Introduction

Increased and repeated use of β-lactam antibiotics (e.g. penicillins and cephalosporins) leads to them becoming ineffective, principally as a result of the onset and worldwide spread of enzymatic resistance via β-lactamase production by bacteria. Two strategies have been employed to counter this resistance problem. Firstly, new β-lactam drugs have been developed that are inherently less susceptible to β-lactamases. A second approach utilizing combinations of a mechanism-based inactivator for β-lactamases (e.g. clavulanic acid, sulbactam and tazobactam) and a penicillin has also been used. The rationale for such combined therapy is based on a synergic effect of the two molecules: the inactivator destroys the β-lactamase activity, whereby the penicillin is protected from inactivation. Combinations of hydrolysable penicillins with a β-lactamase inhibitor are a successful strategy to overcome TEM-type mediated resistance. Reports have established that the susceptibility of Escherichia coli isolates to β-lactamase inhibitors can be affected by hyperproduction of unmodified TEM-type β-lactamase,1–3 or by the modification of the outer membrane proteins, or by both.4 Resistance may also be attributable to production of OXA-type enzymes, or to hyperproduction of cephalosporinases.5 Since 1990, the effect of β-lactamase inhibitors has also been compromised by the emergence of mutant TEM-type β-lactamases,6,7 collectively designated inhibitor-resistant TEM or IRT β-lactamases (Table I).

Until now, the IRT β-lactamases have been described only in Europe (France, Spain and the UK),6,17 but also in three clinical strains of Klebsiella pneumoniae8,9 and ten strains of Proteus mirabilis.10 The presence of IRT was also reported in 1993 in strains of E. coli, but also in three clinical strains of Haemophilus influenzae although co-amoxiclav is widely prescribed to treat infections caused by β-lactamase-producing strains of this bacterium. A suggested by Nicolas-Chanoine,21 this could be related to a high intrinsic activity of penicillins against such strains or to an inadequate number of bacteria in the natural reservoir (the oropharynx) to allow for the spontaneous point mutation of the plasmidic TEM gene. Moreover, this species makes only a small amount of β-lactamase.

This review attempts to summarize and to discuss the many available data concerning the IRT β-lactamases.
Phenotypic characteristics

Detection of IRT-producing strains

The IRT phenotype was characterized by resistance to \(\beta\)-lactam–clavulanate combinations with susceptibility to cephalosporins, which is not observed in the overproduced penicillinase phenotype. A number of studies have tried to determine the resistance pattern which allows a reliable detection of IRT-producing strains.\(^{22-25}\) When susceptibilities to amoxycillin, amoxycillin plus clavulanate, ticarcillin, ticarcillin plus clavulanate, piperacillin, piperacillin plus tazobactam and cephalothin were evaluated by a disc diffusion method with the critical diameters interpreted according to French guidelines,\(^{26}\) the phenotype amoxycillin-resistant, ticarcillin-resistant, amoxycillin or ticarcillin plus clavulanate-intermediate or -resistant and cephalothin-susceptible allowed the detection of about 87% of \(E. coli\) strains producing an IRT-\(\beta\)-lactamase alone or in association with a parental TEM-\(\beta\)-lactamase.\(^{24}\) However, this phenotype did not allow the discrimination of OXA-producing strains, which appeared indistinguishable from IRT strains (Table II). Libert et al.\(^{27}\) proposed the measurement of the inhibition diameters to cefepime, mecillinam and ceftazidime for the routine differentiation of strains producing IRT and OXA enzymes.

In a study using an automated and rapid (4–5 h) ATB method (bioMérieux, La Balme-les-Grottes, France) linked to a knowledge-based expert system (ATB Plus System, Golo V.1.5, bioMérieux) detecting ‘IRT’ on the basis of growth indices obtained with \(\beta\)-lactams, it was demonstrated that this system was able to detect 86.7% of IRT-producing \(E. coli\) strains.\(^{28}\) By routine susceptibility tests, IRT production was inconstantly detected when it was present with additional \(\beta\)-lactamases such as AmpC cephalosporinase.\(^{24}\)

