

## Corynebakterier - mikrobiologi og klinik

*Kristian Schønning*

Department of Clinical Microbiology 445  
Hvidovre Hospital  
DK-2650 Hvidovre

# Corynebakterier – difterigene og non-difterigene

## Corynebacterium (>85 arter):

Gram+, irregulære stave

Katalase+

Normal hud og slimhinde flora

## Difterigene:

*C. diphtheriae* (4 biovarer)

*C. ulcerans*

*C. pseudotuberculosis*

Alle kan indeholde det fag-bårne difteri-toxin gen

Alle er **Pyrazinamidase-negative**.

## Non-difterigene:

*Corynebacterium* spp.

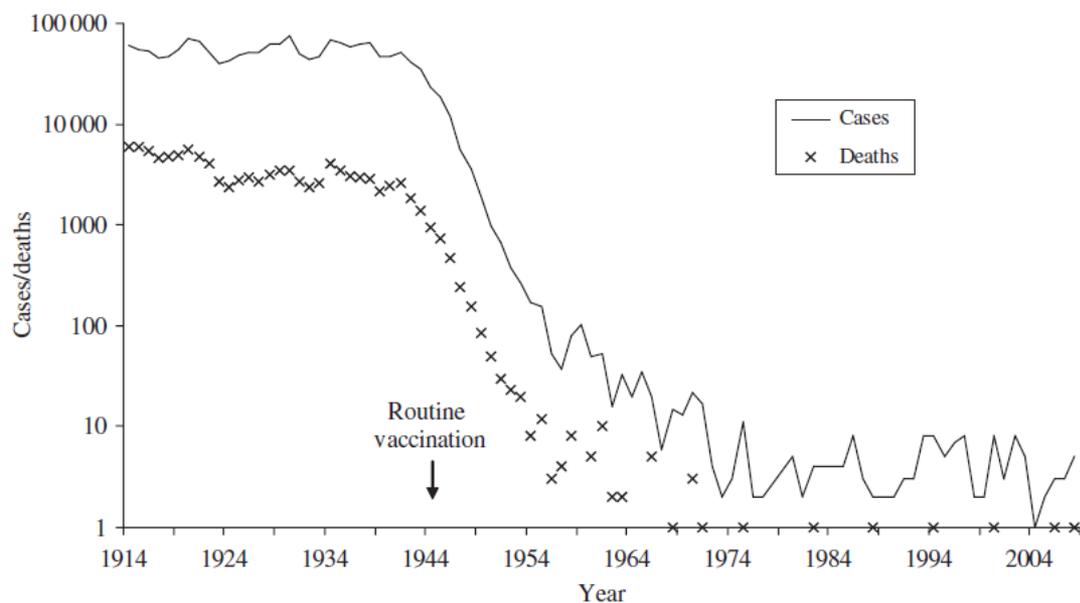
Bakteriæmi, endocardit,

Fremmedlegeme-infektion

Opportunistiske patogener

Med undtagelse af *C. macginleyi* er alle  
**Pyrazinamidase-positive**.

# Difteri i England



**Fig. 1.** Diphtheria notifications and deaths in England and Wales 1914–2008 (notifications up to 1985, laboratory-confirmed cases 1986–2008).

From Wagner et al. (2010);  
Epidemiol. Infect. 138:1519-1530.

# 102 toxigene isolater fra UK:

42 *C. diphtheriae*, 59 *C. ulcerans*, 1 *C. pseudotuberculosis*

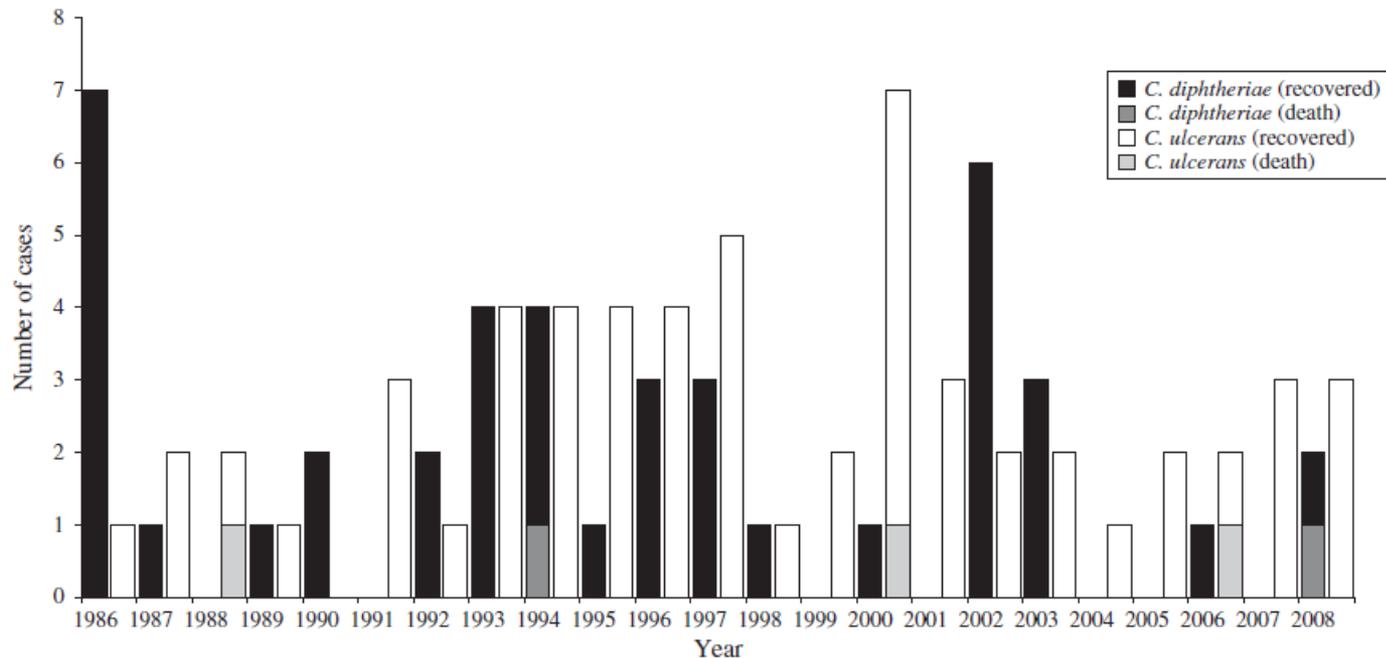


Fig. 2. Toxigenic cases of diphtheria and outcome in the UK, 1986–2008.

From Wagner et al. (2010);  
Epidemiol. Infect. **138**:1519-1530.

# Symptomatologi og vaccinationsstatus

Table 2. *Toxigenic C. diphtheriae* and *C. ulcerans* isolates by clinical presentation and immunization status

Immunization status at the time of infection/clinical presentation	<i>C. diphtheriae</i>				<i>C. ulcerans</i>				Grand total <i>C. diphtheriae</i> and <i>C. ulcerans</i> (%)		
	Fully immunized for age	Partially immunized	Vaccination history not known or not reported	Unimmunized	Total (%)	Fully immunized for age	Partially immunized	Vaccination history not known or not reported		Unimmunized	Total (%)
Classic respiratory presentation with adherent pseudomembrane observed in tonsils, pharynx, or larynx		1*		4* (includes 2 fatal cases)	5 (8%)		2	1	7 (includes 3 fatal cases)	10 (16%)	15 (12%)
Respiratory presentation with exudate†		2		1	3 (5%)	3			1	4 (6%)	7 (6%)
Respiratory presentation (sore throat) with no pseudomembrane or exudate	5	4	4	1	14 (23%)	16	1	16	4	37 (60%)	51 (41%)
Respiratory and cutaneous lesions‡			1	3	4 (6%)					—	4 (3%)
Cutaneous lesions	4	4	2	5	15 (24%)			6	2	8 (13%)	23 (19%)
Other (bacterial endocarditis)	1				1 (2%)					—	1 (1%)
Asymptomatic§	8		11	1	20 (32%)	2		1		3 (5%)	23 (19%)
<b>Total</b>	<b>18</b>	<b>11</b>	<b>18</b>	<b>15</b>	<b>62</b>	<b>21</b>	<b>3</b>	<b>24</b>	<b>14</b>	<b>62</b>	<b>124</b>

\* One patient from each of these groups also had cutaneous lesions but has been assigned to this group since this is the most serious presentation.

† Observation of tonsillar exudate, although not a solid membrane, could indicate the early stages of membrane formation.

‡ Toxigenic organism isolated from both sites.

§ May have been swabbed due to another illness or may be a contact (with no symptoms) of a confirmed case.

# Corynebakterier – difterigene og non-difterigene

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# Identifikation af Corynebakterier

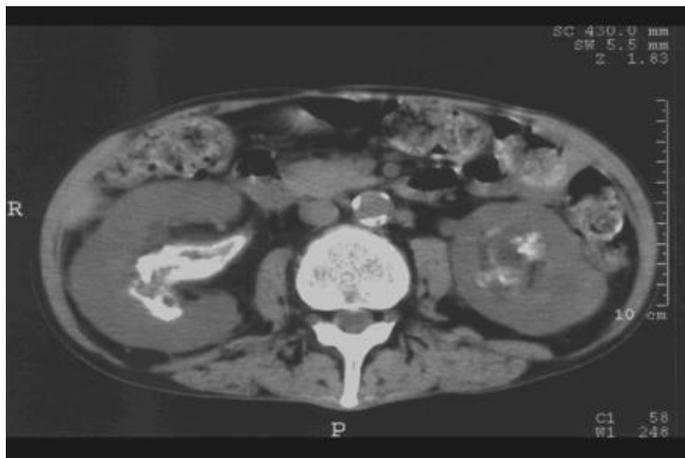
- Fænotypiske metoder, e.g.
  - API coryne panel; i et studium >30% misidentifikationer
  - VITEK 2 ANC kort.
- Genotypiske metoder
  - *rrn* (16S rRNA) (og evt. *rpoB*).
  - Flere species tæt relaterede; e.g. *C. ulcerans* og *C. pseudotuberculosis* <1% afstand.
- MALDI-TOF-MS
  - Diskriminerer difterogene spp. (99.1%; Konrad et al., 2010).
  - Identificerer non-difterogene spp. korrekt med undtagelse af *C. aurimocosum/ minutissimum* og *C. pseudodiphtheriticum/propinquum* (Bruker Biotyper; Alatoon et al., 2012).
- Fuldgenomsekventering

