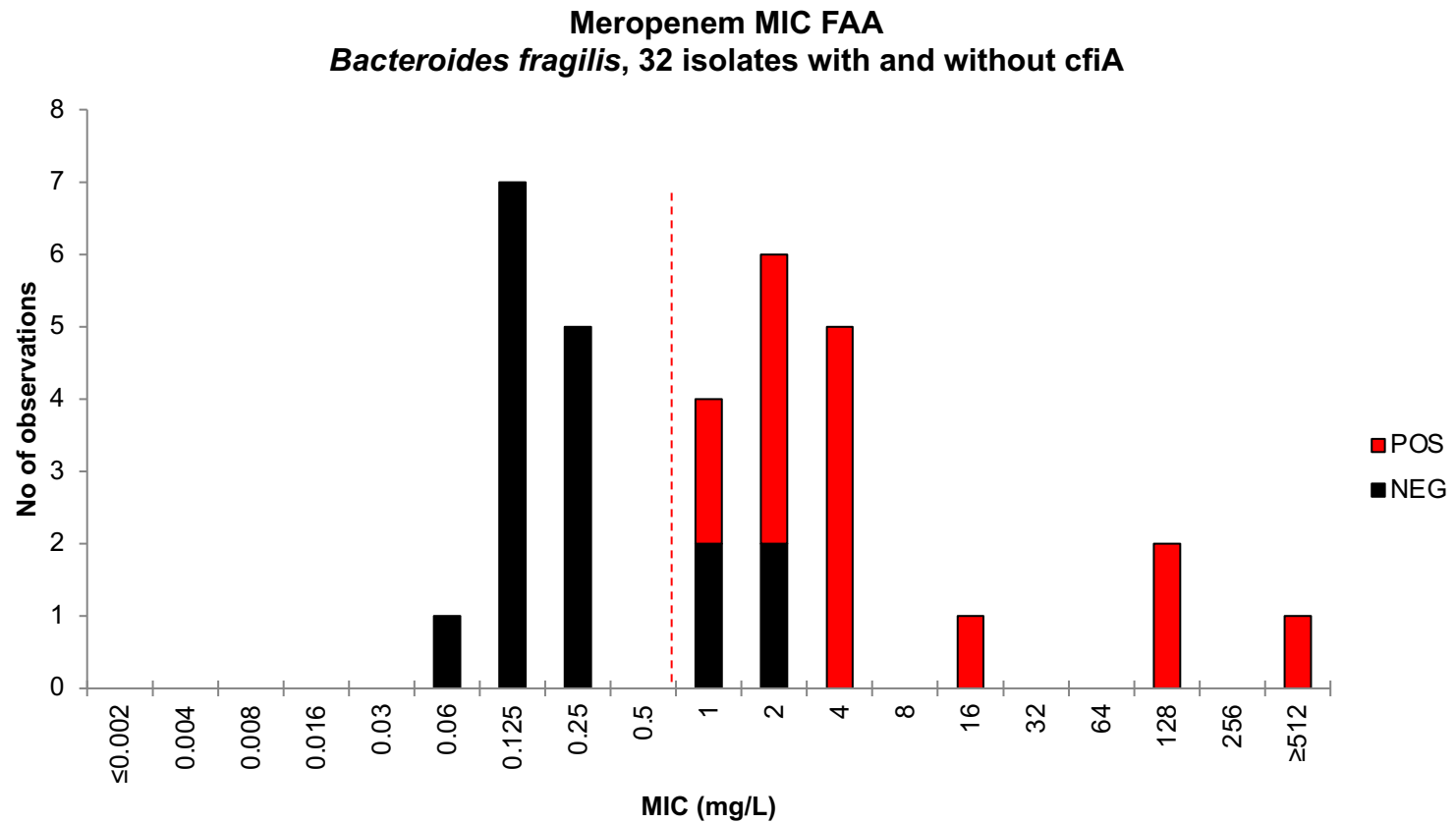


MIC
Epidemiological cut-off (ECOFF): 0.25 mg/L
Wildtype (WT) organisms: ≤ 0.25 mg/L

3284 observations (48 data sources)

Meropenem

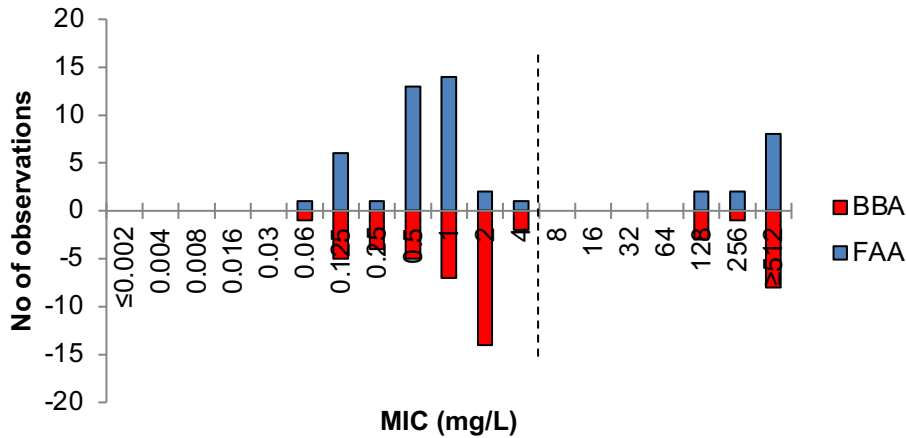
Proposed breakpoints: 1/1 mg/L.



Clindamycin

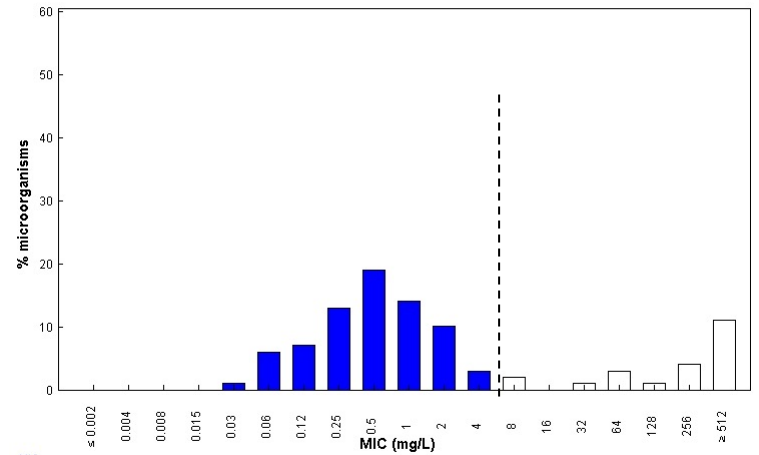
Proposed breakpoints: (4/4)* mg/L

Clindamycin MIC FAA vs. BBA
Bacteroides spp., 50 isolates



Clindamycin / *Bacteroides fragilis*
International MIC Distribution - Reference Database 2020-09-18

MIC distributions include collated data from multiple sources, geographical areas and time periods and can never be used to infer rates of resistance



MIC Epidemiological cut-off (ECOFF): 4 mg/L
Wildtype (WT) organisms: ≤ 4 mg/L

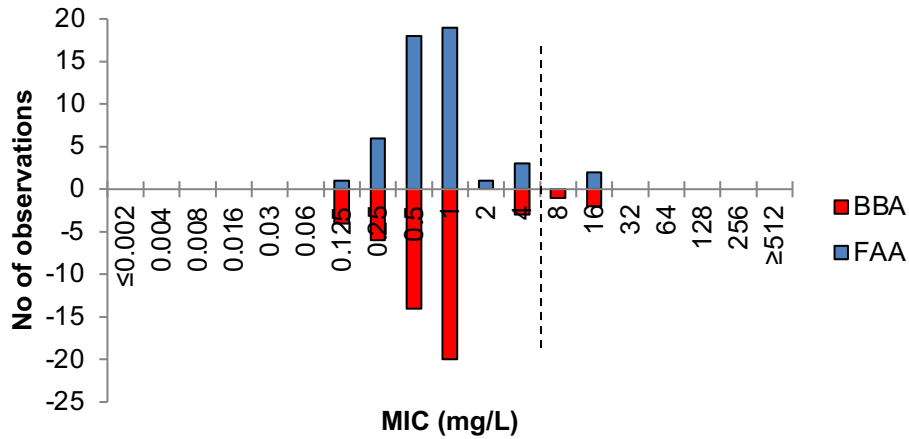
1579 observations (5 data sources)

*Breakpoints in brackets: Note 10 in breakpoint tables: Breakpoints in brackets distinguish between isolates without and with phenotypically detectable resistance mechanisms. They are based on ECOFFs but since they may serve more than one species, the value may represent a best fit. For these agents, clinical evidence as monotherapy is usually lacking but for a specific indication or in combination with another active agent or measure they may still be used. Isolates with resistance can be reported R (resistant) but if S or I are reported, there should be a comment to explain the caveat mentioned above..

Metronidazole

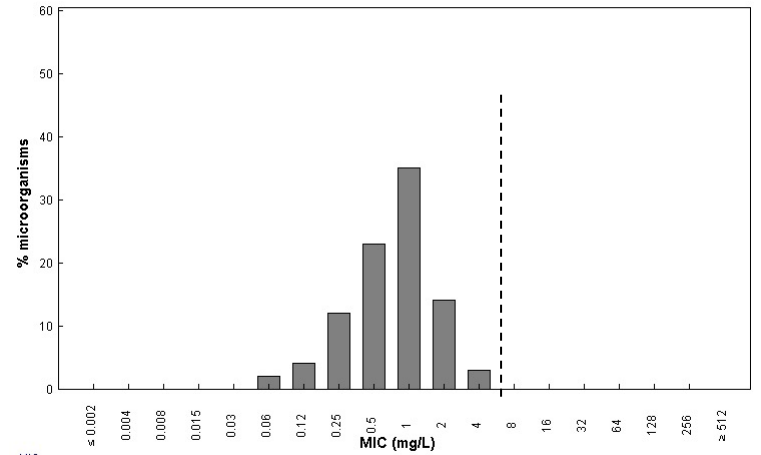
Proposed breakpoints: 4/4 mg/L

Metronidazole MIC FAA vs. BBA
Bacteroides spp., 50 isolates



Metronidazole / *Bacteroides fragilis*
International MIC Distribution - Reference Database 2020-09-18

MIC distributions include collated data from multiple sources, geographical areas and time periods and can never be used to infer rates of resistance



MIC
Epidemiological cut-off (ECOFF): -
Wildtype (WT) organisms:

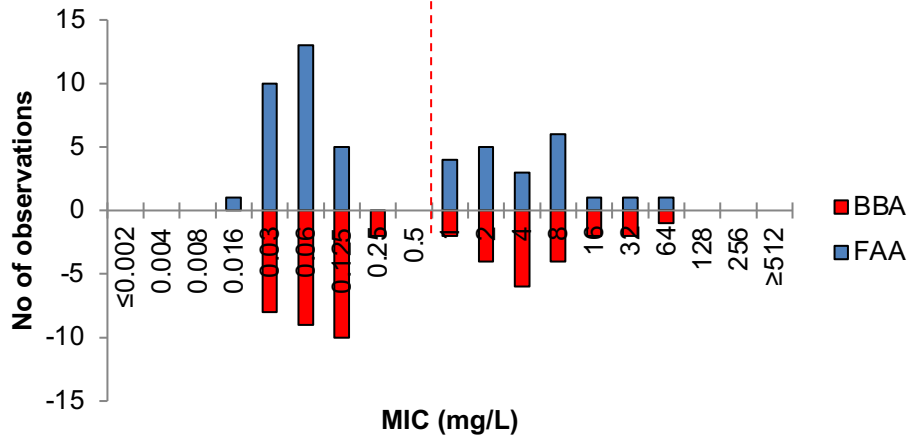
2332 observations (5 data sources)

Prevotella spp.