\(\beta\)-Lactam susceptibility of IRT-producing \(E. coli\) strains

The susceptibility of 98 IRT-producing isolates of \(E. coli\), collected in 1993 in the teaching hospital of Clermont Ferrand (France), was assessed by determination of \(\beta\)-lactam MICs by the agar dilution method (Table III). The isolates selected produced nine different IRT enzymes: TEM-30/IRT-2 \((n = 19)\), TEM-32/IRT-3 \((n = 4)\), TEM-33/IRT-5 \((n = 16)\), TEM-34/IRT-6 \((n = 13)\), TEM-35/IRT-4 \((n = 13)\), TEM-36/IRT-7 \((n = 11)\), TEM-37/IRT-8 \((n = 19)\), TEM-38/IRT-9 \((n = 1)\), and TEM-39/IRT-10 \((n = 2)\).

For all the IRT-producing isolates high-level resistance to amoxycillin and ticarcillin (90% inhibitory concentration >4096 mg/L) was observed. A addition of clavulanic acid (2 mg/L) reduced the MICs of amoxycillin and ticarcillin to intermediate levels.
Inhibitor-resistant TEM \( \beta \)-lactamases

**Table II.** \( \beta \)-Lactam resistance phenotypes of amoxycillin–clavulanate-intermediate (I) and -resistant (R) E. coli strains

<table>
<thead>
<tr>
<th>( \beta )-Lactamase</th>
<th>Amoxycillin</th>
<th>Amoxycillin + clavulanate</th>
<th>Ticarcillin</th>
<th>Ticarcillin + clavulanate</th>
<th>Cephalothin</th>
<th>Cefoxitin</th>
</tr>
</thead>
<tbody>
<tr>
<td>IRT</td>
<td>R</td>
<td>I/R</td>
<td>I/R</td>
<td>S/I</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>OXA</td>
<td>R</td>
<td>I/R</td>
<td>R</td>
<td>I/R</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>TEM-type penicillinase</td>
<td>R</td>
<td>I/R (^b)</td>
<td>R</td>
<td>S/I (^b)/R (^b)</td>
<td>S/I</td>
<td>S</td>
</tr>
<tr>
<td>Cephalosporinase(^c)</td>
<td>I/R</td>
<td>I/R</td>
<td>S/I</td>
<td>I/R</td>
<td>I/R</td>
<td>I/R</td>
</tr>
</tbody>
</table>

\(^a\)Data obtained from references 23 and 24.

\(^b\)Overproduced penicillinase.

\(^c\)Enhanced production of chromosomal cephalosporinase.

**Table III.** Distribution of \( \beta \)-lactam MICs (mg/L) for 98 IRT-producing E. coli isolates

<table>
<thead>
<tr>
<th>( \beta )-Lactams</th>
<th>No. of isolates inhibited at following concentration (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2</td>
</tr>
<tr>
<td>Amoxycillin</td>
<td>-</td>
</tr>
<tr>
<td>+ clavulanic acid(^a)</td>
<td>-</td>
</tr>
<tr>
<td>Ticarcillin</td>
<td>-</td>
</tr>
<tr>
<td>+ clavulanic acid(^a)</td>
<td>-</td>
</tr>
<tr>
<td>Piperacillin</td>
<td>-</td>
</tr>
<tr>
<td>+ tazobactam(^b)</td>
<td>2</td>
</tr>
</tbody>
</table>

\(^a\)Clavulanic acid at fixed concentration of 2 mg/L.

\(^b\)Tazobactam at fixed concentration of 4 mg/L.

