New ß-lactamase in *Staphylococcus aureus* with increased resistance towards penicillin and oxacillin/cloxacillin

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REGION

BACKGROUND

ß-lactamases in *S. aureus* belong to the molecular class A which are encoded by *blaZ*. The enzymes are serine hydrolases which cleaves the ß-lactam ring in ß-lactam antibiotics.

Today, four variants of class A ß-lactamases (A, B, C and D) have been characterised and vary in their ß-lactam susceptibility and enzyme kinetics.
The variants are distinguished based on the amino acids on position 119 and 207 which corresponds to position 128 and 216 in Nannini *et al.*

AIM

The purpose of this study was to investigate the distribution of ß-lactamase variants in clinical isolates and their phenotypic importance.

METHOD

The genome sequence from 500 clinical isolates derived from blood were analysed to determine the prevalence of *blaZ* and the distribution of the four ß-lactamase variants. Subsequently, we selected MSSA isolates representing the various types of ß-lactamases and strains lacking the *blaZ* gene (n = 51) with different ST-types to exclude possible clones. The classification of each ß-lactamase variant was defined as:
For type A, threonine on position 119 and serine on position 207. For type B, lysine on position 119 and asparagine on position 207. For type C, threonine on position 119 and asparagine on position 207. For type D, alanine on position 119 and serine on position 207.

The strains were tested against a range of antibiotics by disk diffusion and E-test according to EUCAST guidelines.

RESULTS

We detected 359 isolates harbouring *blaZ*.
124 of variant A, 83 of B, 141 of C, 8 of D. The whole genome sequencing revealed a new variant in 3 isolates with a previously uncharacterized threonine mutation (TT) on position 119 and 207 in the protein sequence. The three TT isolates belong to two different ST- types (118 and 1) and they share up to 99% identity with the known variants, A and C.

Disk diffusion (Table and Figure 1) and E-test (Table 2) revealed that TT has lower susceptibility towards penicillin, oxacillin, cloxacillin and cefuroxime compared to the four characterized variants.

Disk diffusion tests with cefoxitin (Table 1) revealed that all variants were susceptible to cefoxitin i.e., zone diameter above 22 mm for all isolates.

CONCLUSION

A new class A ß-lactamase in *S. aureus* (possible E variant) with a threonine residue on position 119 and 207 (TT) shows lower susceptibility towards penicillin, oxacillin, cloxacillin and cefuroxime.

The increased resistance towards commonly used antibiotics in clinical setting could provide a benefit for the new variant, thus, becoming a more prevalent type of ß-lactamase in the future.

Due to the small oxacillin zone diameter, TT strains could be confused with MRSA strains in a clinical setting. These results emphasize the importance of cefoxitin testing.

TABLE 1. ZONE OF INHIBITION FOR ß-LACTAMASE VARIANTS

Antibiotics	ß-lactamase types					Control
	A	B	C	D	TT	S
Penicillin	**13.7** (2.8)	**10.5** (2.2)	**7.5** (2.0)	**11.8** (4.4)	**6*** (0)	**29.9** (5.7)
Oxacillin	**22.1** (2.2)	**18.9** (1.9)	**17.6** (1.6)	**21.3** (1.6)	**8.5** (2.1)	**23.9** (2.0)
Cefuroxime	**33.4** (2.4)	**31.6** (0.9)	**31.0** (2.4)	**31.7** (2.2)	**23** (1.1)	**32.7** (1.2)
Cefoxitin	**30.4** (1.5)	**29.1** (1.6)	**29.1** (1.4)	**31.1** (1.2)	**29.3** (0.5)	**30.6** (1.2)

Table 1. Average of zones of inhibition (mm) with standard deviation for the four known ß-lactamase types (n_A = 10, n_B = 10, n_C = 10, n_D = 8), the new ß-lactamase, TT (n_{TT} = 3) and *S. aureus* strains with no antibiotic resistance genes (n_S = 10). * : No zone of inhibition detected

TABLE 2. MINIMUM INHIBITORY CONCENTRATION FOR ß-LACTAMASE VARIANTS

Antibiotics	ß-lactamase types					Control
	A	B	C	D	TT	S
Penicillin	**0.25** (0.125-0.5)	**0.5** (0.25-2)	**0.5** (0.25-2)	**0.38** (0.125-0.5)	**4** (1-32)	**0.064**
Oxacillin	**0.25** (0.125-0.5)	**0.5** (0.25-0.5)	**0.5** (0.25-1)	**0.38** (0.25-0.5)	**2** (2-4)	**0.125**
Cefuroxime	**1** (0.5-1)	**1** (0.5-2)	**1** (1-2)	**1** (1-2)	**2** (1-2)	**1**
Cloxacillin	**0.125** (0.125-0.25)	**0.25** (0.125-0.25)	**0.25** (0.25-0.5)	**0.25** (0.125-0.25)	**1** (0.5-2)	**0.125**