## *C. urealyticum*: Et overset urinvejspatogen?

Langsomt voksende; lipofil; inkubation >24h ofte nødvendig

Kraftig urease-danner; alkaniserer urin; danner struvit/apatit-sten

Ofte multiresistent



- Asymptomatisk bakteriuri (40%).
- Typisk bestående urinvejspatologi og immunsuppression (e.g. RTX)
- Akut UTI
- Kronisk UTI
- Encrusted cystitis/pyelitis

# Corynebakterier som luftvejspatogener?

- Nhan et al. (2012) identificerede 27 corynebakterier isoleret fra luftvejssekreter genetisk
  - 15/27 (56%) skønnedes inficerede
  - 8/15 (60%) *C. pseudodiphtheriticum*
  - 4/15 (27%) *C. striatum*
  - 2/15 (13%) *C. accolens*
- Diez-Aguilar et al. (2013) identificerede 10 ptt. (MALDI-TOF-MS)
  - 4/10 (40%) *C. pseudodiphtheriticum*
  - 4/10 (40%) *C. propinquum*
  - 2/10 (20%) *C. striatum*
- *C. pseudodiphtheriticum* adhærer, internaliseres og overlever intracellulært i humane epithel-cellelinier

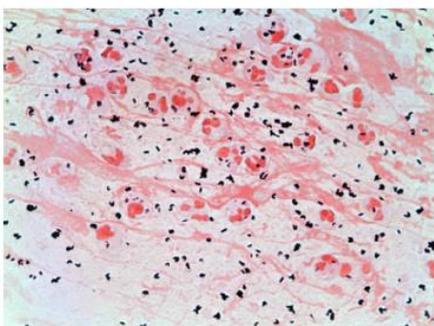


Fig. 1 Gram-stained sputum showing polymorphonuclear leucocytes and abundant Gram-positive bacilli extra- and intra-celularly located

## Konklusioner

- Corynebakterier kan være årsag til klinisk infektion; specielt hos disponerede individer
- Artsidentifikation kan være vejledende for klinisk tolkning af fund og behandling
- MALDI-TOF-MS giver mikrobiologiske laboratorier mulighed for korrekt identifikation
- Resistensforhold er artsafhængige og er ofte komplicerede.



# Resistensbestämning av Corynebakterier

Erika Matuschek

NordicAST Workshop 2013

# *Corynebacterium* spp.

- Inga brytpunkter
- Få artspezifika MIC-distributioner
  - Problem med artidentifiering
- Ingen lappdiffusionsmetod

# Resistensbestämning och artidentifiering av Corynebakterier

- Kliniska isolat av olika geografiskt ursprung
  - SENTRY-kollektionen (R. Jones, JMI Labs, USA)
  - Spanien (L. Martinez-Martinez, Spanien)
  - Kronobergs län
- Artidentifiering med Maldi-tof
- Resistensbestämning
  - Buljongspädning (BMD)
  - Etest
  - Lappdiffusion

# Validering av Maldi-tof MS

## EUCAST Laboratory for AST

Organism*	ID med Maldi-tof Växjö	Score-värde
<i>C. amycolatum</i>	<i>C. amycolatum</i> (12/12)	≥2.0
<i>C. jeikeium</i>	<i>C. jeikeium</i> (12/12)	≥2.0
<i>C. pseudodiphtheriticum</i>	<i>C. pseudodiphtheriticum</i> (11/12) <i>C. lipophile</i> grpe F1 (1/12)	≥2.0 1.85
<i>C. striatum</i>	<i>C. striatum</i> (12/12)	≥2.0
<i>C. urealyticum</i>	<i>C. urealyticum</i> (12/12)	≥2.0 (10/12) 1.91-1.98 (2/12)

\*Enligt API Coryne och/eller 16SrRNA eller tidigare utförd Maldi-tof.

### Meaning of Score Values

Range	Description
2.300-3.000	highly probable species identification
2.000-2.299	secure genus identification, probable species identification
1.700-1.999	probable genus identification
0.000-1.699	not reliable identification

## **Corynebacterium spp.**

C. striatum	76
C. amycolatum	63
C. jeikeium	35
C. pseudodiphtheriticum	20
C. urealyticum	18
C. afermentans	9
C. aurimucosum	8
C. propinquum	5
C. singulare	4
C. coyleae	3
C. imitans	3
C. striatum	2
C. glucuronolyticum	2
C. minutissimum	2
C. xerosis	2
C. diphtheriae	1
C. freneyi	1
C. mucifaciens	1
C. riegelii	1
C. simulans	1
C. ulcerans	1
<b>Total</b>	<b>258</b>

- 21 olika species
- 88 % score  $\geq 2.000$
- ID med score 1.700-1.999 bedömdes som riktiga om fyra upprepningar gav identiska resultat
- Score  $< 2.000$  vanligast för *C. afermentans* och *C. amycolatum*

# Resistensbestämning

Metod	Medium	Inokulat	Inkubering
BMD	MH-F-buljong	$5 \times 10^5$ cfu/mL	16-20 h (44 h) 35±1°C Luft (förseglade paneler)
Etest	MH-F	McFarland 0.5	16-20 h (44 h) 35±1°C 5% CO <sub>2</sub>
Lapp-diffusion	MH-F	McFarland 0.5	16-20 h (44 h) 35±1°C 5% CO <sub>2</sub>

**MH-F-buljong:** Mueller-Hintonbuljong med 5% lyserat hästblod och 20 mg/L β-NAD

# Inkuberingstid

- Lappdiffusion
  - 10% av isolaten inkuberades 44 h
    - *C. jeikeium* (6/35)
    - *C. pseudodiphtheriticum* (6/20)
    - *C. urealyticum* (9/18)
- MIC (broth microdilution)
  - 30% av isolaten inkuberades 44 h
    - *C. amycolatum* (18/63)
    - *C. jeikeium* (22/35)
    - *C. pseudodiphtheriticum* (8/20)
    - *C. urealyticum* (18/18)

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... Media preparation

## Preparation of MH plates and broth

EUCAST antimicrobial susceptibility testing is based on MH agar and MH broth, without supplements for non-fastidious organisms and with supplements for fastidious organisms (Streptococci, Haemophilus influenzae, Campylobacter and others). The plate and broth for fastidious organisms are named MH-F plates and MH-F broth.

 [Preparation of plates and broth for EUCAST AST.](#) (v 3.0, 2013)

 [Zubereitung der Medien](#) (v 2.0, 2012)

 [Preparación del medio para el estudio de sensibilidad con discos](#) (v 1.1, 2010)

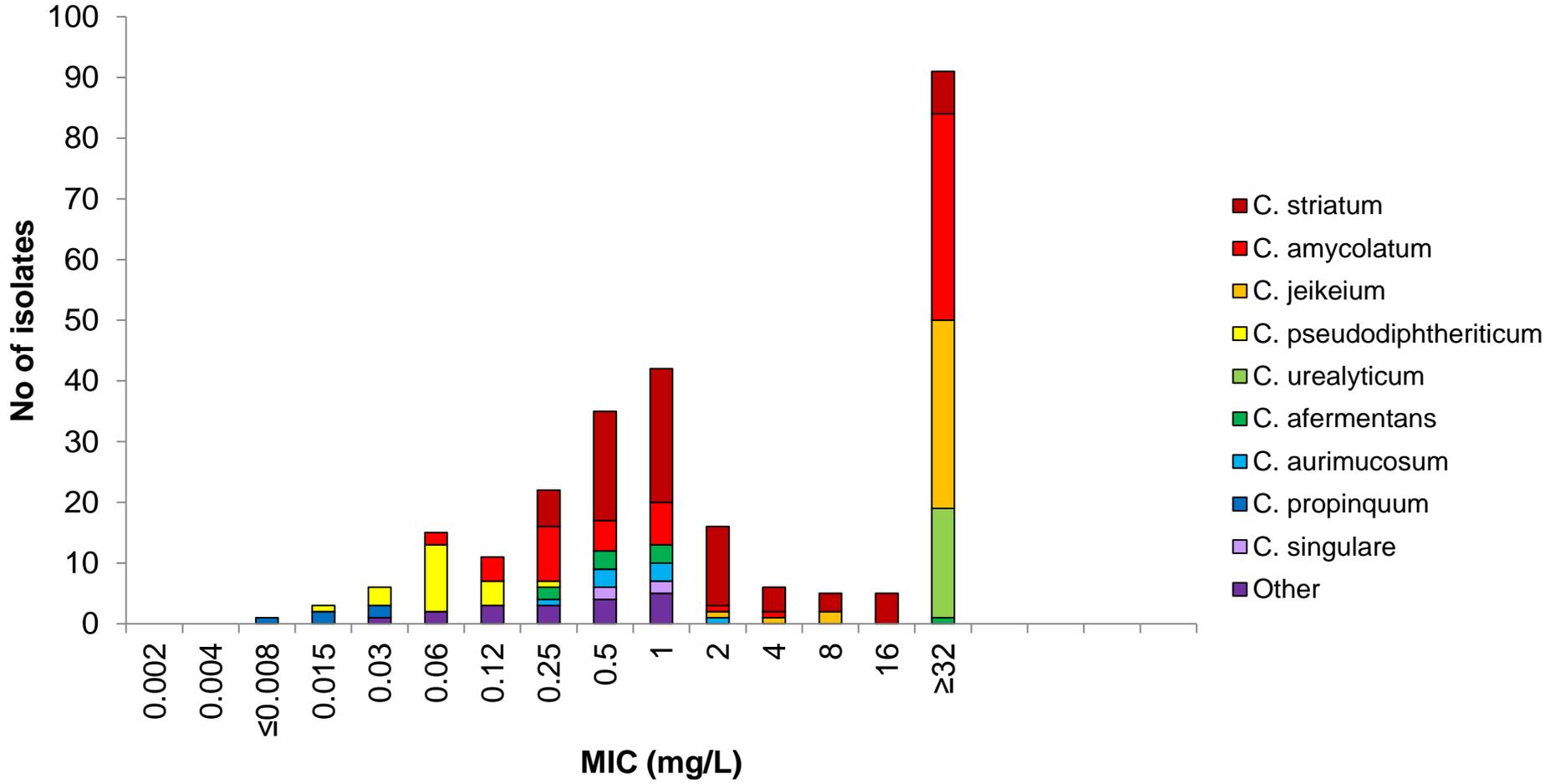
 [Méthodes EUCAST de diffusion en gélose et de détermination des CMI par microdilution en milieu liquide: préparation des milieux.](#) (v 2.0, 2012)