Genetic characteristics

Three \( \text{bla} \) gene sequences designated TEM-1A, TEM-1B and TEM-2 encode two different \( \beta \)-lactamases, namely TEM-1 and TEM-2 (Table IV). There are a total of nine nucleotide sequence differences between these genes at positions 32, 175, 226, 317, 346, 436, 604, 682 and 925 (numbering according to Sutcliffe\(^29\)). Caniça et al.\(^13\) have explored the molecular diversity of IRT enzymes by using a strategy which involved DNA amplification by polymerase chain reaction (PCR), analysis of restriction fragment length polymorphism (RFLP) and direct nucleotide sequencing. Study of the primary structure of the genes encoding these enzymes with altered phenotype is expected to provide insight into the molecular basis of the phenotype and to help in tracing their evolution. Table V shows that the IRT \( \beta \)-lactamase genes can be grouped in three sequence linkage groups: ‘TEM-1A like’, ‘TEM-1B like’ and ‘TEM-2 like’. It should be noted that a given mutation conferring an IRT phenotype can be found in two different gene frameworks: e.g. TEM-30/IRT-2, TEM-31/IRT-1 and TEM-34/IRT-6 are found independently in both ‘TEM-1B like’ and ‘TEM-2 like’ gene sequences. As suggested by Caniça...
**Table IV.** Sequence differences between $\text{bla}_{\text{TEM-1A}}, \text{bla}_{\text{TEM-1B}}$ and $\text{bla}_{\text{TEM-2}}$ genes

<table>
<thead>
<tr>
<th>$\text{bla}$ gene</th>
<th>Enzyme</th>
<th>Nucleotide$^a$ (amino acid)$^b$ in:</th>
<th>coding region</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\text{bla}_{\text{TEM-1A}}$</td>
<td>TEM-1</td>
<td>C A C C (Gln) A C G T G</td>
<td>32 175 226 (6) 317 (39) 346 (48) 436 (78) 604 (134) 682 (160) 925 (242)</td>
</tr>
<tr>
<td>$\text{bla}_{\text{TEM-1B}}$</td>
<td>TEM-1</td>
<td>C G T C A T T T G</td>
<td>30, 75</td>
</tr>
<tr>
<td>$\text{bla}_{\text{TEM-2}}$</td>
<td>TEM-2</td>
<td>T A C A (Lys) G T G C A</td>
<td>30, 75</td>
</tr>
</tbody>
</table>

$^a$Nucleotide positions are numbered according to Sutcliffe.$^{29}$

$^b$The amino acid is indicated when a point mutation leads to amino acid substitution compared with the sequence of TEM-1. Numbering is according to the ABL numbering system of Ambler$^{50}$.

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**Table V.** Isolated or associated amino acid substitutions present in the IRT $\beta$-lactamases; distribution among sequence linkage groups

<table>
<thead>
<tr>
<th>Amino acid position$^a$</th>
<th>Mutation$^a$</th>
<th>Linkage group$^b$</th>
<th>TEM $\beta$-lactamase classification$^c$ (IRT)</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Met-69 (ATG)</td>
<td>Leu (CTG)</td>
<td>1B</td>
<td>TEM-33 (IRT-5)</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>Val (GTG)</td>
<td>2</td>
<td>TEM-34 (IRT-6)</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>Ile (ATT)</td>
<td>2</td>
<td>TEM-40 (IRT-11)</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>Ile + (ATA)</td>
<td>Thr-182 (ACG)</td>
<td>TEM-32 (IRT-3)</td>
<td>16</td>
</tr>
<tr>
<td>Arg-244 (CGC)</td>
<td>Ser (AGC)</td>
<td>1B</td>
<td>TEM-30 (IRT-2)</td>
<td>8, 18, 19</td>
</tr>
<tr>
<td></td>
<td>Cys (TG C)</td>
<td>2</td>
<td>TEM-31 (IRT-1)</td>
<td>8, 18</td>
</tr>
<tr>
<td></td>
<td>His (CA C)</td>
<td>1A</td>
<td>TEM-51 (IRT-15)</td>
<td>14</td>
</tr>
<tr>
<td>Arg-275 (CGA)</td>
<td>Leu + (CTA)</td>
<td>Val-69 (GTG)</td>
<td>TEM-38 (IRT-9)</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>Gin + (CAA)</td>
<td>Leu-69 (TTG)</td>
<td>TEM-45 (IRT-14)</td>
<td>13</td>
</tr>
<tr>
<td>Asn-276 (AAT)</td>
<td>Asp (GAT)</td>
<td>Leu-69 (CTG)</td>
<td>TEM-35 (IRT-4)</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>Asp (GAT)</td>
<td>Leu-69 (TTG)</td>
<td>TEM-35 (IRT-4)</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>Asp (GAT)</td>
<td>Leu-69 + (CTG)</td>
<td>TEM-39 (IRT-10)</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>Asp (GAT)</td>
<td>Val-69 (GTG)</td>
<td>TEM-36 (IRT-7)</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>Asp (GAT)</td>
<td>Ile-69 (ATA)</td>
<td>TEM-37 (IRT-8)</td>
<td>11</td>
</tr>
</tbody>
</table>

$^a$ABL numbering system of Ambler$^{50}$. The nucleotide triplet codons are indicated in parentheses.