Benzylpenicillin

Proposed breakpoints: 0.5/0.5 mg/L

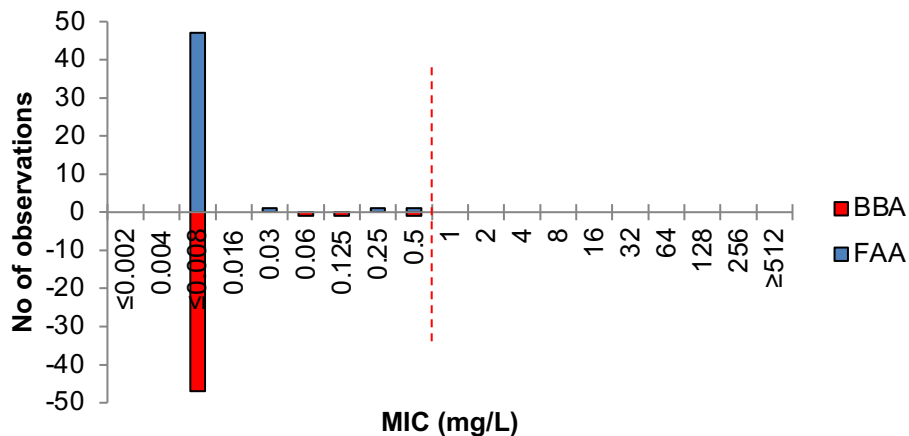
**Benzylpenicillin MIC FAA vs. BBA
Prevotella spp., 50 isolates**



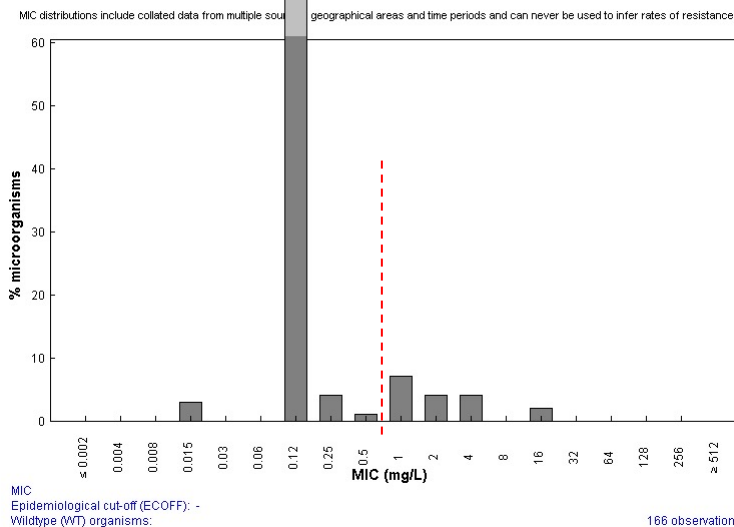
Piperacillin-tazobactam

Proposed breakpoints: 0.5/0.5 mg/L

Piperacillin-tazobactam MIC FAA vs. BBA
Prevotella spp., 50 isolates



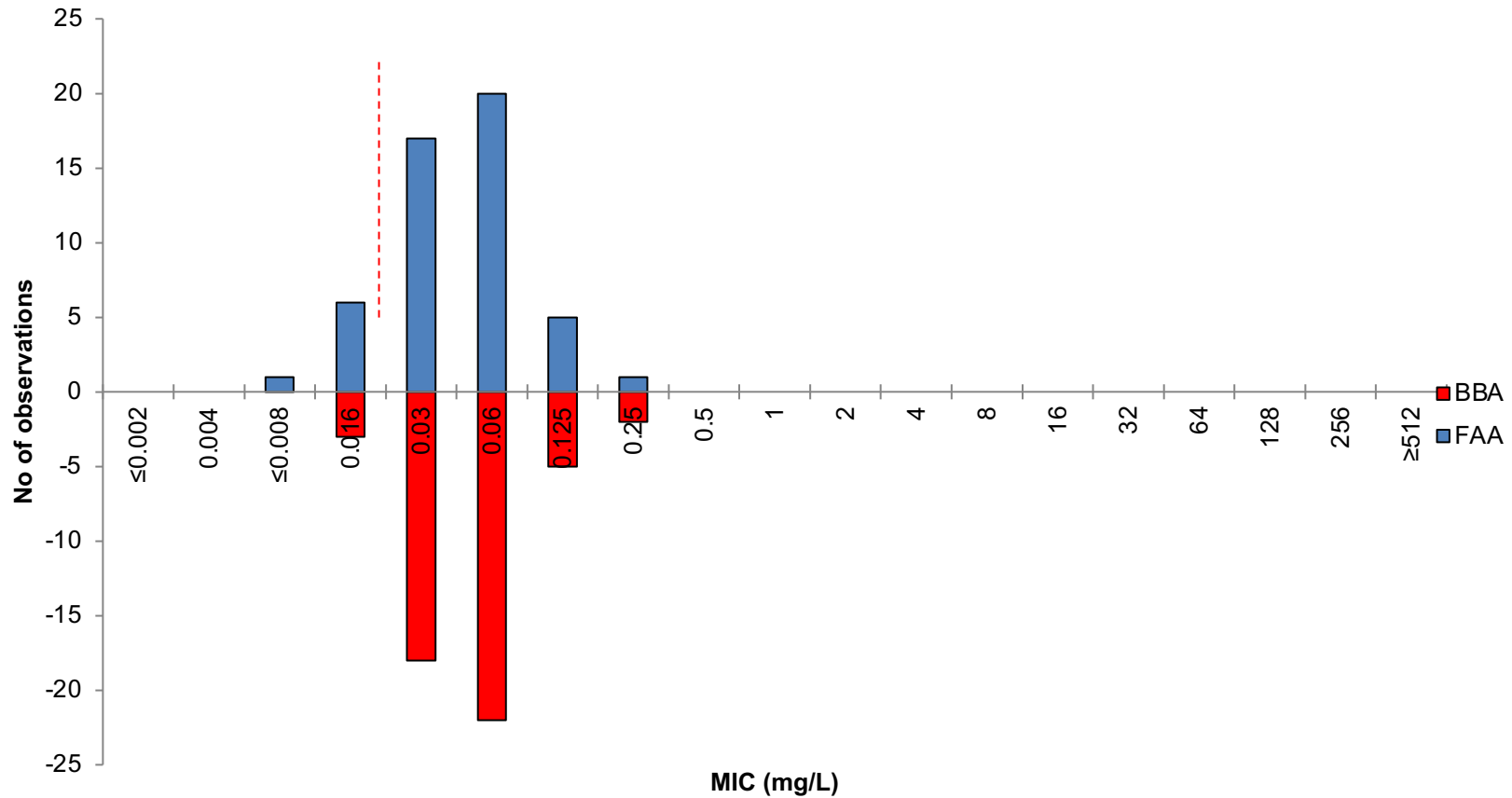
Piperacillin-tazobactam / *Prevotella* spp
International MIC Distribution - Reference Database 2020-02-18



Meropenem

Proposed breakpoints: 0.25/0.25 mg/L

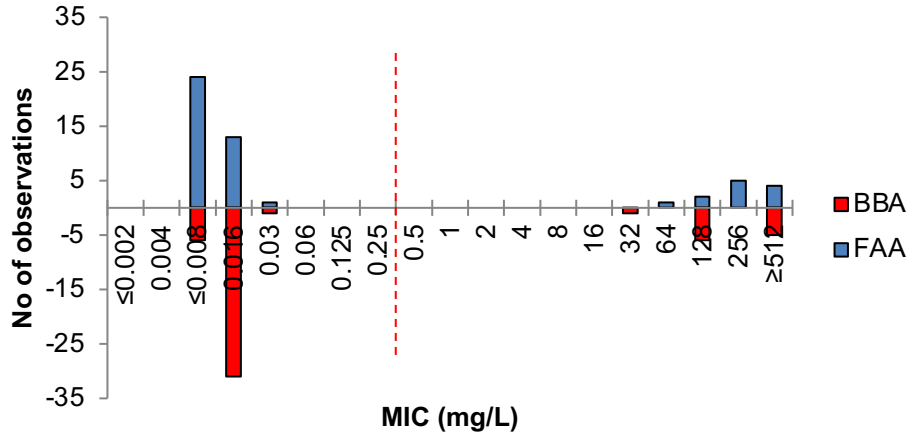
Meropenem MIC FAA vs. BBA
Prevotella spp., 50 isolates



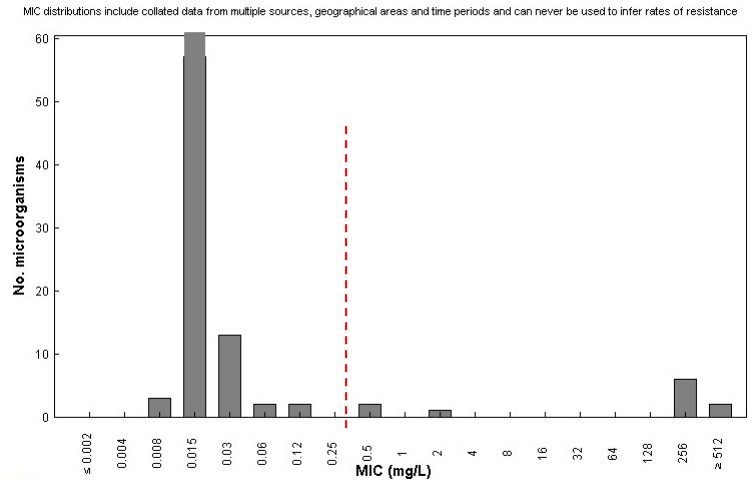
Clindamycin

Proposed breakpoints: 0.25/0.25 mg/L

Clindamycin MIC FAA vs. BBA
Prevotella spp., 50 isolates

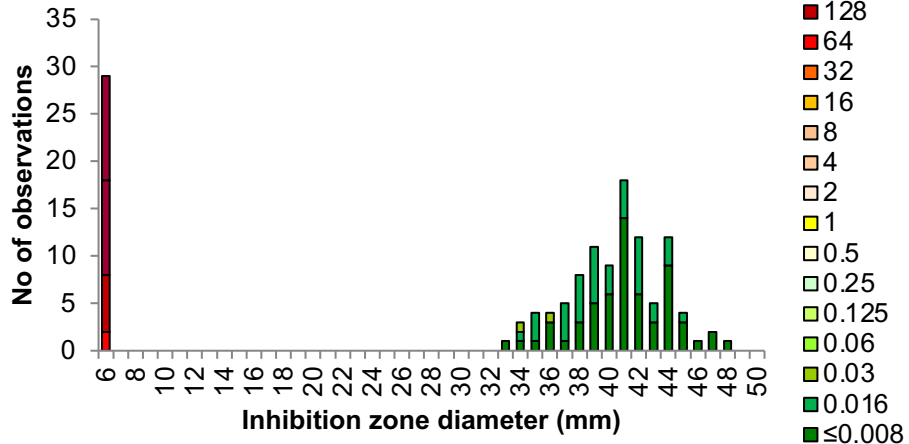


Clindamycin / *Prevotella* spp
International MIC Distribution - Reference Database 2020-02-18