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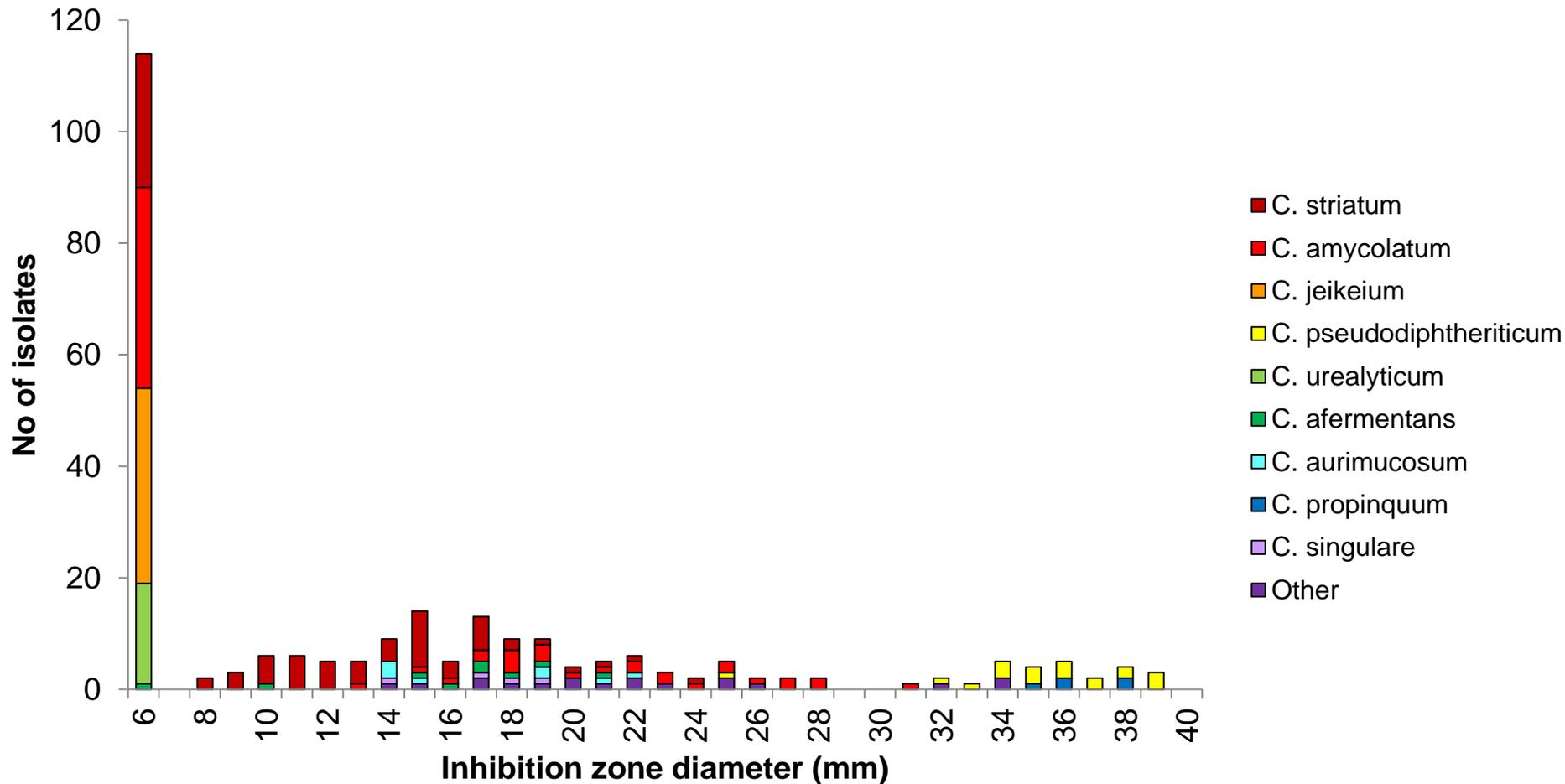
# Testade antibiotika

- **Bensylpenicillin**
- Ciprofloxacin
- **Moxifloxacin**
- Gentamicin
- **Vankomycin**
- Klindamycin
- **Tetracyklin**
- Linezolid
- Rifampicin
- Trimetoprim-sulfametoxazol

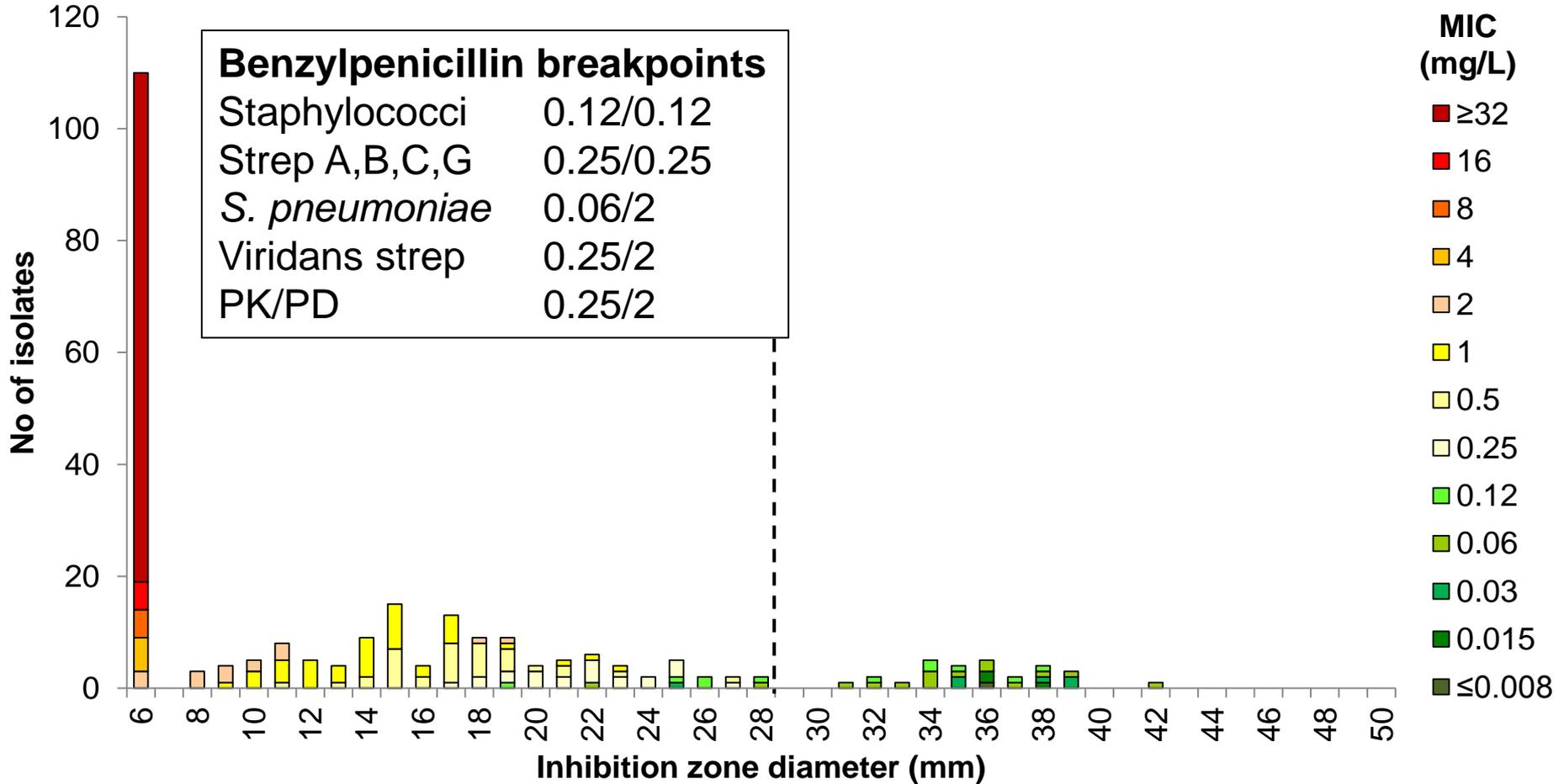
# Benzylopenicillin MIC vs. species *Corynebacterium* spp., 258 clinical isolates