$^b$The $\text{bla}$ gene framework(s) in which the mutation is found.

$^c$TEM $\beta$-lactamase classification.$^{72}$
et al.\textsuperscript{13} these mutations can be considered as either recurrent (and hence at hot spots for mutations), or much more ancient than others, allowing their occurrence on two different gene matrices. Contrasting with this situation, \(\beta\)-lactamases other than IRT-1, IRT-2 and IRT-6 are associated only with one of the sequence linkage groups, 'TEM-1A like', 'TEM-1B like' or 'TEM-2 like'.

It is significant to note that the IRT \(\beta\)-lactamases can be hyperproduced by mutation at the level of their promoter genes. Indeed, Caniça et al.\textsuperscript{13} have described two nucleotide substitutions in the promoter region of the \(\text{bla}\text{IRT}\) genes: C32 → T and G162 → T. The first substitution, C32 → T, characteristic of the gene promoter of TEM-2,\textsuperscript{30} was found in the promoter region of the TEM-1 gene of 15 clinical isolates of \(\text{E. coli}\).\textsuperscript{3} The second substitution, G162 → T, was found in the \(\beta\)-lactamase genes of TEM-30/IRT-2, TEM-35/IRT-4, TEM-34/IRT-6, TEM-36/IRT-7, TEM-37/IRT-8, TEM-39/IRT-10 and TEM-45/IRT-14.\textsuperscript{13,15} This G162 → T transversion falls within the functional \textsuperscript{10} Pribnow box and consequently renders the \textsuperscript{10} consensus region of the IRT \(\beta\)-lactamase genes more similar to the optimal promoter of \(\text{E. coli}\).\textsuperscript{31} The G162 → T transversion has been also described as the mechanism of TEM-1 hyperproduction in two ampicillin–sulbactam-resistant \(\text{Shigella flexneri}\) isolates from Hong Kong.\textsuperscript{32} These data are clear evidence for the convergent evolution of IRT enzymes because mutations have occurred independently on different gene frameworks (ancestor sequence), but all confer an identical IRT phenotype in response to selective pressure imposed by the clinical use of \(\beta\)-lactamase inhibitors.\textsuperscript{13}

### Biochemical data

**Kinetic parameters**

Kinetic parameters (\(k_{\text{cat}}\) and \(K_{m}\)) and catalytic efficiency (\(k_{\text{cat}}/K_{m}\)) of TEM-1 and IRTs are shown in Table VI. Generally, most IRT \(\beta\)-lactamases have lower catalytic efficiency values for all substrates than those of TEM-1. This results from a decrease of \(k_{\text{cat}}\) values and an increase of \(K_{m}\) values. The mutants which have one amino acid substitution at position 69 show catalytic efficiency values higher than those of other IRT mutants. This indicates the important contribution of residues 244, 275 and 276 in the enzyme–substrate interaction. It is noteworthy that all IRTs have high \(K_{m}\) values for ticarcillin (a carboxypenicillin). Similar results are obtained with carbenicillin, another carboxypenicillin (data not shown). For all IRTs this characteristic may be related to electrostatic interactions, as they have low \(K_{m}\) values for carfecillin (a phenyl ester of carbenicillin) (data not shown). The structures of ticarcillin, carbenicillin and carfecillin are shown in Figure 1. For the mutants at position 69, modelling suggests repulsion between the carboxylate of the side chain of ticarcillin (or carbenicillin) and a carboxylate of side chains of Glu-104 and Glu-240.\textsuperscript{33} Other residue(s) required for these electrostatic interactions are still to be found.