MIC distributions include collated data from multiple sources, geographical areas and time periods and can never be used to infer rates of resistance
Epidemiological cut-off (ECOFF): -
Wildtype (WT) organisms: -
88 observations

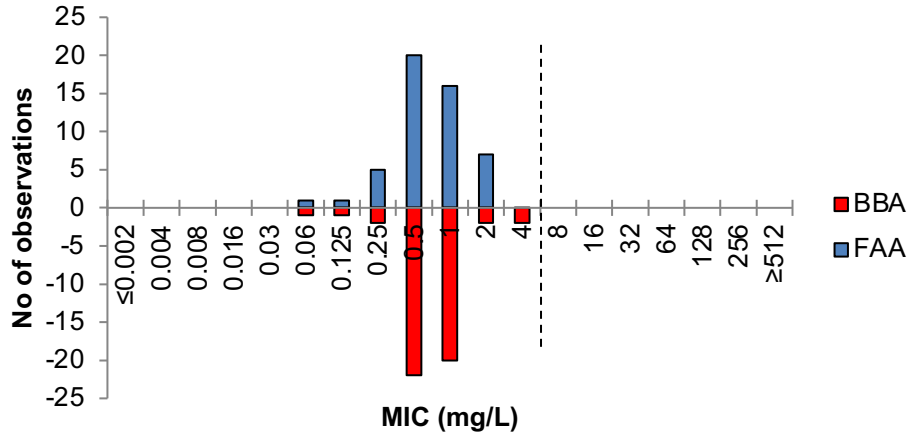
Clindamycin 2 µg vs. FAA MIC
Prevotella spp., 50 isolates (129 correlates)



Metronidazole

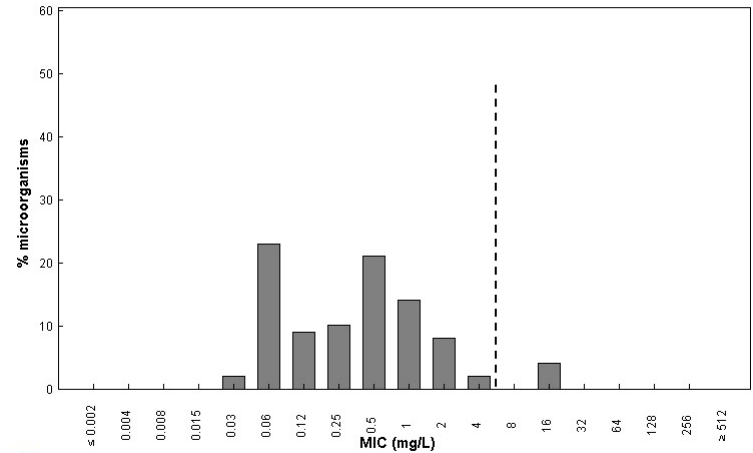
Proposed breakpoints: 4/4 mg/L

Metronidazole MIC FAA vs. BBA
***Prevotella* spp., 50 isolates**



Metronidazole / *Prevotella* spp
International MIC Distribution - Reference Database 2020-02-18

MIC distributions include collated data from multiple sources, geographical areas and time periods and can never be used to infer rates of resistance



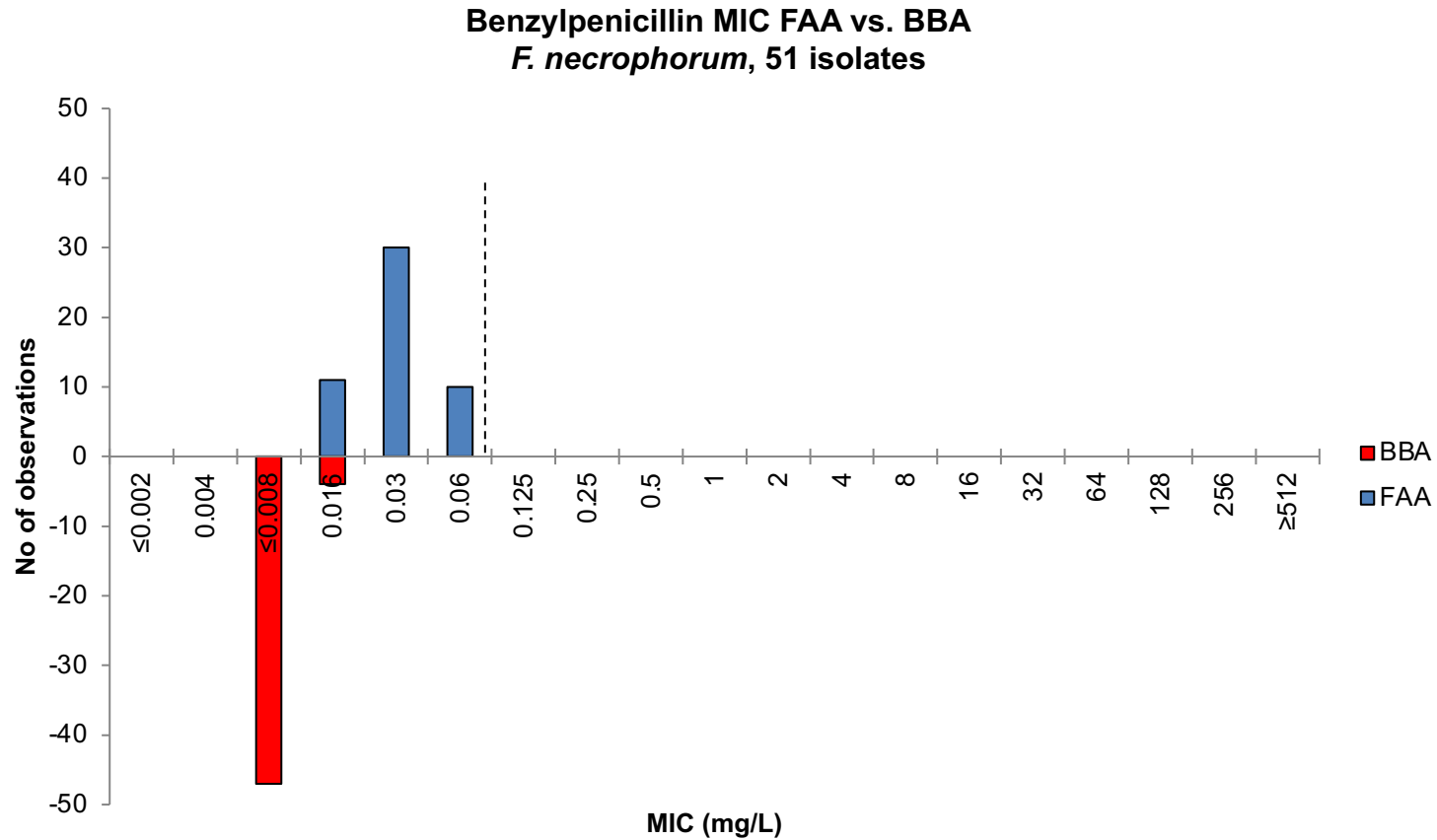
MIC
Epidemiological cut-off (ECOFF): -
Wildtype (WT) organisms:

725 observations (2 data sources)