# Benzylpenicillin 1 unit vs. species *Corynebacterium* spp., 258 clinical isolates



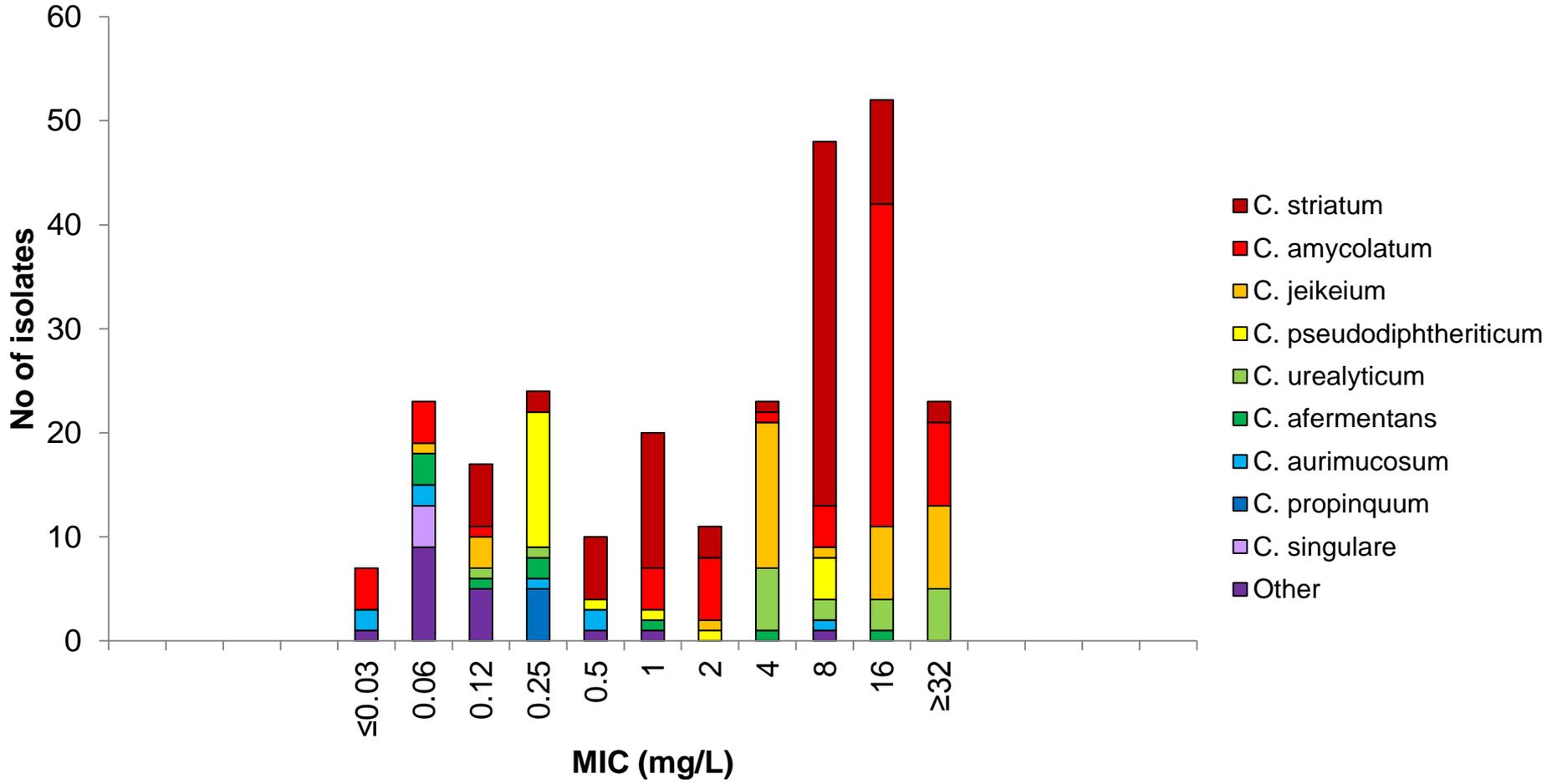
## Benzylpenicillin 1 unit vs. MIC *Corynebacterium* spp., 258 clinical isolates



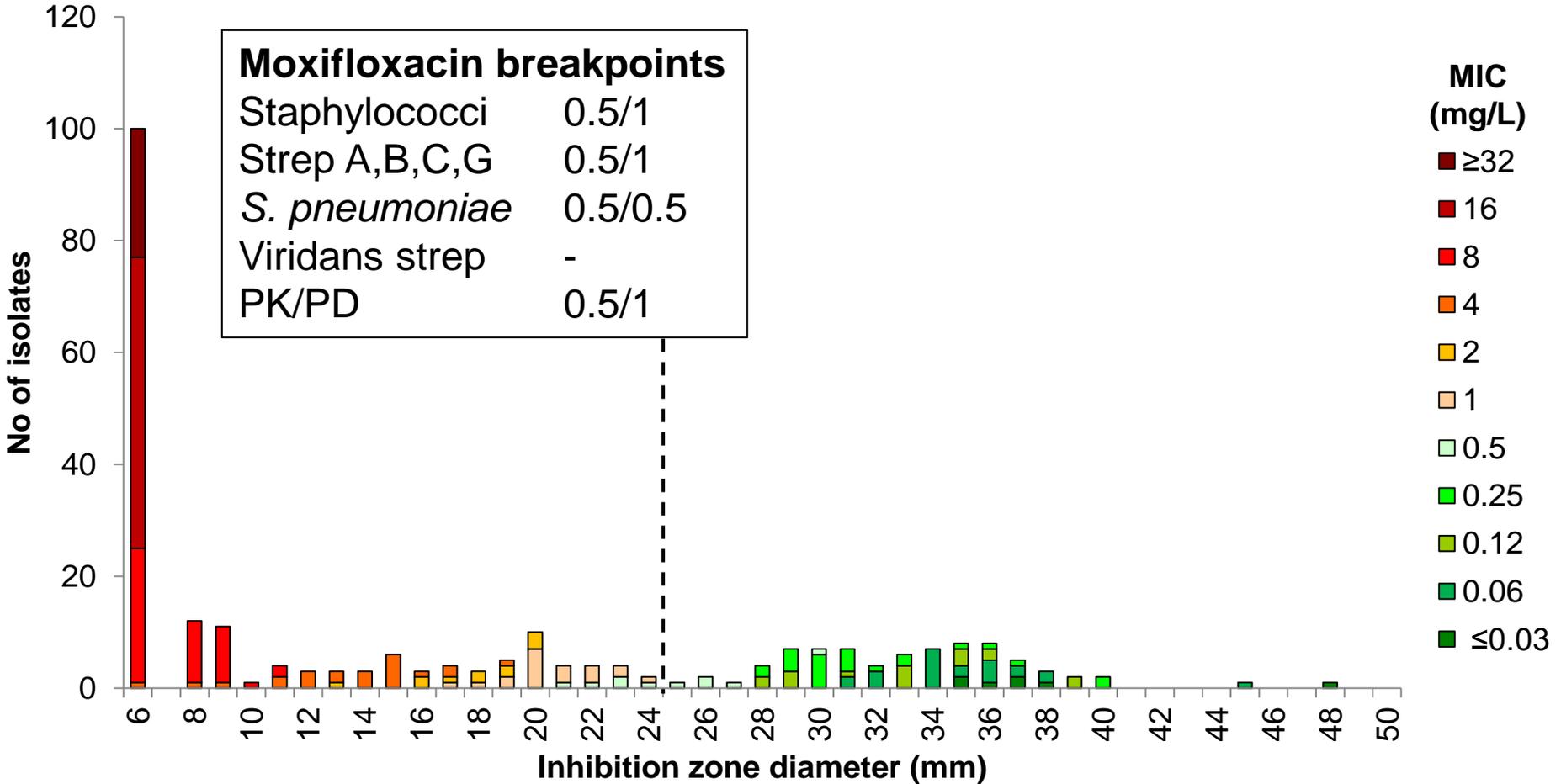
**Suggested breakpoints:** S ≤ 0.12, R > 0.12 mg/L and S ≥ 29, R < 29 mm

# Moxifloxacin MIC vs. species

## *Corynebacterium* spp., 258 clinical isolates



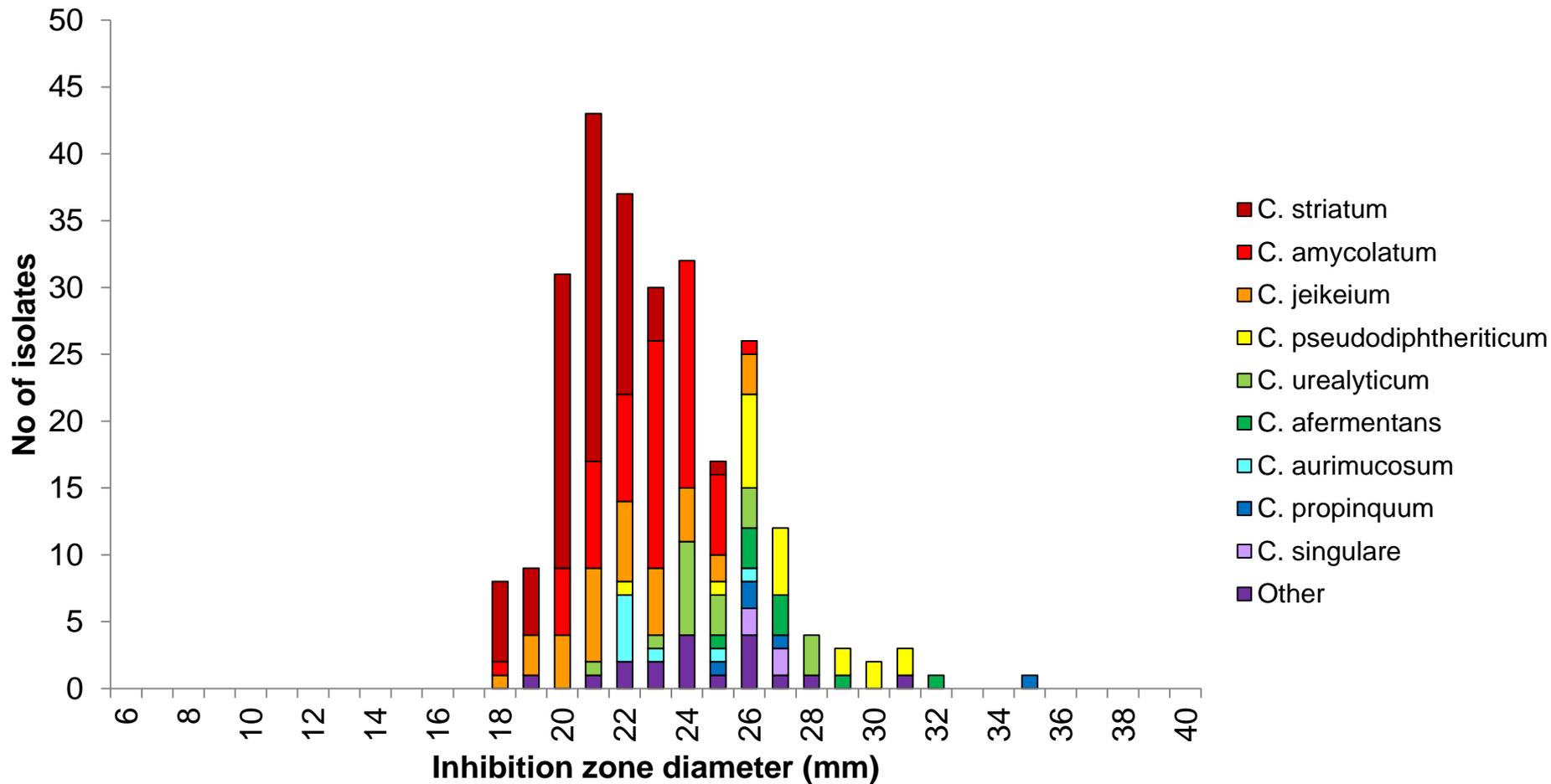
## Moxifloxacin 5 $\mu$ g vs. MIC *Corynebacterium* spp., 258 clinical isolates