**Interaction with clavulanic acid, sulbactam and tazobactam**

The structures of \(\beta\)-lactamase inhibitors are shown in Figure 1. All the IRTs have IC\textsubscript{50} and \(K_{i}\) values for

![Figure 1. Structures of some substrates and \(\beta\)-lactamase inhibitors.](image-url)
Table VI. Kinetic data of TEM-1 and IRT β-lactamases

<table>
<thead>
<tr>
<th>β-Lactamase</th>
<th>Benzylpenicillin</th>
<th>Amoxycillin</th>
<th>Ticarcillin</th>
<th>Cephalothin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$k_{cat}$ (s$^{-1}$)</td>
<td>$K_m$ (μM)</td>
<td>$k_{cat}/K_m$ (μM$^{-1}$ s$^{-1}$)</td>
<td>$k_{cat}$ (s$^{-1}$)</td>
</tr>
<tr>
<td>TEM-1</td>
<td>1200</td>
<td>25</td>
<td>48</td>
<td>920</td>
</tr>
<tr>
<td>TEM-30 IRT-2$^a$</td>
<td>650</td>
<td>330</td>
<td>2</td>
<td>1300</td>
</tr>
<tr>
<td>TEM-31 IRT-1$^a$</td>
<td>790</td>
<td>460</td>
<td>2</td>
<td>1500</td>
</tr>
<tr>
<td>TEM-32 IRT-3</td>
<td>230</td>
<td>9</td>
<td>25</td>
<td>390</td>
</tr>
<tr>
<td>TEM-33 IRT-5</td>
<td>1653</td>
<td>45</td>
<td>37</td>
<td>1256</td>
</tr>
<tr>
<td>TEM-34 IRT-6</td>
<td>867</td>
<td>17</td>
<td>51</td>
<td>832</td>
</tr>
<tr>
<td>TEM-35 IRT-4</td>
<td>1011</td>
<td>155</td>
<td>6.5</td>
<td>910</td>
</tr>
<tr>
<td>TEM-36 IRT-7</td>
<td>900</td>
<td>60</td>
<td>15</td>
<td>1030</td>
</tr>
<tr>
<td>TEM-37 IRT-8</td>
<td>190</td>
<td>30</td>
<td>6</td>
<td>300</td>
</tr>
<tr>
<td>TEM-38 IRT-9</td>
<td>800</td>
<td>60</td>
<td>13</td>
<td>860</td>
</tr>
<tr>
<td>TEM-39 IRT-10</td>
<td>2100</td>
<td>220</td>
<td>10</td>
<td>2270</td>
</tr>
<tr>
<td>TEM-40 IRT-11</td>
<td>200</td>
<td>8</td>
<td>25</td>
<td>280</td>
</tr>
<tr>
<td>TEM-45 IRT-14</td>
<td>700</td>
<td>140</td>
<td>5</td>
<td>725</td>
</tr>
<tr>
<td>TEM-51 IRT-15</td>
<td>272</td>
<td>160</td>
<td>1.7</td>
<td>267</td>
</tr>
</tbody>
</table>

$^a$k$_{cat}$ values were determined in this study.

All kinetic parameters were determined by computerized micro-acidimetry as described in reference 76.
Inhibitor-resistant TEM \(\beta\)-lactamases

\(\beta\)-lactamase inhibitors higher than those of TEM-1 (Table VII). Those with mutations at position 69 exhibit lower \(IC_{50}\) and \(K_i\) values than those of other mutants. Sulbactam is a poor inhibitor of all the IRT \(\beta\)-lactamases (high \(IC_{50}\) and \(K_i\) values), whereas tazobactam was the most active inhibitor (low \(IC_{50}\) and \(K_i\) values), except against those mutations at position 69, indicating a more favourable interaction with the triazole ring-substituted penicillanic acid sulphone than with the naked sulphone. This finding is consistent with work published recently by Bonomo et al.\(^{34}\) This study complements and extends previous investigations in which clavulanic acid and tazobactam have been shown to be more effective \(\beta\)-lactamase inhibitors than sulbactam against extended-spectrum and conventional-spectrum enzymes and that clavulanic acid had activities equivalent to those of tazobactam.\(^{35,36}\)