Table 2. Median of minimum inhibitory concentrations (MIC) (mg/L) with range for the four known ß-lactamase types (n_A = 10, n_B = 10, n_C = 10, n_D = 8), the new ß-lactamase, TT (n_{TT} = 3) and one *S. aureus* strain with no antibiotic resistance genes .

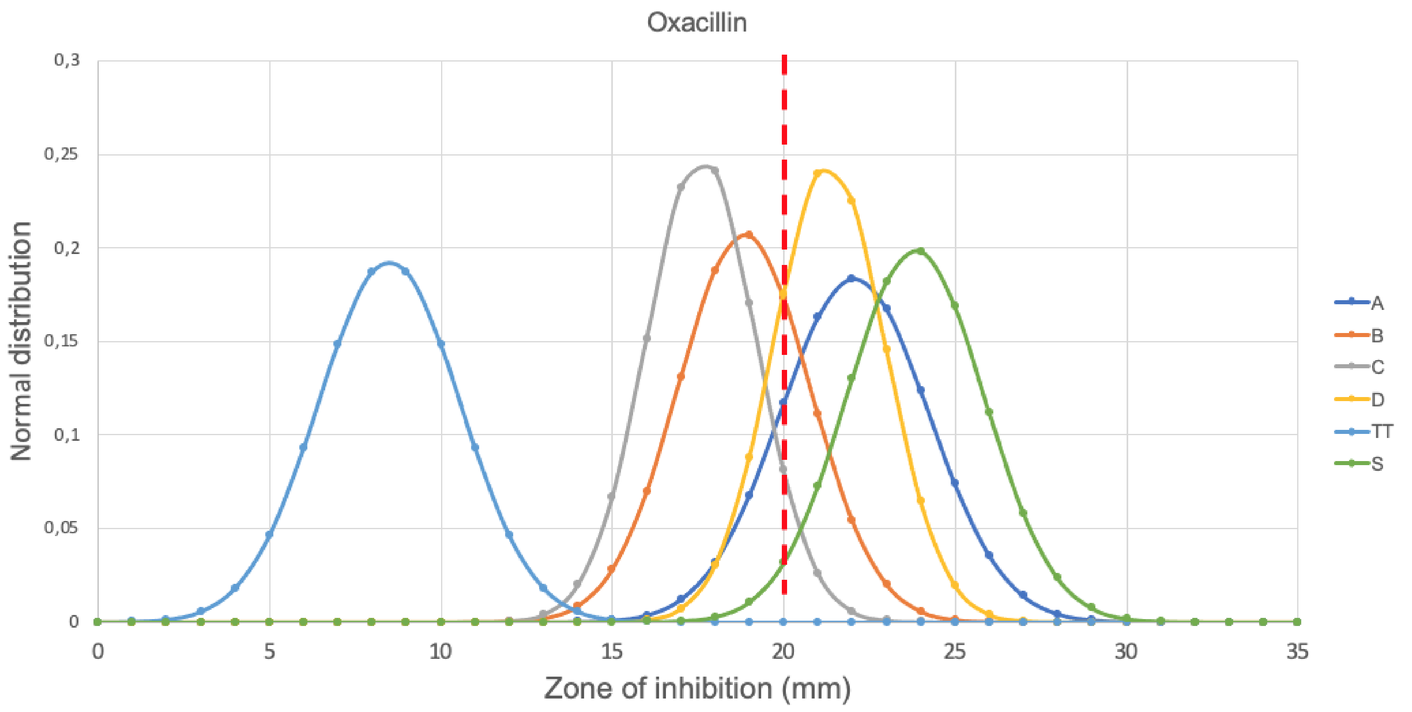

Figure 1. Normal distribution of the zones of inhibition (mm) for oxacillin based on the data in Table 1. The ß-lactamase variants illustrated is A (dark blue), B (orange), C (grey), and D (yellow), the new ß-lactamase variant, TT (light blue) and susceptible *S. aureus* strains (green). The red, vertical line represents zone diameter breakpoint for oxacillin in staphylococcal strains according to EUCAST.

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