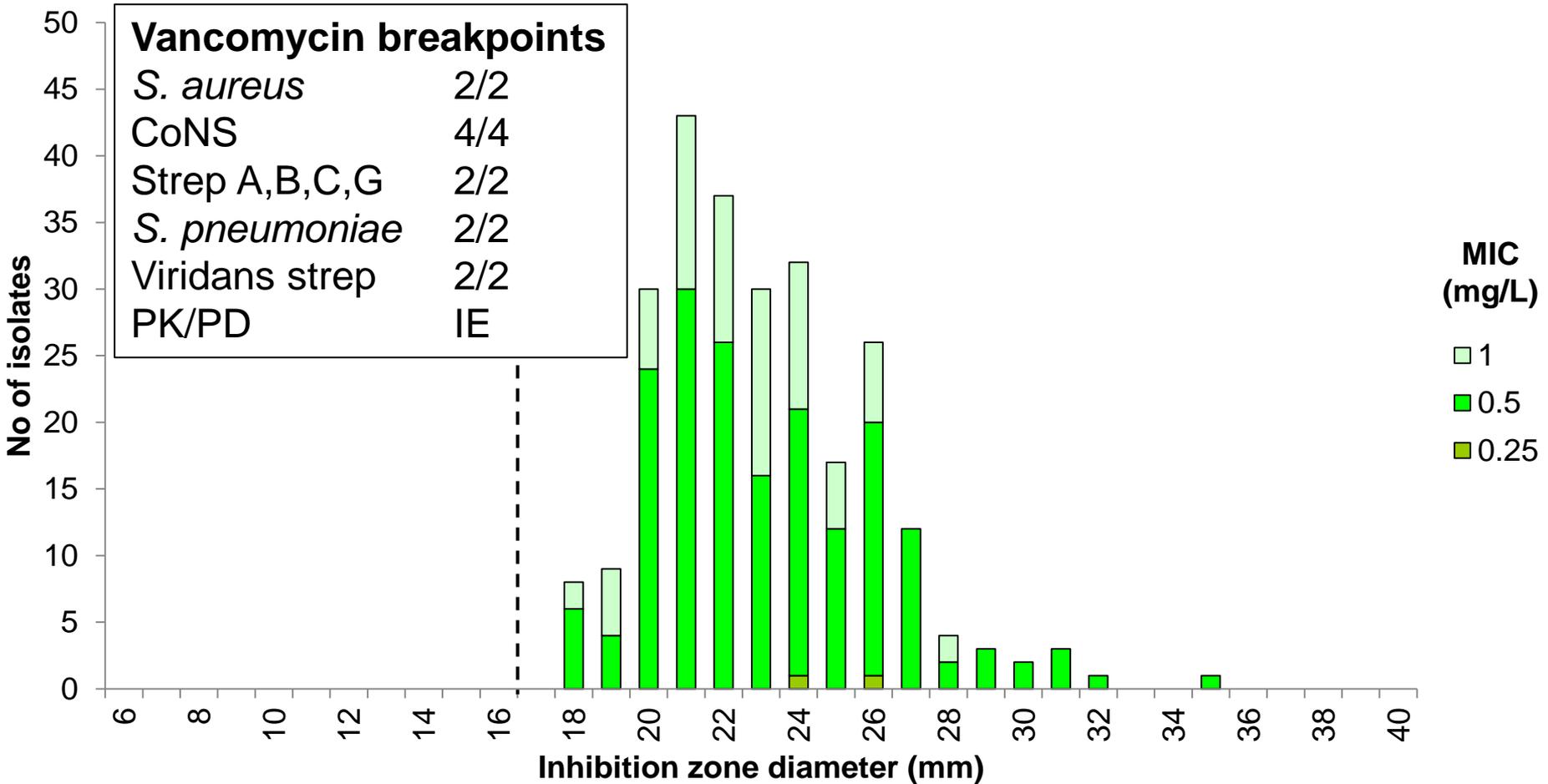
**Suggested breakpoints:**  $S \leq 0.5$ ,  $R > 0.5$  mg/L and  $S \geq 25$ ,  $R < 25$  mm