### Relationship between structure and function

In the absence of crystal structures, most structure-function relationships of the IRT \(\beta\)-lactamases have been studied by molecular modelling. Two excellent reviews have recently discussed these relationships.\(^{37,38}\) Figure 2 shows the ribbon representation of the three-dimensional structure of a class A TEM-type \(\beta\)-lactamase, established at the atomic level by X-ray crystallography,\(^{39,40}\) together with the location of each of the point mutations. All the IRT variants arise from point mutations in the gene encoding either TEM-1 or TEM-2. At amino acid position 39, located at the end of the N-terminal \(\alpha\)-2 helix, TEM-1 enzymes have a glutamine and TEM-2 a lysine. The catalytic properties of these parental enzymes are slightly, but significantly, different.\(^{41,42}\)

### Residue 69

Residue 69 is rather variable in size and character among class A \(\beta\)-lactamases but is always in high conformational energy.\(^{39,40,43,44}\) The importance of this amino acid is not its closeness to the reactive Ser-70, but rather the position of its side chain behind the \(\beta\)-3 and \(\beta\)-4 strands. It is adjacent to the oxyanion binding pocket formed by the amides of Ser-70 and Ala-237. The molecular modelling showed that the methyl of Val-69 (C\(_1\)) and Ile-69 (C\(_2\)) produced steric constraints with the side chain of Ser-70 and Asn-170. The hydrophobicity could be the main factor responsible for the kinetic properties of the variant Met-69 Leu (TEM-33/IRT-5), as no steric effects could be detected by molecular modelling.\(^{33}\) Thus, hydrophobicity and steric constraints could be combined in the variants Met-69 Val (TEM-34/IRT-6) and Met-69 Ile (TEM-40/IRT-11). In addition, we speculate that residue 69 could interfere with the guanidinium group of Arg-244, as previously suggested for the Met-69 Ile mutant of the SHV-type OHIO-1 \(\beta\)-lactamase.\(^{45}\)
Residue 165
Located at the beginning of the \(\Omega\)-loop (position 161 to 179), the side chain of this residue is solvent-oriented. A change of Trp-165→Arg is found in the TEM-39/IRT-10 \(\beta\)-lactamase, but associated with two other substitutions at positions 69 and 276. The TEM-type variant Trp-165→Arg made by site-directed mutagenesis exhibited a slight decrease to the inhibitory effect of clavulanic acid. Molecular modelling suggests that the side chain of Arg-165 is able to form a salt bond with the \(\Omega\)-loop Glu-168 (unpublished data).

Residue 182
Located just before the \(\alpha-8\) helix (position 183 to 195), this residue is rather far from the binding site. A threonine is present in the TEM-32/IRT-3 \(\beta\)-lactamase. However, the enzyme contains a second change at position 69 that was shown to be the dominant factor in the resistance to \(\beta\)-lactamase inhibitors. Molecular modelling showed a novel hydrogen bond between the hydroxyl of Thr-182 and the carbonyl of the amide bond of Glu-64. That strengthens the dense hydrogen bond network that stabilizes the active site, and therefore was expected to be responsible for the increase in the catalytic activity of the TEM-32/IRT-3 \(\beta\)-lactamase compared with that of TEM-40/IRT-11. Moreover, Huang & Palzkill have recently demonstrated that the addition of the Met-182→Thr substitution to the TEM-1 variant Met-69→Ile increased the stability of the Met-69→Ile enzyme. The Met-182→Thr substitution may have been selected in natural isolates as a suppressor of folding or stability defects resulting from mutations associated with drug resistance. It is noteworthy that with the sequences of 28 class A \(\beta\)-lactamases previously aligned, TEM-1 was the sole protein exhibiting a Met at position 182, a position that generally has hydrogen bond-forming residues such as threonine, serine or cysteine.

Residue 244
Arg-244 is a relatively conserved residue on the \(\beta-4\) strand of class A \(\beta\)-lactamases, but when absent a basic residue (Arg or Lys) is found at position 220 or at position 276. It is anchored in place by two hydrogen bonds to Asn-276. Via a well-ordered, structurally conserved water molecule, it may interact with the C-3 (C-4) carboxylic acid group of \(\beta\)-lactams. However, Delaire et al. believe there are no direct interactions with the acid group and that the role of Arg-244 is to destabilize the enzyme product complex and optimize the turnover rate.