Fusobacterium necrophorum

Benzylpenicillin

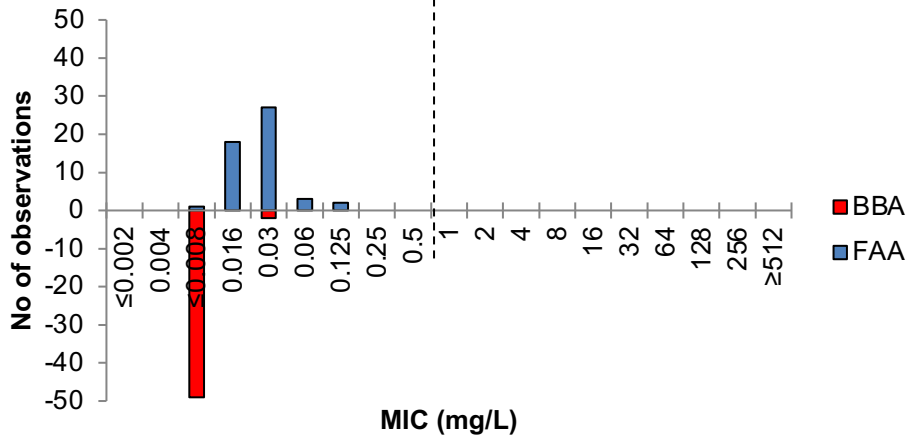
Proposed breakpoints: 0.06/0.06 mg/L



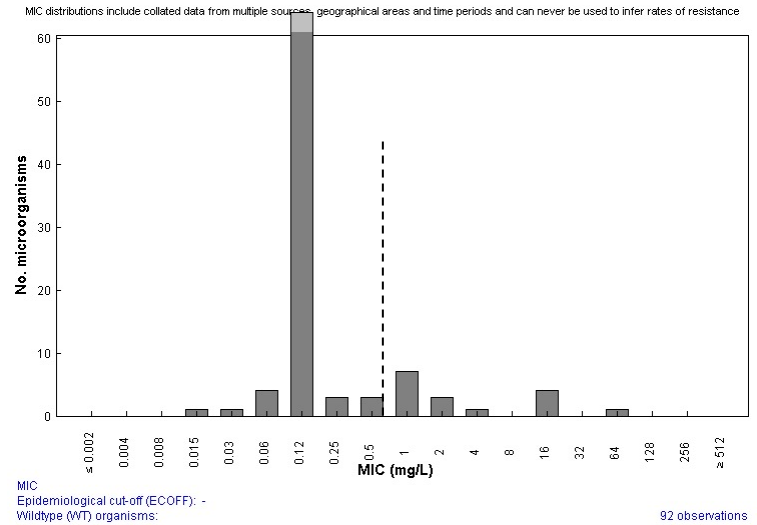
Piperacillin-tazobactam

Proposed breakpoints: 0.5/0.5 mg/L

Piperacillin-tazobactam MIC FAA vs. BBA
F. necrophorum, 51 isolates



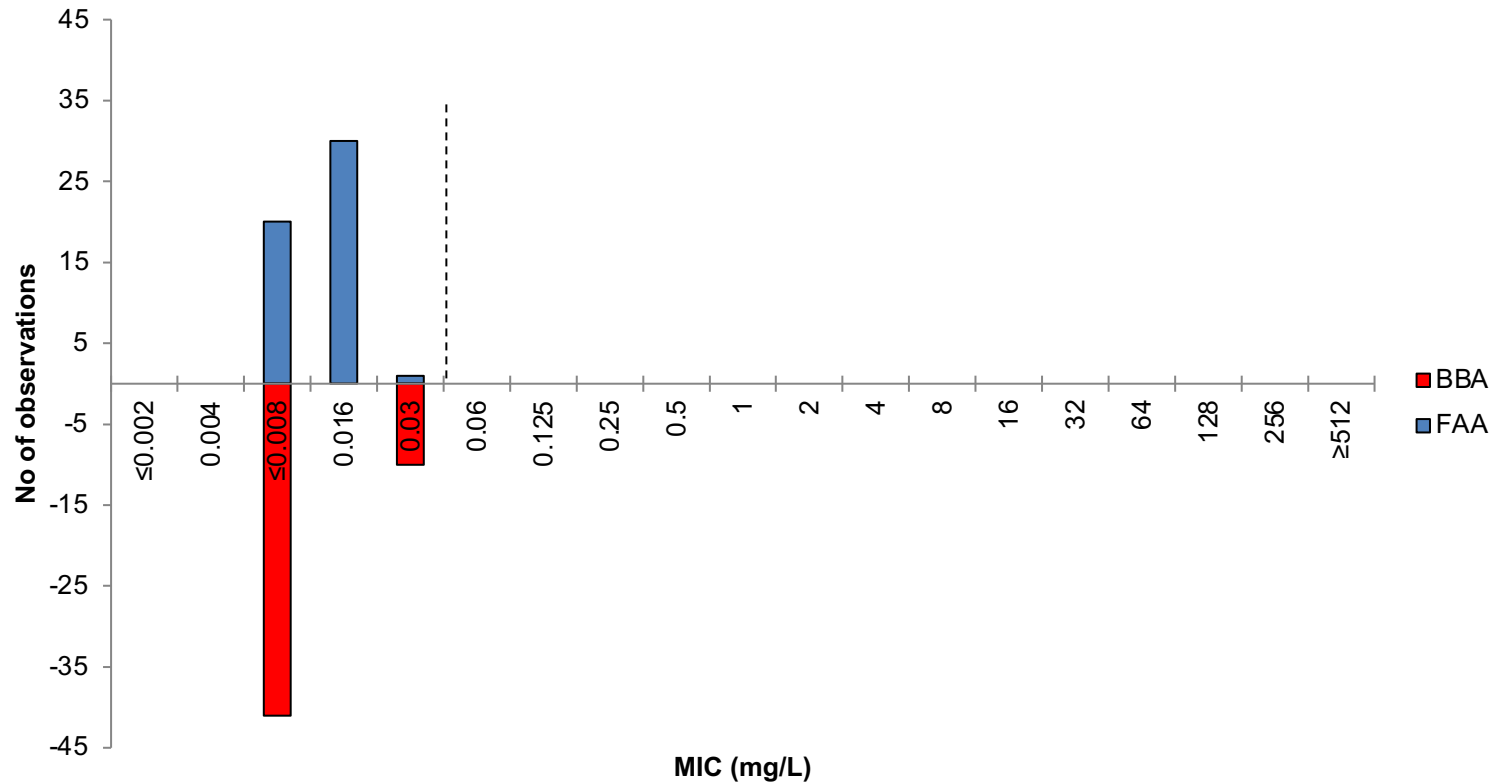
Piperacillin-tazobactam/ *Fusobacterium* spp
International MIC Distribution - Reference Database 2020-02-18



Meropenem

Proposed breakpoints: 0.03/0.03 mg/L

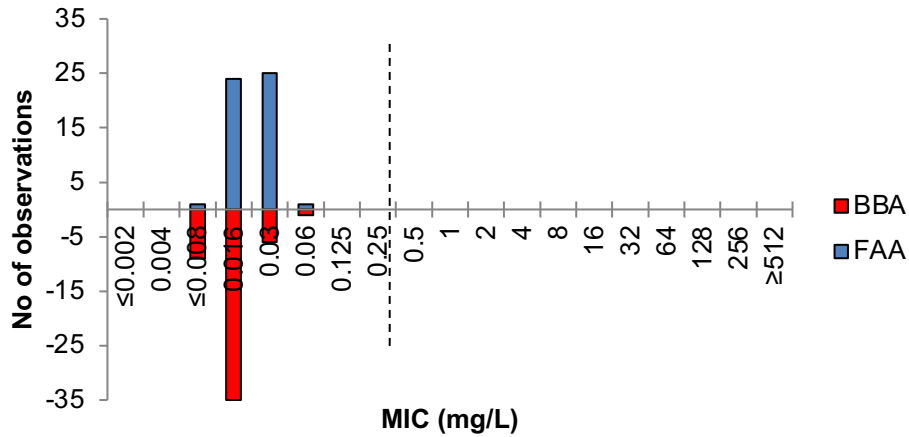
Meropenem MIC FAA vs. BBA
F. necrophorum, 51 isolates



Clindamycin

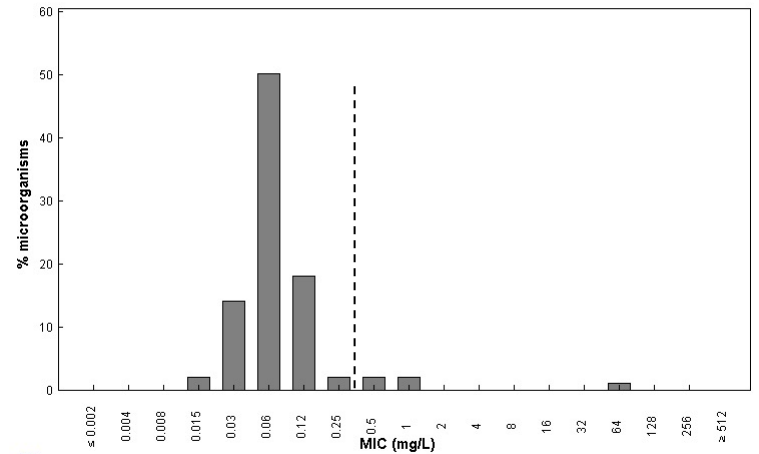
Proposed breakpoints: 0.25/0.25 mg/L

Clindamycin MIC FAA vs. BBA
F. necrophorum, 51 isolates



Clindamycin *Fusobacterium spp*
International MIC Distribution - Reference Database 2020-02-18

MIC distributions include collated data from multiple sources, geographical areas and time periods and can never be used to infer rates of resistance



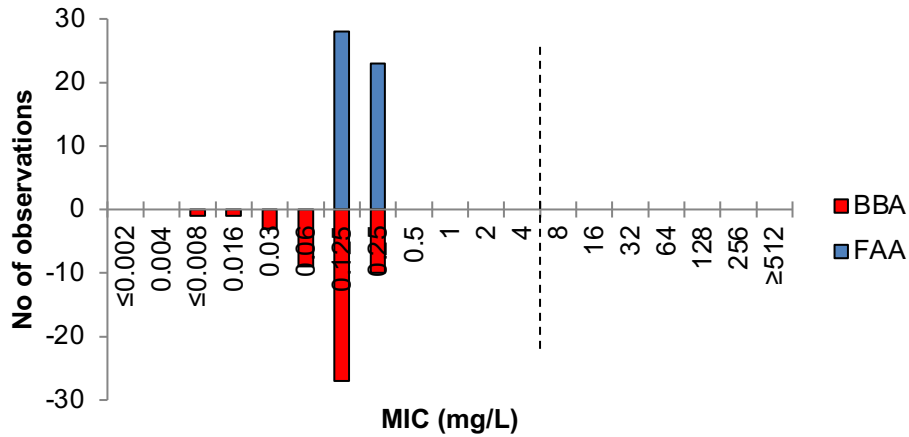
MIC
Epidemiological cut-off (ECOFF): -
Wildtype (WT) organisms:

206 observations (2 data sources)

Metronidazole

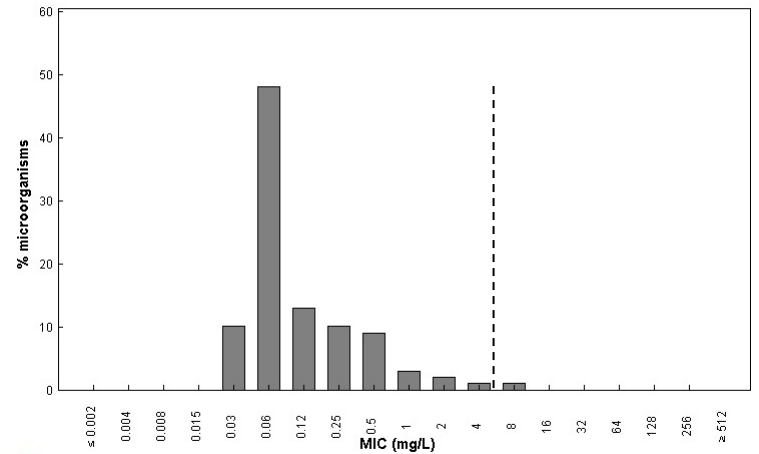
Proposed breakpoints: 0.5/0.5 mg/L

Metronidazole MIC FAA vs. BBA
F. necrophorum, 51 isolates



Metronidazole (*Fusobacterium spp*)
International MIC Distribution - Reference Database 2020-02-18

MIC distributions include collated data from multiple sources, geographical areas and time periods and can never be used to infer rates of resistance



MIC
Epidemiological cut-off (ECOFF): -
Wildtype (WT) organisms:

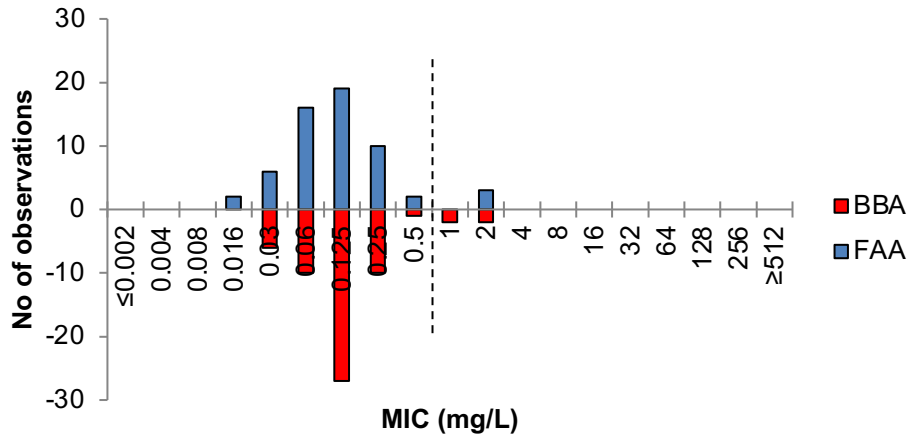
166 observations

Clostridium perfringens

Benzylpenicillin

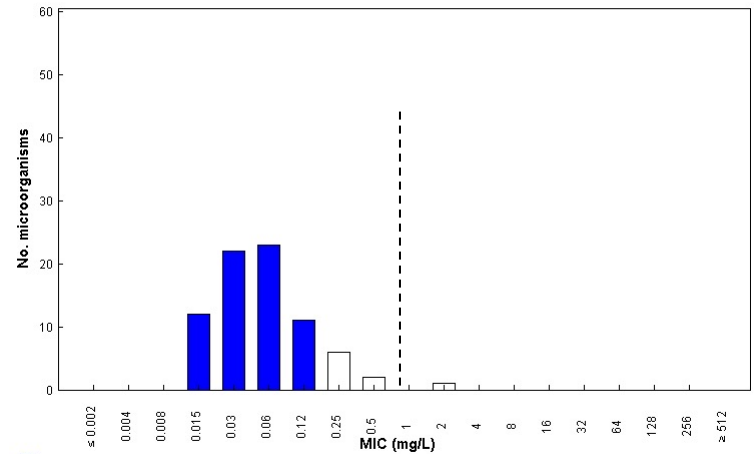
Proposed breakpoints: 0.5/0.5 mg/L

**Benzylpenicillin MIC FAA vs. BBA
C. perfringens, 58 isolates**



**Benzylpenicillin / *Clostridium perfringens*
International MIC Distribution - Reference Database 2020-02-18**

MIC distributions include collated data from multiple sources, geographical areas and time periods and can never be used to infer rates of resistance



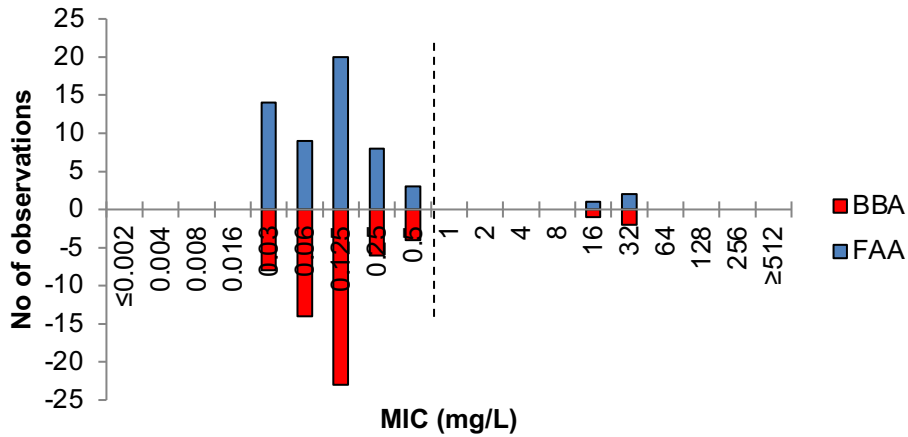
MIC
Epidemiological cut-off (ECOFF): 0.125 mg/L
Wildtype (WT) organisms: ≤ 0.125 mg/L

77 observations (3 data sources)

Piperacillin-tazobactam

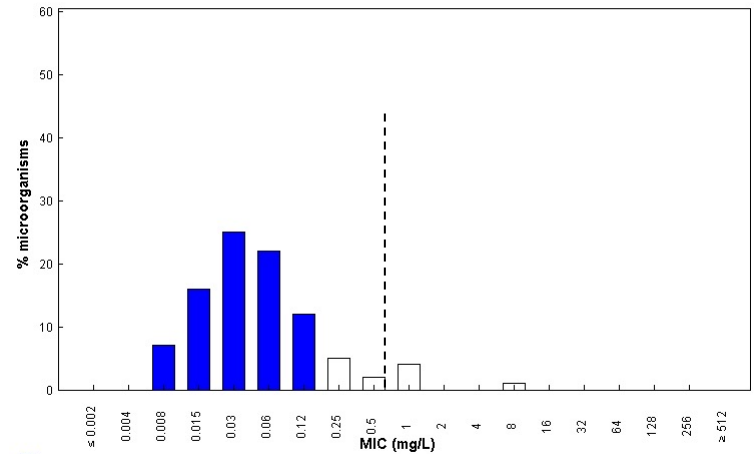
Proposed breakpoints: 0.5/0.5 mg/L

Piperacillin-tazobactam MIC FAA vs. BBA
C. perfringens, 58 isolates



Piperacillin-tazobactam / Clostridium perfringens
International MIC Distribution - Reference Database 2020-02-18

MIC distributions include collated data from multiple sources, geographical areas and time periods and can never be used to infer rates of resistance



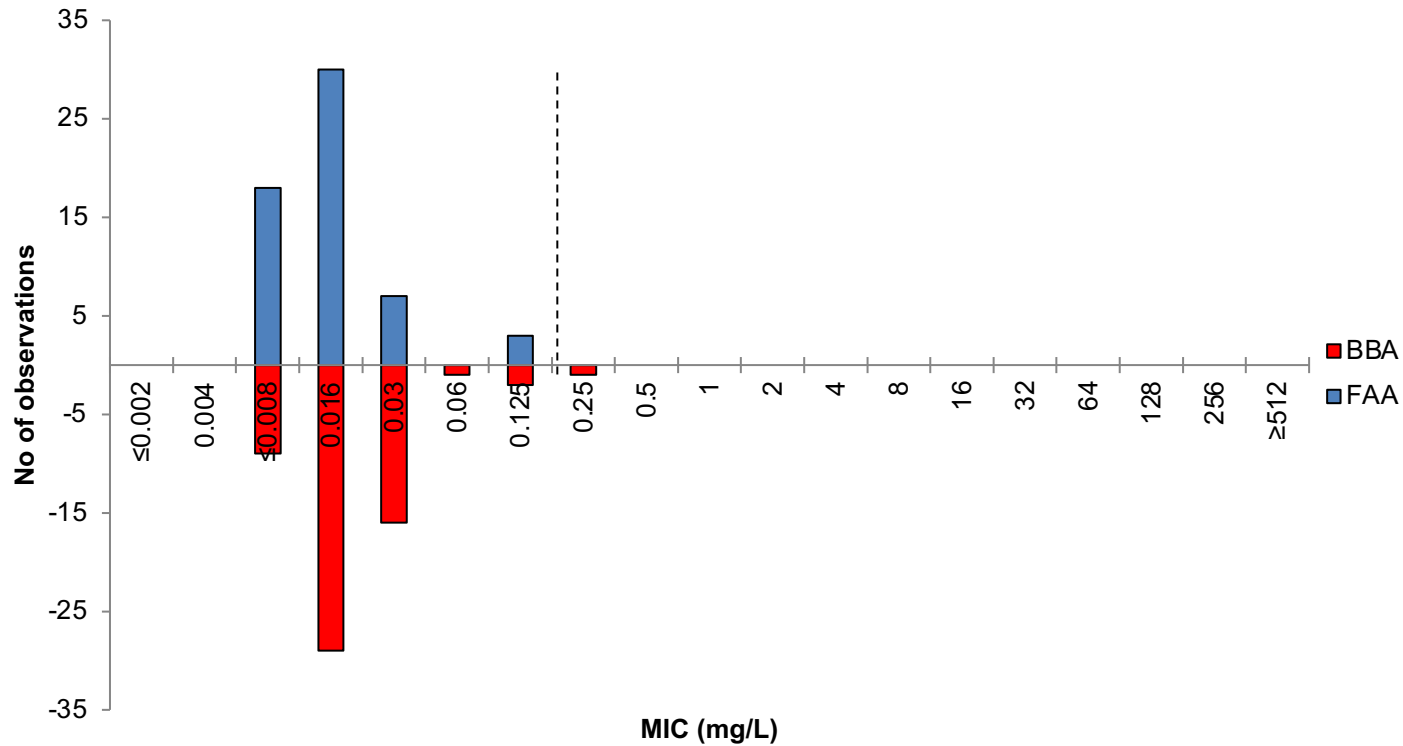
MIC
Epidemiological cut-off (ECOFF): 0.125 mg/L
Wildtype (WT) organisms: ≤ 0.125 mg/L

144 observations (3 data sources)

Meropenem

Proposed breakpoints: 0.125/0.125 mg/L

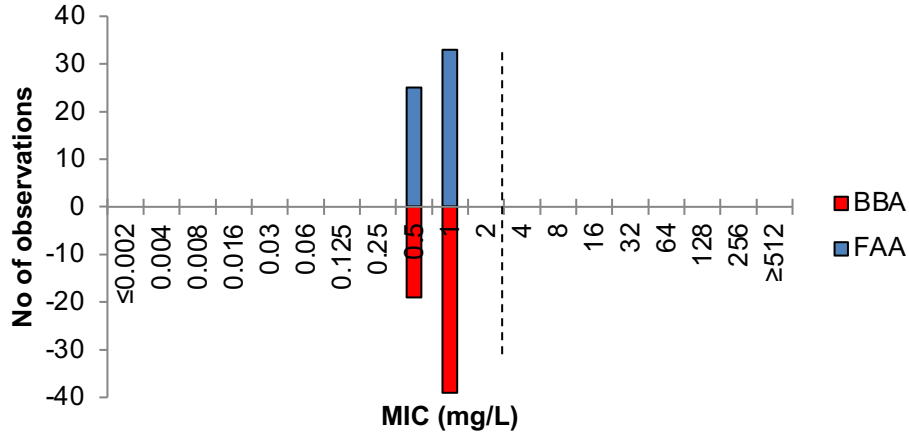
Meropenem MIC FAA vs. BBA
C. perfringens, 58 isolates