# Vancomycin 5 µg vs. species *Corynebacterium* spp., 259 clinical isolates



## Vancomycin 5 µg vs. MIC *Corynebacterium* spp., 258 clinical isolates

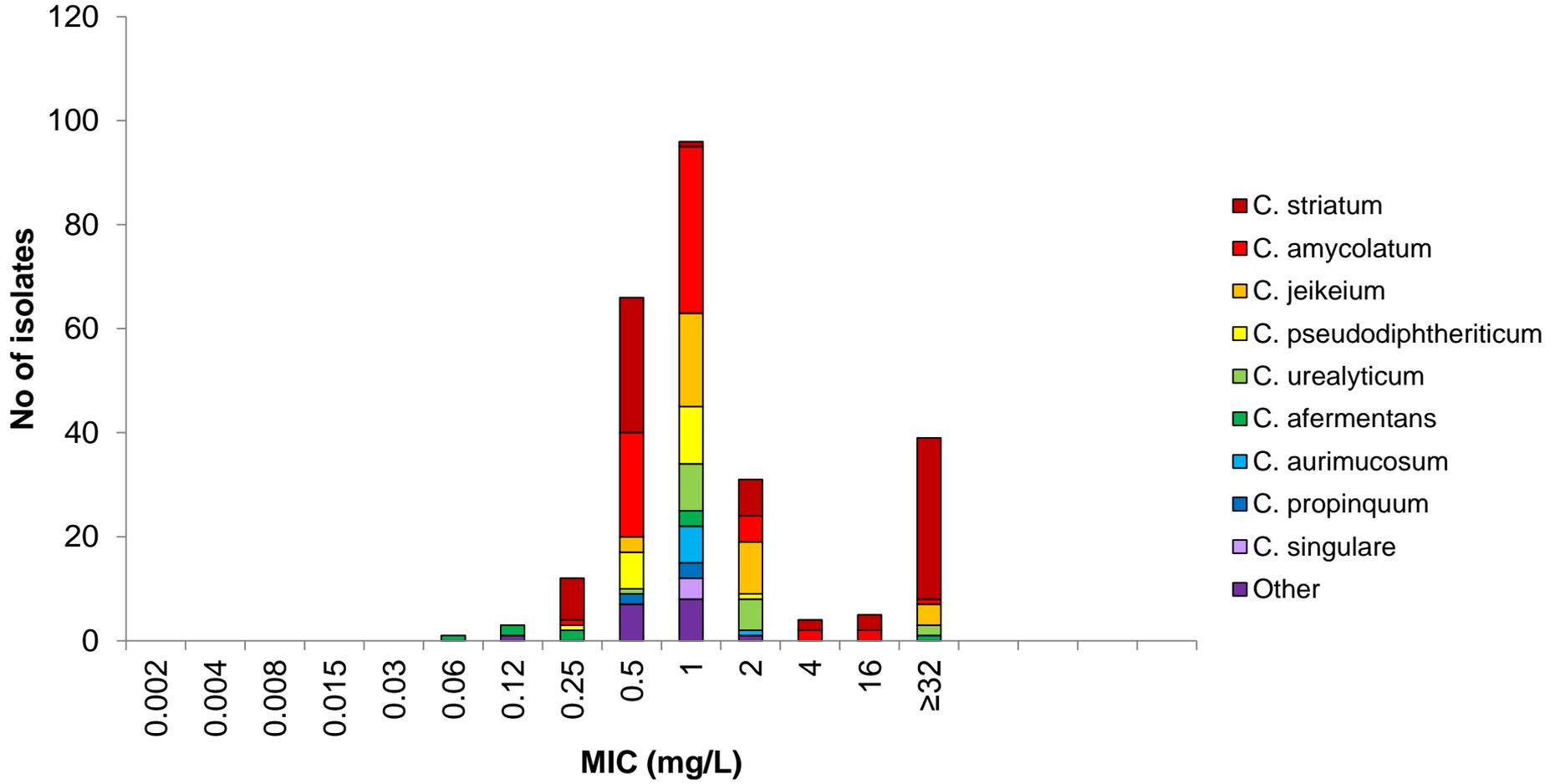


**Suggested breakpoints:**

$S \leq 2$ ,  $R > 2$  mg/L and  $S \geq 17$ ,  $R < 17$  mm

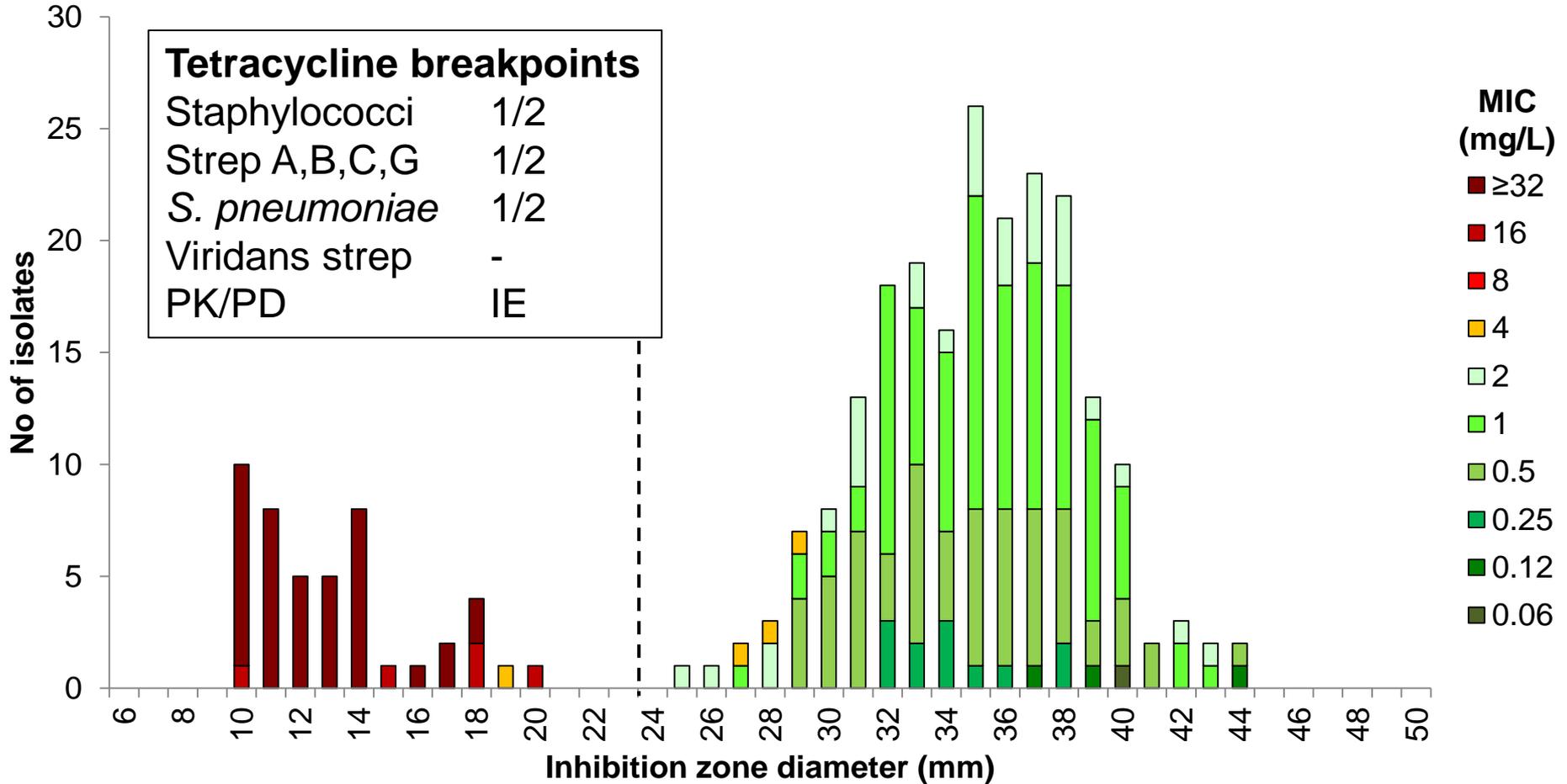
# Tetracycline MIC vs. species

## *Corynebacterium* spp., 258 clinical isolates





## Tetracycline 30 µg vs. MIC *Corynebacterium* spp., 258 clinical isolates

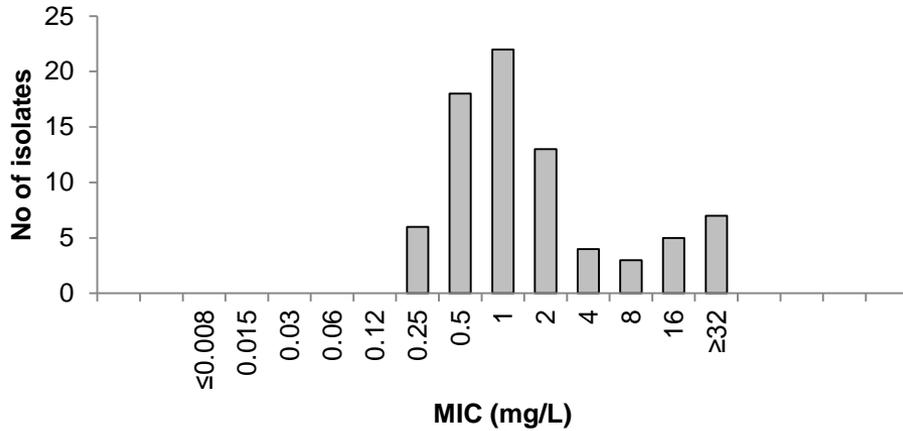


**Suggested breakpoints:**

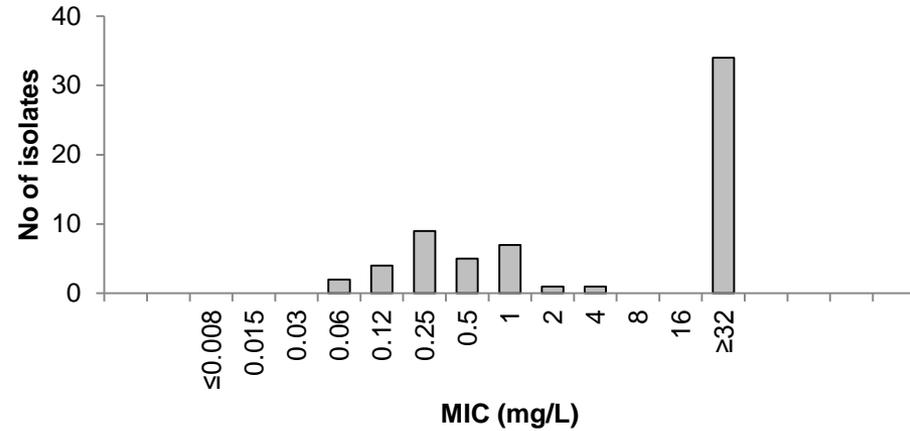
**S ≤ 2, R > 2 mg/L and S ≥ 24, R < 24 mm**

# Artspecifika MIC-distributioner

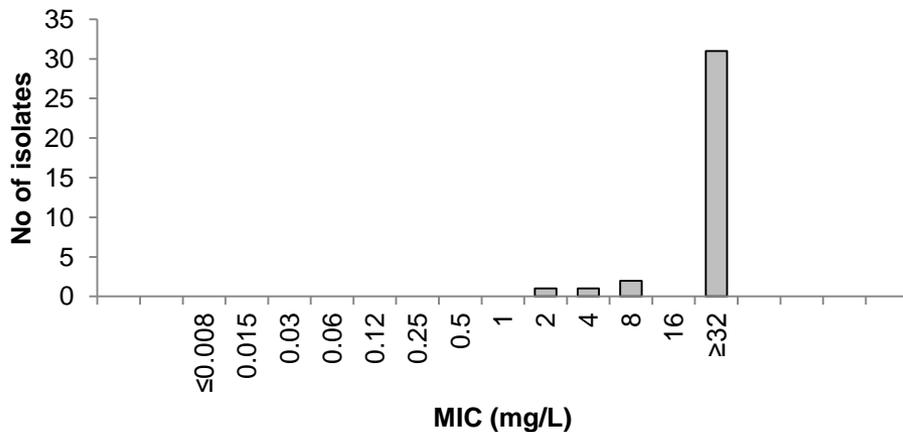
***C. striatum* with benzypenicillin**  
78 clinical isolates



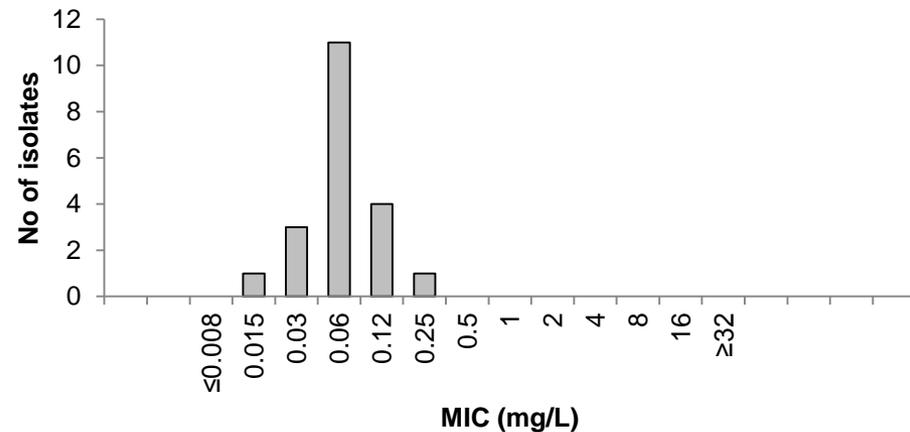
***C. amycolatum* with benzylpenicillin**  
63 clinical isolates