When Arg-244 is replaced by an amino acid with a short side chain such as cysteine, serine or histidine, the enzyme-substrate interaction is modified and affinity for the substrate decreases (Table V I). Moreover, the shorter side chains of these residues would be unable to activate the water molecule involved in the inactivation process of clavulinate. Sulbactam and tazobactam are thought to

Figure 2. Ribbon representation of a class A TEM-type \(\beta\)-lactamase. In the \(\beta\)-lactam binding site, the reactive Ser-70 is located at the N-terminus of the \(\alpha-2\) helix.\(^{39,40}\)
use a different mechanism and are not dependent on the structurally conserved water molecule. An unexpected finding is that the doubly mutated derivative of the TEM-1 enzyme (Ser-164/Ser-244) retains the characteristics of the Ser-164 mutant enzyme, e.g., enhanced activity against ceftazidime and sensitivity to inactivation by clavulinate, is perhaps due in part to structural changes resulting from the disruption of the Ω-loop. A rg-244 or a water molecule coordinated to its side chain also plays an essential role in the carbapenem tautomerization in the β-lactamase TEM-1 active site.

Residue 261

Located at the β-5 strand, its side chain is buried at the hydrophobic region far from the active site. The amino acid substitution Val-261→Ile is found in TEM-58,51 but is associated with the change A rg-244→Ser which is involved in the resistance of TEM-30/I RT-2 to β-lactamase inhibitors.

Residue 275

Located at the C-terminal of the α-11 helix, its side chain is in close vicinity to the guanidinium group of A rg-244. Substitution of A rg-275 by leucine or glutamine is found in the β-lactamases TEM-38/I RT-9 and TEM-45/I RT-14, respectively. However, these enzymes contain a second change at position 69 (V al or L eu). Kinetic study of the A rg-275→L eu variant of the TEM-type β-lactamase has shown the involvement of this change in the resistance to inactivation by clavulanic acid.60 This could be related to electrostatic interactions with A rg-244 and/or to a possible displacement of the water molecule involved in the inactivation.

Residue 276

The partially exposed side chain at residue 276 is on the C-terminal α-11 helix. In the TEM-1 β-lactamase the carbonyl group of A sn-276 accepts two hydrogen bonds from A rg-244 that orient the guanidinium group. The amino acid substitution A sn-276→A sp is found in the natural variants TEM-35/I RT-4, TEM-37/I RT-8 and TEM-39/I RT-10, but associated with another change at position 69. B run et al.,10 by comparing the kinetic properties of the TEM-35/I RT-4 enzyme and the M et-69→L eu variant of the TEM-type enzyme, have suggested a direct or an indirect role of A sp-276 in the catalytic mechanism. Thus, the TEM-type variant A sn-276→A sp made by site-directed mutagenesis exhibited decreased affinity and catalytic efficiency for β-lactam substrates, as well as a 20-fold higher K for clavulinate.61 The resistance to the inactivation process of clavulanic acid could be linked to electrostatic interactions with A rg-244 and/or to a possible displacement of the water molecule involved in the inactivation.

Conclusions

The emergence of I RT-producing strains might be related to the frequent use of clavulanate-containing formulations in hospitals and in general practice. The I RT-producing strains cannot, however, be detected reliably by routine susceptibility tests. Thus, this characterization must be completed by iso-electric points of β-lactamases, determination of kinetic parameters,64,65 and the use of molecular biology techniques.

Genetic studies argue in favour of the convergent evolution of the blaR T genes. It seems that such evolution of the parent TEM β-lactamase to resistance to β-lactamase inhibitors involves both forward and backward mutations,66 as previously suggested for TEM- and SHV-derived extended-spectrum β-lactamases.67 Recently the extended-spectrum β-lactamases TEM-AQ and TEM-50 (CMT-1) derived from TEM-1, which also have reduced susceptibility to clavulanic acid, provided a new example of convergence in this evolution process.68,69 On the other hand, inhibitor-resistant β-lactamases have also been reported in the SHV family.70,71

References


Inhibitor-resistant TEM β-lactamases

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