Vancomycin

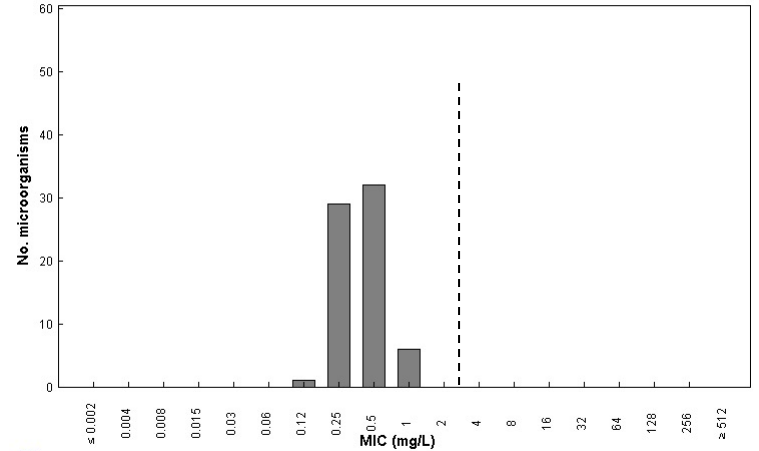
Proposed breakpoints: 2/2 mg/L

Vancomycin MIC FAA vs. BBA
C. perfringens, 58 isolates



Vancomycin Clostridium spp
International MIC Distribution - Reference Database 2020-02-18

MIC distributions include collated data from multiple sources, geographical areas and time periods and can never be used to infer rates of resistance



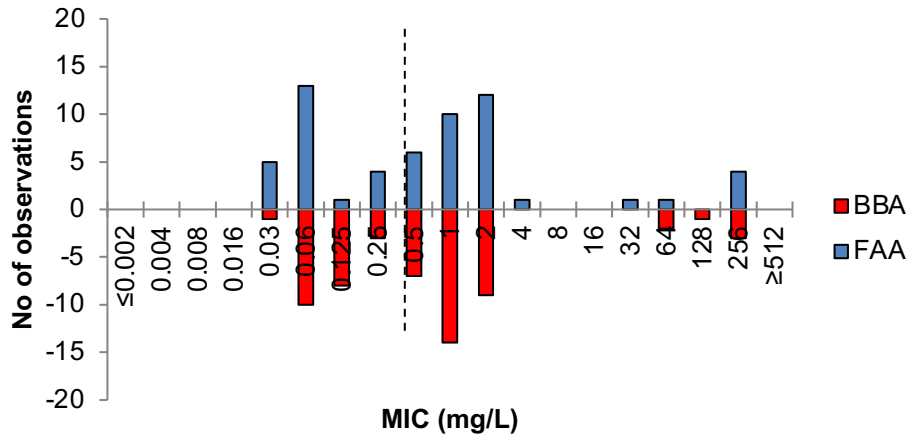
MIC
Epidemiological cut-off (ECOFF): -
Wildtype (WT) organisms:

68 observations

Clindamycin

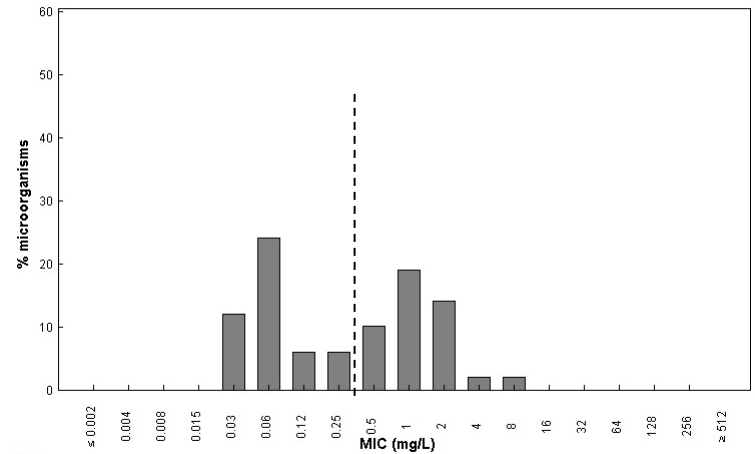
Current breakpoints: 0.25/0.25 mg/L

**Clindamycin MIC FAA vs. BBA
C. perfringens, 58 isolates**



**Clindamycin / Clostridium perfringens
International MIC Distribution - Reference Database 2020-02-18**

MIC distributions include collated data from multiple sources, geographical areas and time periods and can never be used to infer rates of resistance



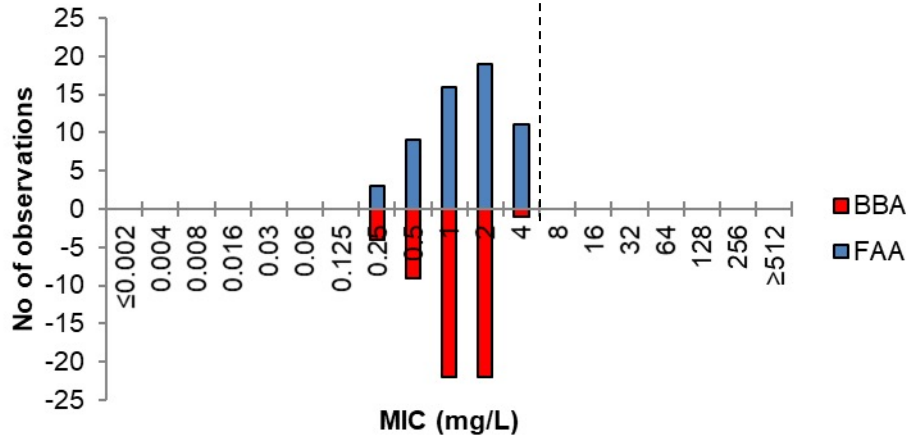
MIC
Epidemiological cut-off (ECOFF): -
Wildtype (WT) organisms:

148 observations (3 data sources)

Metronidazole

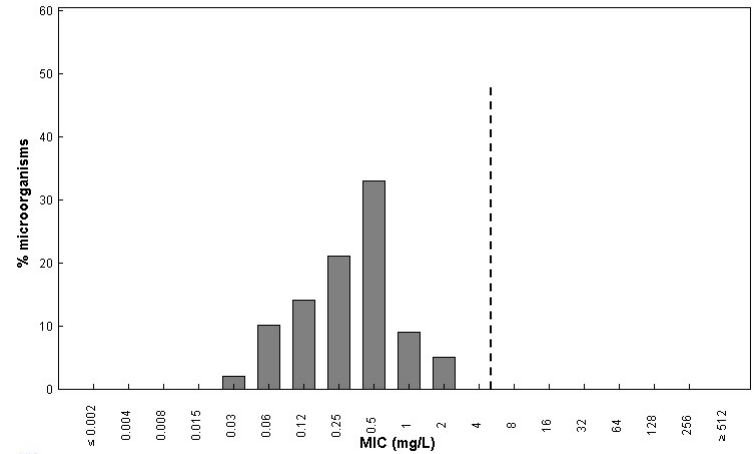
Proposed breakpoints: 4/4 mg/L

**Metronidazole MIC FAA vs. BBA
C. perfringens, 58 isolates**



**Metronidazole / Clostridium spp
International MIC Distribution - Reference Database 2020-02-18**

MIC distributions include collated data from multiple sources, geographical areas and time periods and can never be used to infer rates of resistance



MIC
Epidemiological cut-off (ECOFF): -
Wildtype (WT) organisms:

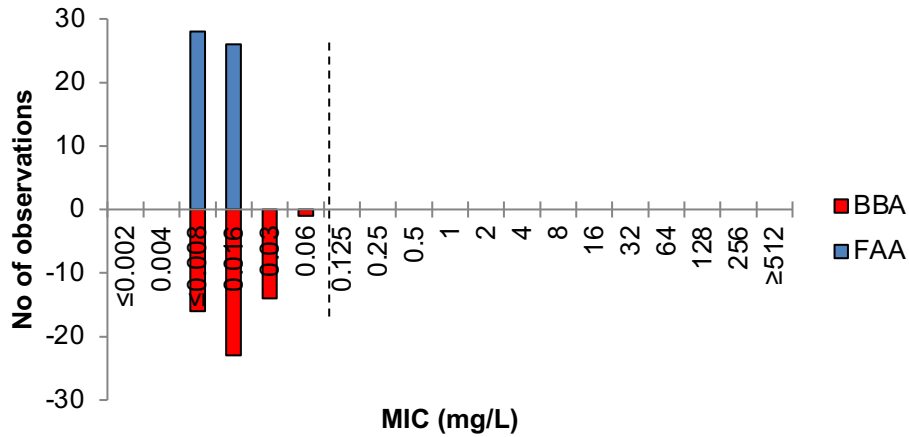
145 observations (4 data sources)

Cutibacterium acnes

Benzylpenicillin

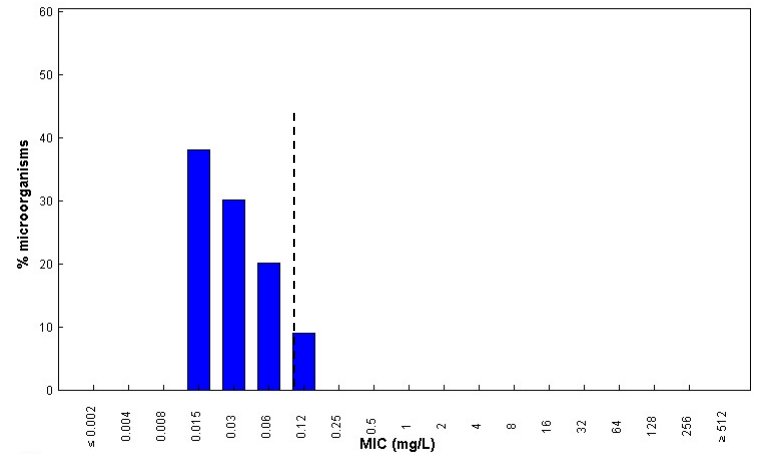
Proposed breakpoints: 0.06/0.06 mg/L

Benzylpenicillin MIC FAA vs. BBA
***C. acnes*, 54 isolates**



Benzylpenicillin / *Propionibacterium acnes*
International MIC Distribution - Reference Database 2020-02-18

MIC distributions include collated data from multiple sources, geographical areas and time periods and can never be used to infer rates of resistance