***C. jeikeium* with benzylpenicillin**  
35 clinical isolates

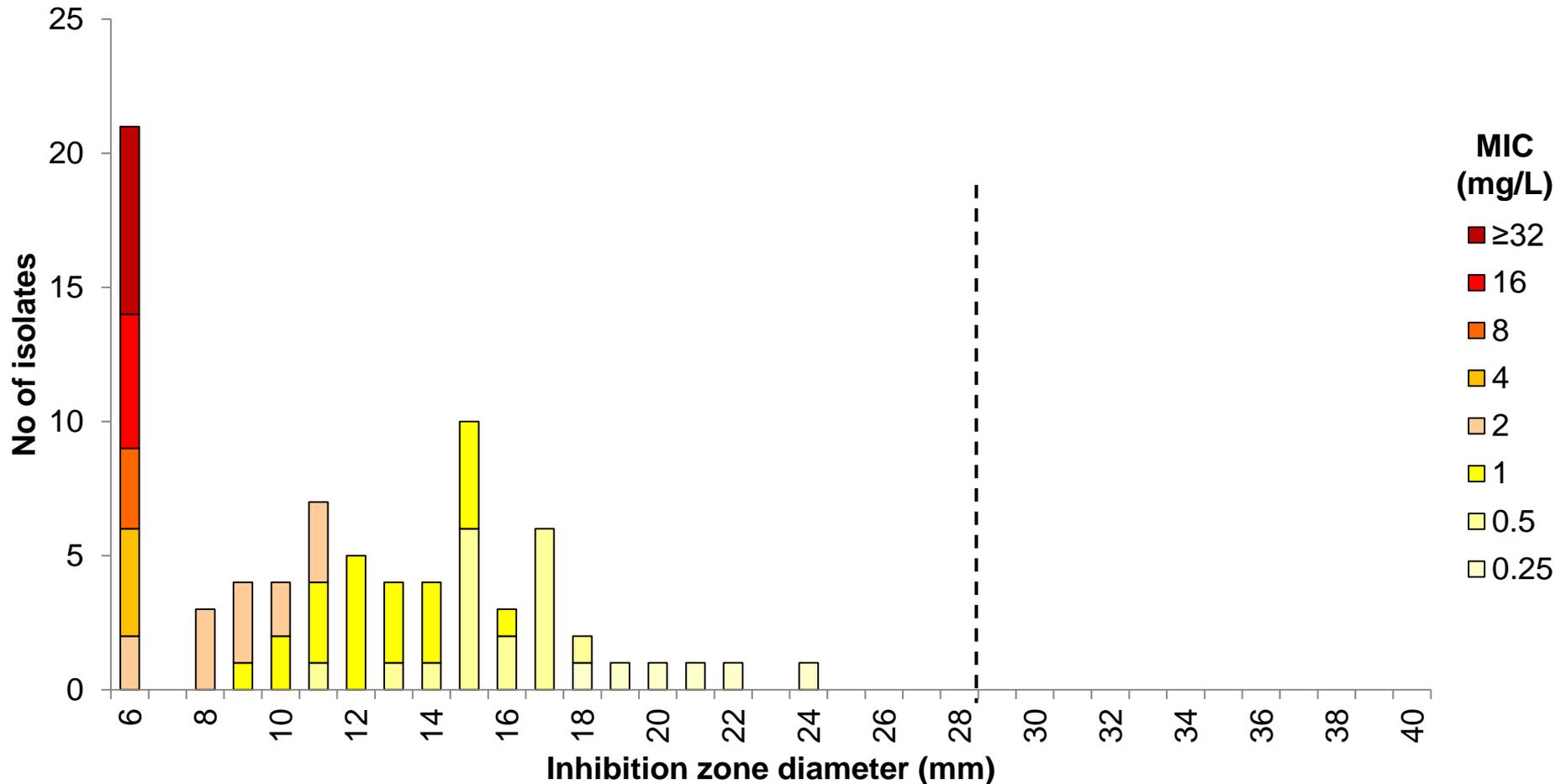


***C. pseudodiphtheriticum* with benzylpenicillin**  
20 clinical isolates



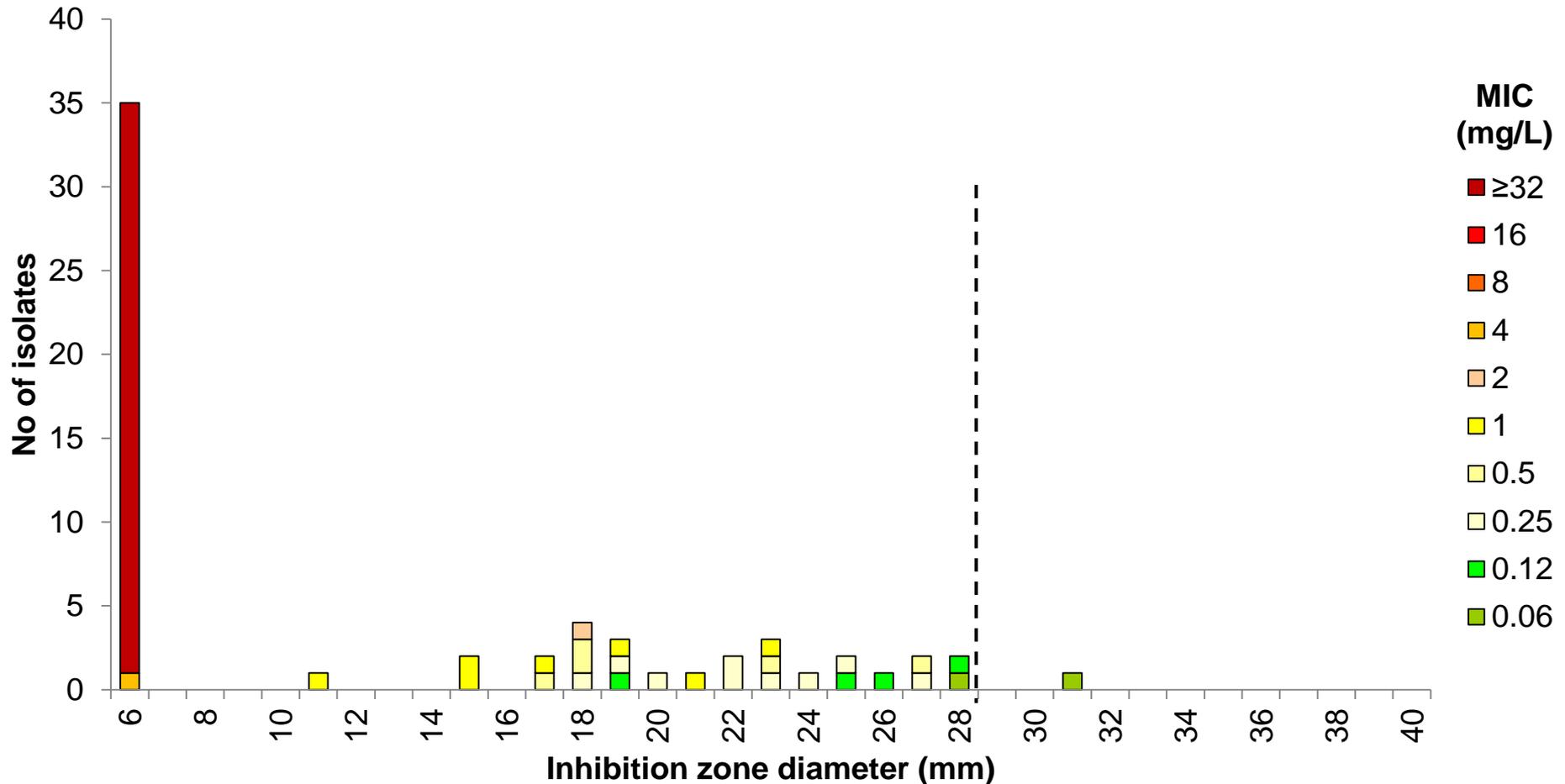
# Artspecifika zondistributioner

**Benzylpenicillin 1 unit vs. MIC  
*C. striatum*, 78 clinical isolates**



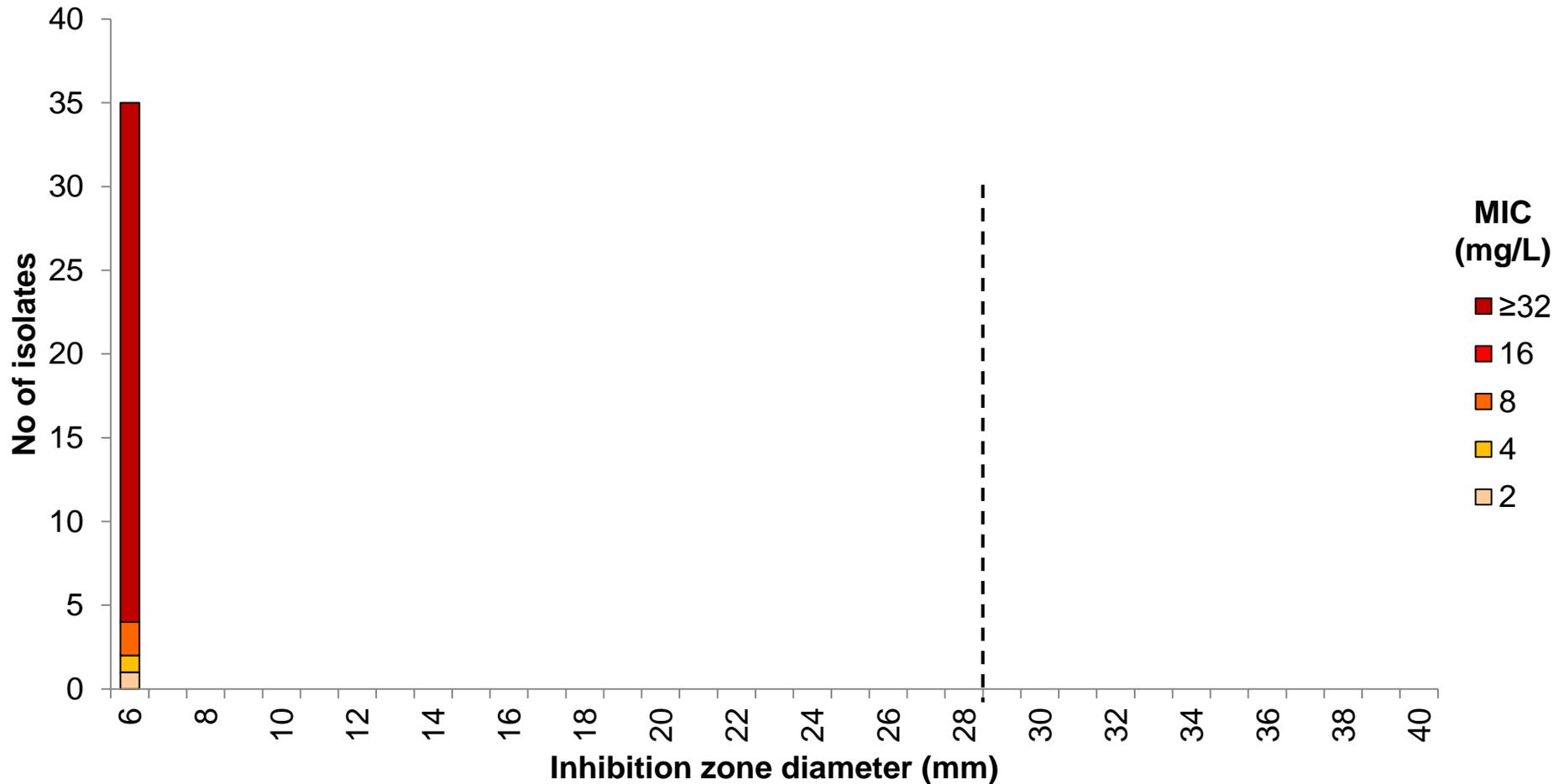
# Artspecifika zondistributioner

Benzylpenicillin 1 unit vs. MIC  
*C. amycolatum*, 63 clinical isolates



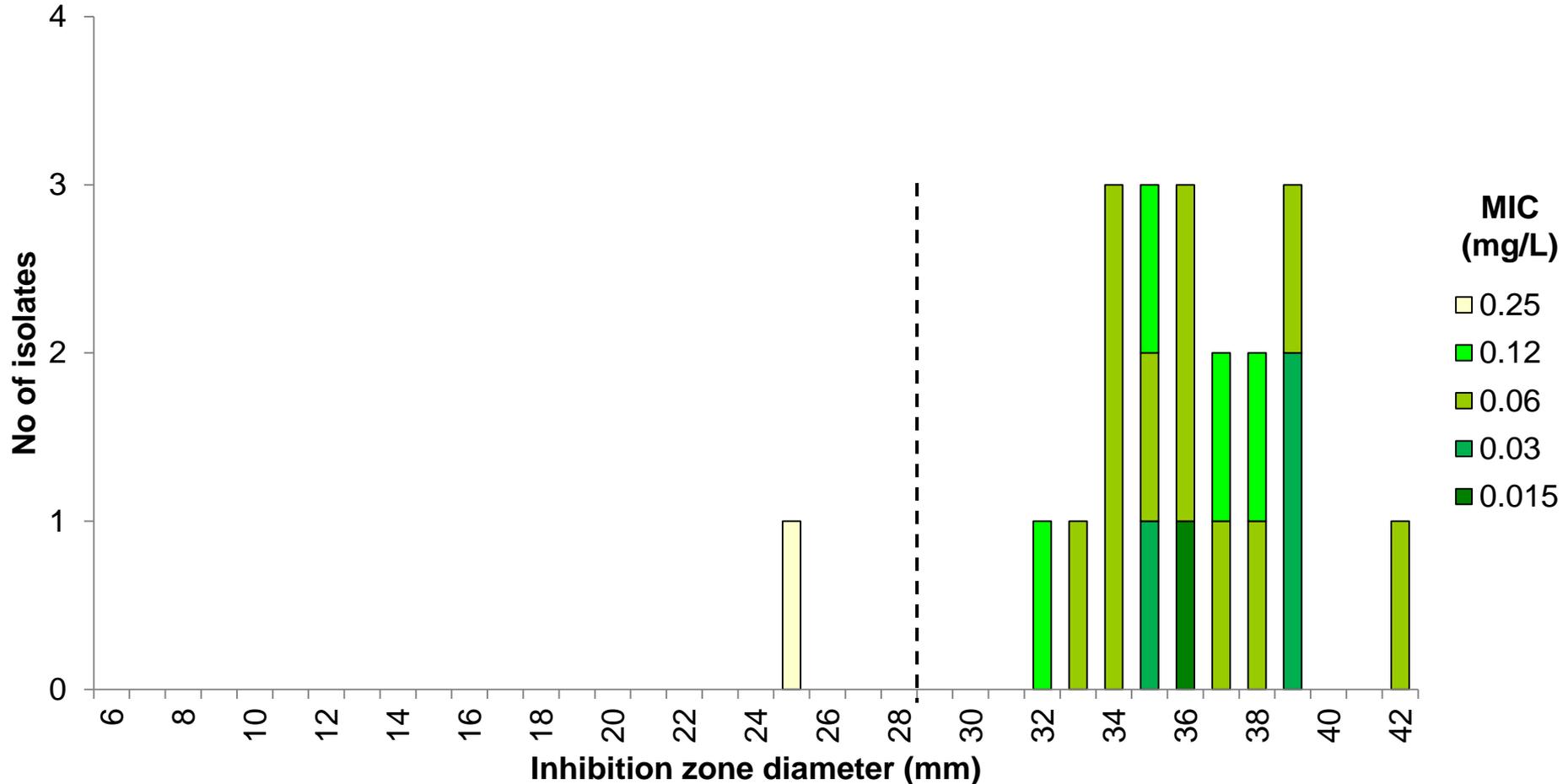
# Artspecifika zondistributioner

**Benzylpenicillin 1 unit vs. MIC  
*C. jeikeium*, 35 clinical isolates**



# Artspecifika zondistributioner

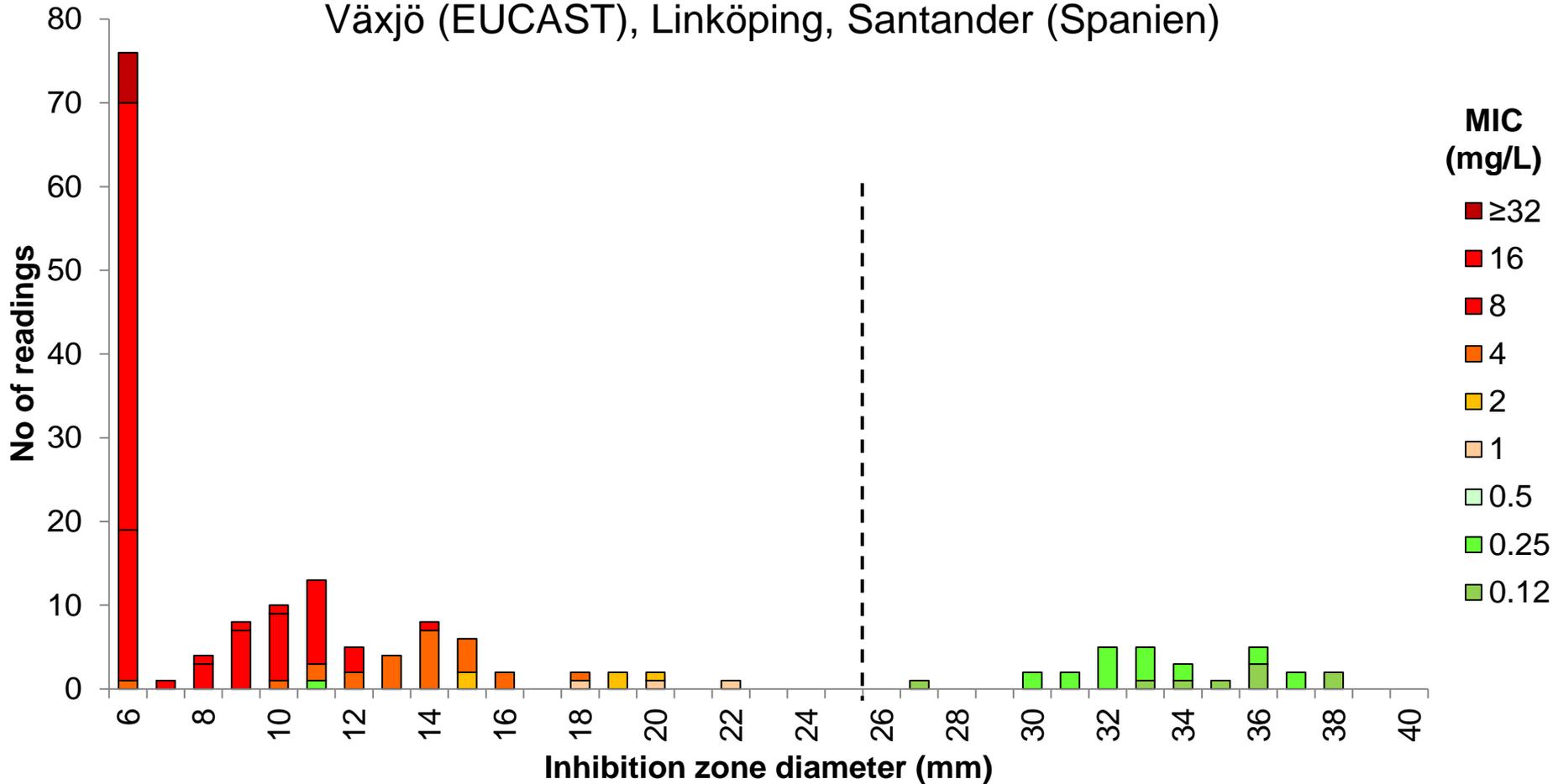
Benzylpenicillin 1 unit vs. MIC  
*C. pseudodiphtheriticum*, 20 clinical isolates



# Variation mellan laboratorier

## Moxifloxacin 5 µg vs. MIC *Corynebacterium* spp., 60 clinical isolates tested at 3 sites

Växjö (EUCAST), Linköping, Santander (Spanien)





# Skillnader i spänningssteg mellan Etest och BMD (referens)

<b>Benzylpenicillin</b>	<b>&gt;-2</b>	<b>-2</b>	<b>-1</b>	<b>=</b>	<b>+1</b>	<b>+2</b>	<b>&gt;+2</b>
Corynebacterium amycolatum		1	2	8	1		
Corynebacterium jeikeium				11	1		
Corynebacterium pseudodiphtheriticum	1	8	2	1			
Corynebacterium striatum			4	7	1		
Corynebacterium urealyticum				12			
<b>Sum</b>	<b>1</b>	<b>9</b>	<b>8</b>	<b>39</b>	<b>3</b>		