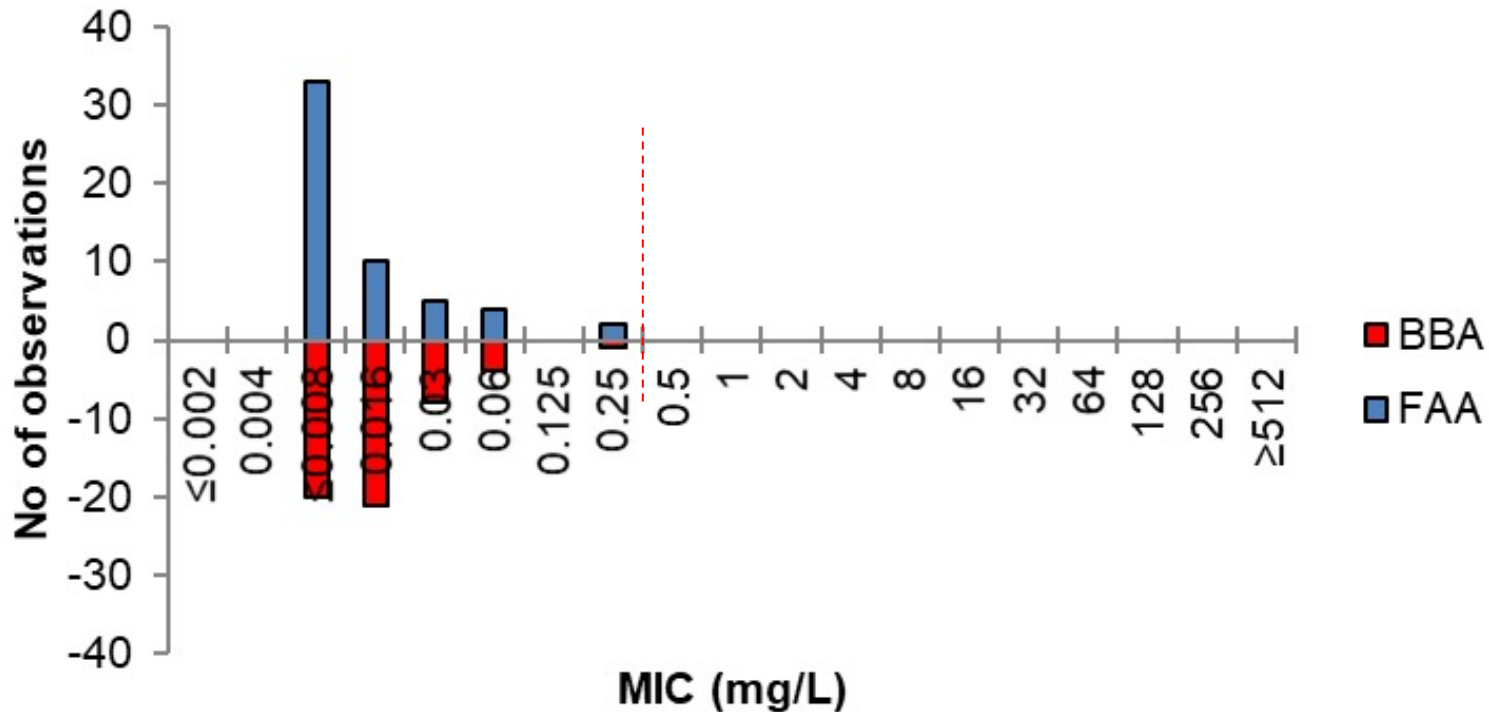
MIC
Epidemiological cut-off (ECOFF): 0.125 mg/L
Wildtype (WT) organisms: ≤ 0.125 mg/L

303 observations

Piperacillin-tazobactam

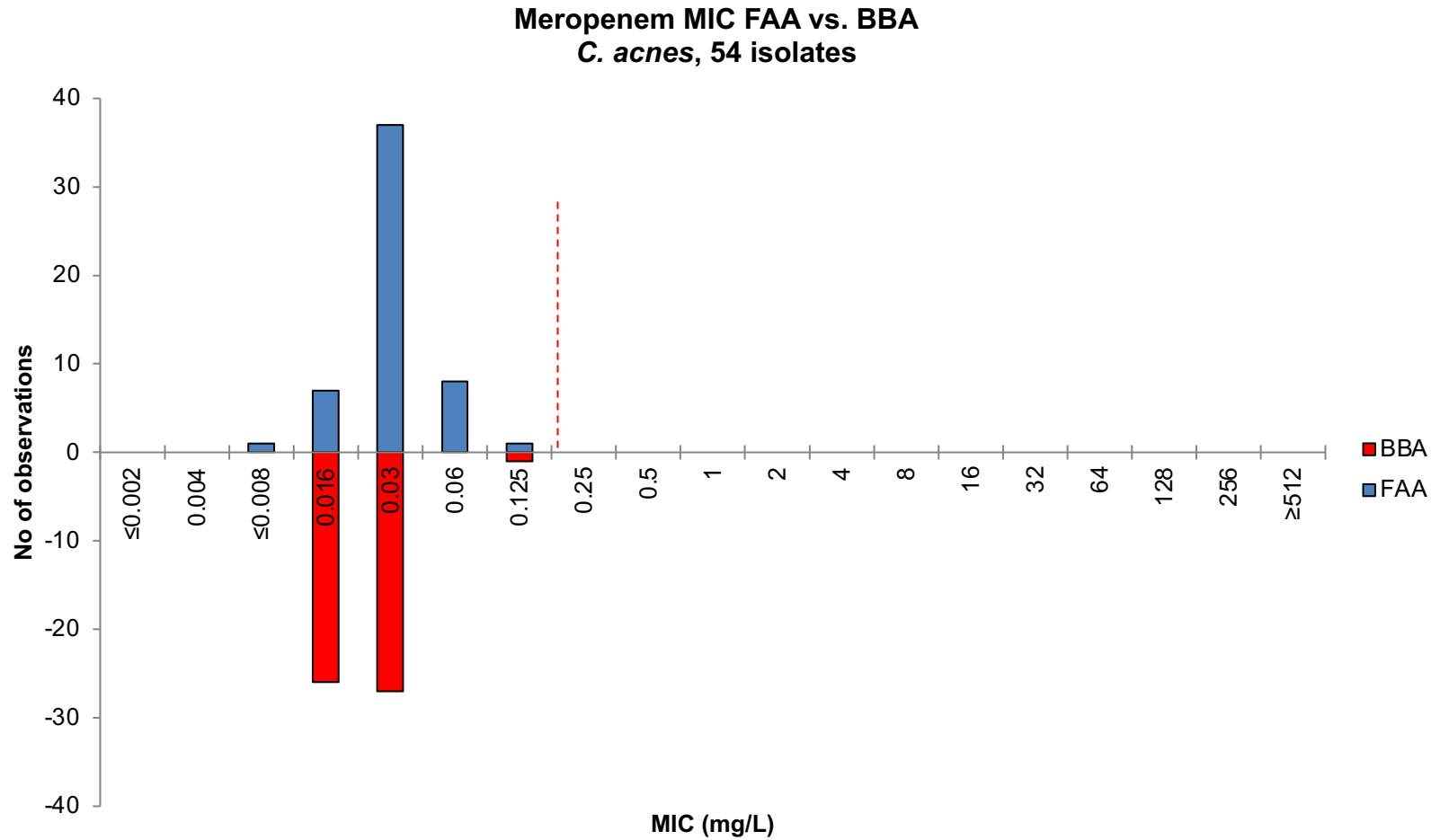
Proposed breakpoints: 0.25/0.25 mg/L

Piperacillin-tazobactam MIC FAA vs. BBA
C. acnes, 54 isolates



Meropenem

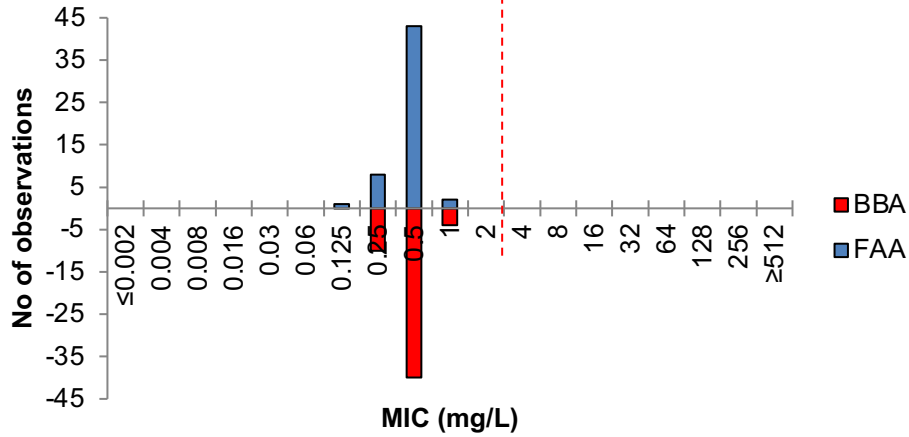
Proposed breakpoints: 0.125/0.125 mg/L



Vancomycin

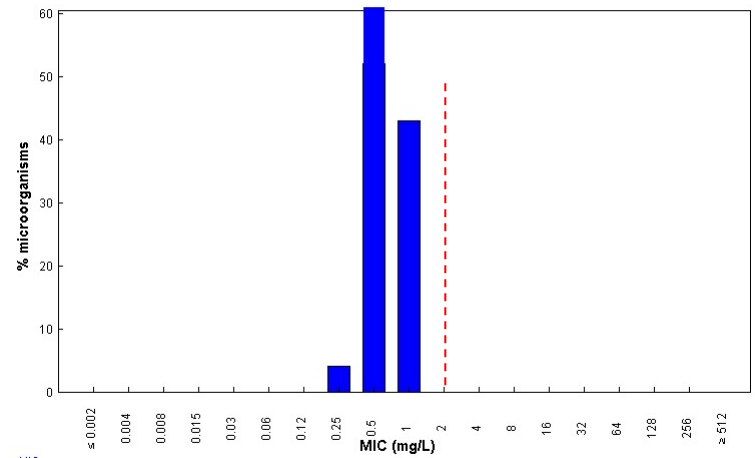
Proposed breakpoints: 2/2 mg/L

**Vancomycin MIC FAA vs. BBA
C. acnes, 54 isolates**



**Vancomycin / Propionibacterium acnes
International MIC Distribution - Reference Database 2020-02-18**

MIC distributions include collated data from multiple sources, geographical areas and time periods and can never be used to infer rates of resistance



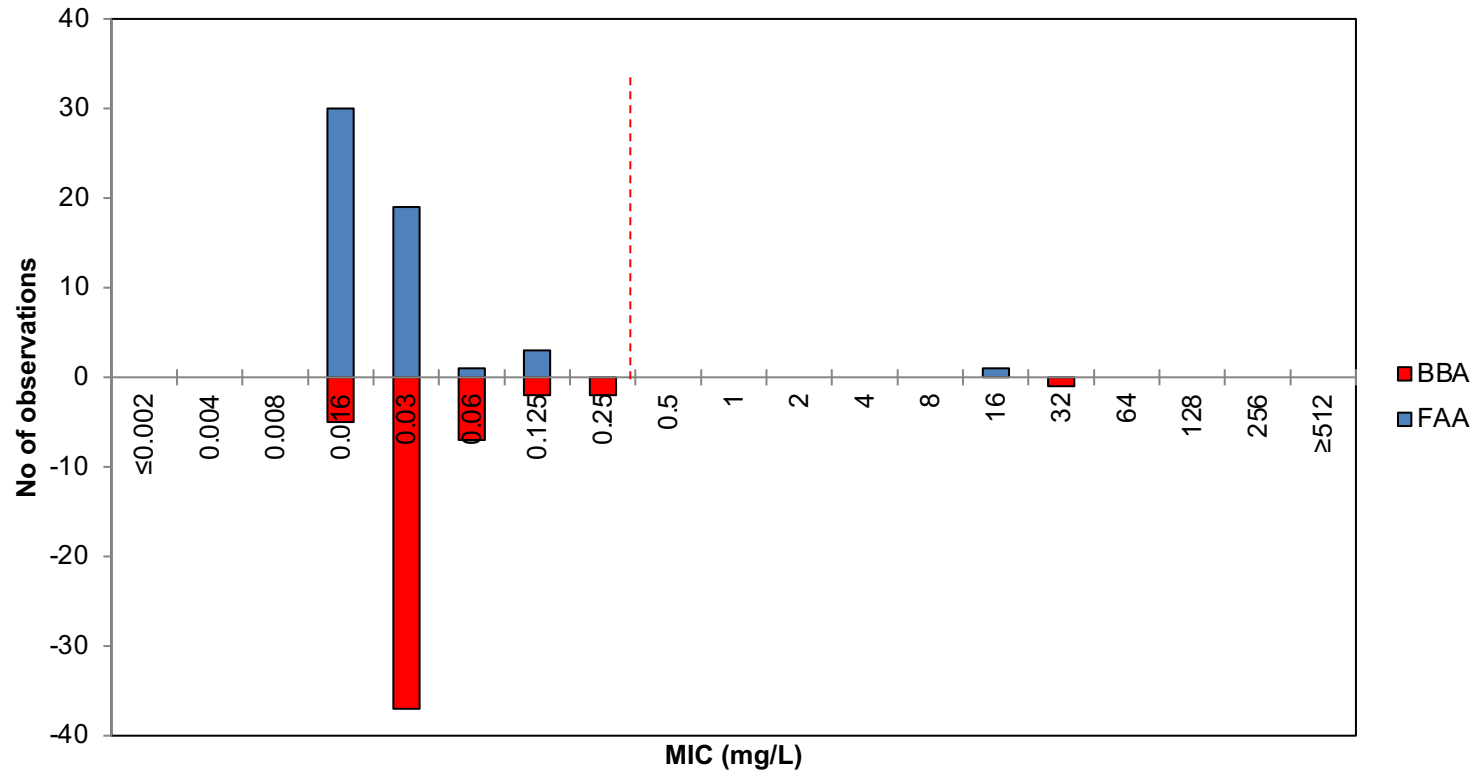
MIC
Epidemiological cut-off (ECOFF): 2 mg/L
Wildtype (WT) organisms: ≤ 2 mg/L

303 observations

Clindamycin

Proposed breakpoints: 0.25/0.25 mg/L

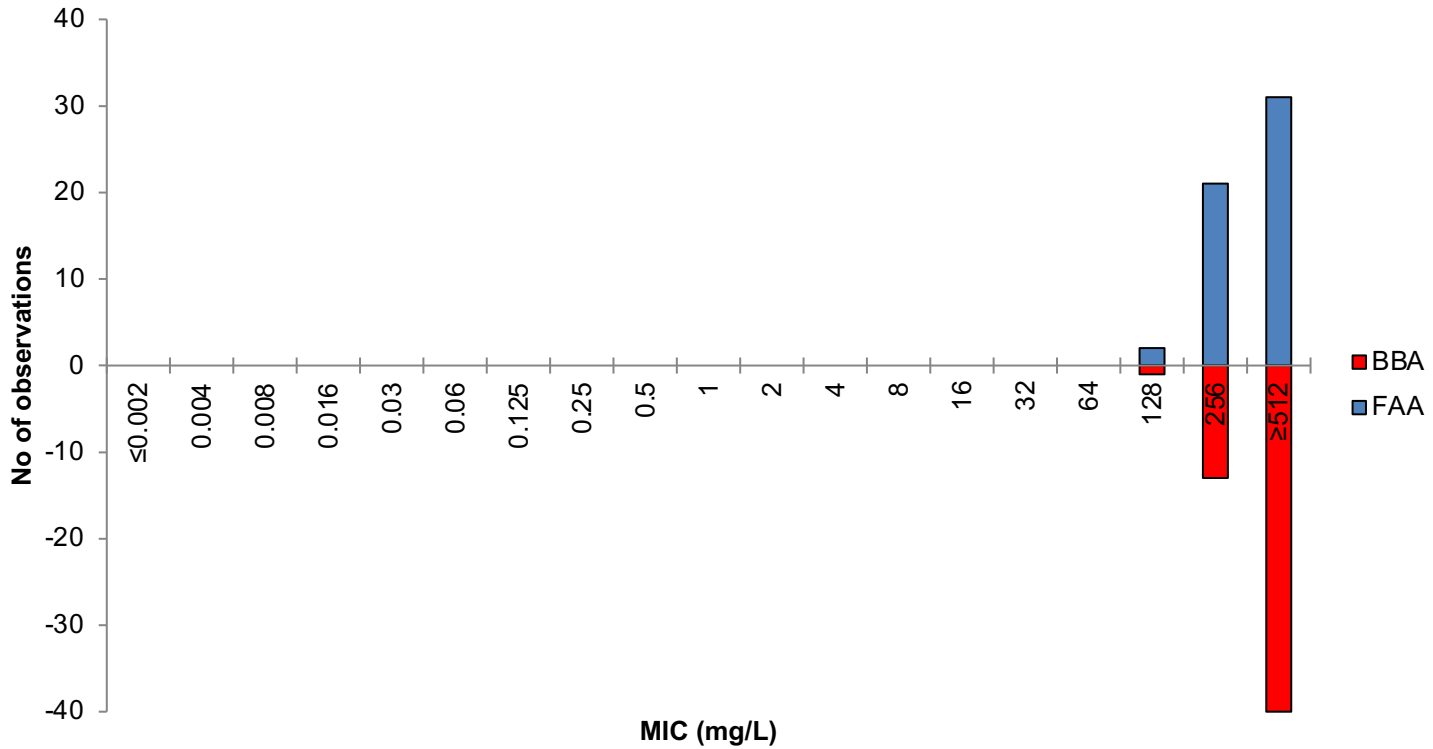
Clindamycin MIC FAA vs. BBA
C. acnes, 54 isolates



Metronidazole

Proposed breakpoints: -

Metronidazole MIC FAA vs. BBA
C. acnes, 54 isolates



45 degree analysis FAA vs. BBA

All species aggregated

Benzylpenicillin

Agar dilution on FAA vs. Brucella

		Brucella																
		<0.008	0.016	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	≥512
FAA	<0.008	14	12	2														
	0.016	11	13	15	1													
	0.03	29	1	8	8													
	0.06	9	1	3	9	17												
	0.125				2	17	5											
	0.25					3	7											
	0.5							1	1									
	1								2	1	1							
	2								1	5	2							
	4										2	1						
	8										1	3	2					
	16												1					
	32													1				
	64														1			
	128															1		
	256																1	
≥512																	1	

MICs identical 83/213 39%

MIC 1 dilution higher or lower 87/213 41%

MICs within ±1 dilution step 170/213 80%

Isolates with lower MICs on Brucella agar are *F. necrophorum*, who are known to grow poorer on Brucella.

Piperacillin-tazobactam

Agar dilution on FAA vs. Brucella

		Brucella																
		<0.008	0.016	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	≥512
FAA	<0.008	67	16															
	0.016	18	6	5														
	0.03	29		13	8													
	0.06	3		2	9	5												
	0.125	2			4	18	4											
	0.25			1		4	7	4										
	0.5						2	4	2									
	1							1	4	5								
	2									2	1							
	4									4	3							
	8										2							
	16										1		3					
	32													2				
	64																	
	128																	1
	256																	1
≥512																		

MICs identical 139/263 53%

MIC 1 dilution higher or lower 87/263 33%

MICs within ±1 dilution step 226/263 86%

Isolates with lower MICs on Brucella agar are *F. necrophorum*, who are known to grow poorer on Brucella.

Meropenem

Agar dilution on FAA vs. Brucella

Gram-positive organisms only

		Brucella																
		<0.008	0.016	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	≥512
FAA	<0.008	28	11	1														
	0.016	21	23	28	1													
	0.03	1	23	33	5													
	0.06		1	9	18	3												
	0.125			1	7	6	2											
	0.25					6	10											
	0.5						2											
	1							2	1	1								
	2									7	2							
	4										1	4						
	8										1							
	16												1					
	32																	
	64																	
	128															2		
	256																	
≥512																	1	

MICs identical 131/263 50%

MIC 1 dilution higher or lower 127/263 48%

MICs within ±1 dilution step 258/263 98%

Vancomycin

Agar dilution on FAA vs. Brucella

		Brucella																
		<0.008	0.016	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	≥512
FAA	<0.008																	
	0.016																	
	0.03																	
	0.06																	
	0.125																	
	0.25																	
	0.5																	
	1																	
	2																	
	4																	
	8																	
	16																	
	32																	
	64																	
	128																	
	256																	
≥512																		

MICs identical 80/112 71%
MIC 1 dilution higher or lower 32/112 29%
 MICs within ±1 dilution step 112/112 100%

Clindamycin

Agar dilution on FAA vs. Brucella

		Brucella																	
		<0.008	0.016	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	≥512	
FAA	<0.008	7	18																
	0.016	3	38	24	2														
	0.03	5	15	20	9	1													
	0.06			1	7	8													
	0.125				1	6	3												
	0.25						4	1											
	0.5						2	8	7	2									
	1							3	10	11									
	2								4	9	1								
	4									1	1								
	8																		
	16														1				
	32															1			
	64														1	1			
	128																3		1
	256																7	4	
≥512																		12	

MICs identical 130/263 49%

MIC 1 dilution higher or lower 122/263 46%

MICs within ±1 dilution step 252/263 96%

Metronidazole

Agar dilution on FAA vs. Brucella

		Brucella																
		<0.008	0.016	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	≥512
FAA	<0.008																	
	0.016																	
	0.03																	
	0.06																	
	0.125	1	1	2	5	20	1											
	0.25			1	4	12	16	4										
	0.5						5	32	8	1								
	1							9	41	1								
	2								11	13	3							
	4								1	9	4	1						
	8																	
	16																	
	32																	
	64																	
	128																	
	256																	
≥512																		

MICs identical 166/263 63%

MIC 1 dilution higher or lower 85/263 32%

MICs within ±1 dilution step 251/263 95%

Isolates with lower MICs on Brucella agar are *F. necrophorum*, who are known to grow poorer on Brucella.

Comments

- Differences seen for *Fusobacterium necrophorum* results may be due to the lack of cystine in the Brucella plates. Cysteine affects the growth of *Fusobacterium* (as published by John Brazier and followed up by UKARU, Cardiff).