**EA 83%**

<b>Moxifloxacin</b>	<b>&gt;-2</b>	<b>-2</b>	<b>-1</b>	<b>=</b>	<b>+1</b>	<b>+2</b>	<b>&gt;+2</b>
Corynebacterium amycolatum		1				11	
Corynebacterium jeikeium			1	4	3	4	
Corynebacterium pseudodiphtheriticum			2	4	5		1
Corynebacterium striatum					7		5
Corynebacterium urealyticum				3	2	4	3
<b>Sum</b>		<b>1</b>	<b>3</b>	<b>11</b>	<b>17</b>	<b>19</b>	<b>9</b>

**EA 52%**

<b>Vancomycin</b>	<b>&gt;-2</b>	<b>-2</b>	<b>-1</b>	<b>=</b>	<b>+1</b>	<b>+2</b>	<b>&gt;+2</b>
Corynebacterium amycolatum			6	6			
Corynebacterium jeikeium			6	6			
Corynebacterium pseudodiphtheriticum			5	7			
Corynebacterium striatum			2	9	1		
Corynebacterium urealyticum			9	3			
<b>Sum</b>			<b>28</b>	<b>31</b>	<b>1</b>		

**EA 100%**

<b>Tetracycline</b>	<b>&gt;-2</b>	<b>-2</b>	<b>-1</b>	<b>=</b>	<b>+1</b>	<b>+2</b>	<b>&gt;+2</b>
Corynebacterium amycolatum	1	6	3	2			
Corynebacterium jeikeium	3	7	1	1			
Corynebacterium pseudodiphtheriticum		1	9	2			
Corynebacterium striatum		1	5	5	1		
Corynebacterium urealyticum	5	4	3				
<b>Sum</b>	<b>9</b>	<b>19</b>	<b>21</b>	<b>10</b>	<b>1</b>		

**EA 53%**

# *Corynebacterium* spp.

## EUCASTs föreslagna brytpunkter

Antibiotika	S $\leq$	R $>$	Lappstyrka	S $\geq$	R $<$
Bensylpenicillin	0.12	0.12	1 unit	29	29
Ciprofloxacin	1	1	5	25	25
Moxifloxacin	0.5	0.5	5	25	25
Gentamicin	1	1	10	23	23
Klindamycin	0.5	0.5	2	20	20
Tetracyklin	2	2	30	24	24
Rifampicin	0.06	0.5	5	30	25
Vankomycin	2	2	5	17	17
Linezolid	2	2	10	25	25
Trim-Sulfa	1	2	1.25-23.75	19	16

