

IRT and CMT β-lactamases and inhibitor resistance

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ABSTRACT

Acquired resistance to penicillin-β-lactamase inhibitor combinations in Escherichia coli is due to: (i) penicillinase hyperproduction due to the presence of the blaTEM-1 gene in small multicopy plasmids or strong promoters; (ii) overproduction of constitutive AmpC cephalosporinase; and (iii) OXA-type and inhibitor-resistant TEM (IRT) β-lactamases. IRT enzymes emerge via mutational events from TEM-1 or TEM-2 β-lactamases that affect substrate affinity for β-lactamase inhibitors. They are mainly isolated in urinary infections from community patients. Prevalence is variable, depending on geographical area, detection methods and potential selection pressure. These enzymes may evolve into complex mutants (CMT enzymes), which also confer resistance to extended-spectrum cephalosporins. CTX-M enzymes with the IRT phenotype have not been detected to date. New studies of IRT enzymes, including population structure, association with virulence traits and plasmid dispersion, are needed.

Keywords Class A β-lactamase inhibitors, clavulanate, inhibitor-resistant β-lactamases, IRT, review

Clin Microbiol Infect 2008; 14 (Suppl. 1): 53–62

INTRODUCTION

Production of β-lactamases is the most important mechanism of resistance against β-lactam antibiotics. These enzymes constitute a family of proteins that degrade or modify the β-lactam drugs before they can reach the penicillin-binding protein target sites. They covalently bind to the carbonyl moiety of the β-lactam ring and hydrolyse its amide bond [1,2]. Although the first recognised β-lactamase was identified in 1940 in Escherichia coli (formerly Bacillus coli), the importance of this resistance mechanism was not perceived until 1944, when penicillinase production in Staphylococcus aureus was associated with clinical failure. At that time, fewer than 10% of S. aureus isolates were able to produce this enzyme, but by the 1950s this proportion had reached as high as 80%. The problem of β-lactamase production in Gram-negatives became apparent in the 1960s with the first description of an E. coli isolate resistant to aminopenicillins due to the production of TEM-1 β-lactamase. The corresponding bla gene encoding this enzyme was carried in a transposable element (Tn3) by plasmids of different incompatibility groups, which subsequently were responsible for the rapid increase in prevalence of TEM-1-producing isolates. Both staphylococcal penicillinase and TEM-1 enzymes belong to Ambler class A of the serine β-lactamases. Other serine β-lactamases belong to classes C and D, whereas zinc metallo-β-lactamases belong to class B.

During the 1970s and 1980s, there were different approaches to circumvent β-lactamase-mediated resistance, including the development of β-lactam compounds resistant to hydrolysis, and of β-lactamase inhibitors. The latter strategy gave rise to a series of different compounds such as boronates, phosphonates, and β-lactam-related compounds that act as suicide inhibitors. This allowed the introduction of commercial class A β-lactamase inhibitors with high affinity for β-lactamases, acting as ‘suicide substrates’ of these enzymes [3]. This group includes clavulanate, sulbactam and tazobactam [2]. All of these are effective in inhibiting broad-spectrum β-lactamases such as TEM-1, TEM-2 and SHV-1 and their extended-spectrum β-lactamase (ESBL) variants, along with the more recently described CTX-M enzymes [4,5]. Nevertheless, bacteria have developed β-lactamase variants that are able to
resist the action of suicide inhibitors. Within this group are the so-called inhibitor-resistant TEM (IRT) β-lactamases, which emerged in the 1990s, being mainly found in urine isolates [6–9]. Although these enzymes have not achieved the prominence of ESBLs, they have been associated with clinical failure with the use of β-lactam–β-lactamase inhibitor combinations [10–12].

**MECHANISMS AFFECTING β-LACTAM–Β-LACTAMASE INHIBITOR COMBINATIONS IN GRAM-NEGATIVE BACTERIA**

Resistance to inhibitors of class A β-lactamases and their commercial combinations with β-lactam antibiotics (amoxycillin–clavulanate, ampicillin–sulbactam, ticarcillin–clavulanate, cefoperazone–sulbactam and piperacillin–tazobactam) may emerge as a consequence of different mechanisms. In some cases, there are intrinsic resistance mechanisms due to natural production of different chromosomal β-lactamases that are not inhibited, or are weakly inhibited, by these β-lactamase inhibitors, such as the AmpC β-lactamases in *Enterobacter*, *Citrobacter*, *Serratia*, *Morganella* and *Pseudomonas aeruginosa*, or metallo-β-lactamases such as L1 in *Stenotrophomonas maltophilia* [2]. In other cases, hyperproduction of constitutive chromosomal β-lactamases can also reduce the activity of β-lactam–β-lactamase inhibitor combinations. This is the case for AmpC hyperproduction in *E. coli* and SHV-1 hyperproduction in *Klebsiella pneumoniae* isolates [13–16].

The hyperproduction of TEM-1 β-lactamase due to the presence of highly efficient promoters or to the presence of the corresponding bla gene in different copies may result in the loss of susceptibility to amoxycillin–clavulanate and other β-lactam–β-lactamase inhibitor combinations. In the first case, strong promoters have been shown with TEM-1 and TEM-2 enzymes and their corresponding ESBL variants, as well as with SHV ESBLs [17–21] In the second case, multiple blaTEM-1 copies may arise as a consequence of their presence in the same plasmid or of being encoded by small plasmids, with at least ten copies per bacterial chromosome [18,22]. Hyperproduction of SHV-1 or SHV ESBL variants has also been described, but with lower frequency than that of TEM-1 [20,23]. Nevertheless, the simultaneous presence of an ESBL and a broad-spectrum β-lactamase is not an infrequent event, thus increasing the net quantity of β-lactamase that needs to be inhibited by the β-lactamase inhibitor. This situation slightly reduces the susceptibility to β-lactam–β-lactamase inhibitor combinations [15].

In *E. coli*, resistance to inhibitor combinations may emerge if susceptible enzymes occur in combination with permeability deficiencies involving OmpF and/or OmpC porins [24]. The lack of one or two of these porins does not significantly affect the susceptibility to either β-lactam agent, alone or combined; however, it becomes relevant when both are associated in the presence of a β-lactamase. Moreover, the concomitant presence of different β-lactamases also affects β-lactam–β-lactamase inhibitor combinations. This is particularly important when OXA-type β-lactamases are expressed, as these enzymes are only weakly inhibited by clavulanate and other inhibitors of class A β-lactamases [25]. The presence of these enzymes consequently reduces the activity of inhibitor combinations [26,27].

**IRT ENZYMES**

**Definition**

IRT enzymes represent an adaptive resistance mechanism specifically developed by bacteria to overcome the activity of β-lactamase inhibitors [7].

IRT enzymes (Bush–Jacoby–Medeiros group 2br) comprise a group of plasmid-encoded variants of TEM-1 and TEM-2 with decreased affinities for amino-, carboxy- and ureido-penicillins and altered interactions with irreversible suicide inhibitors such as clavulanate, sulbactam and tazobactam. IRT-producing isolates remain susceptible to narrow- and extended-spectrum cephalosporins, cephamycins, carbapenems and, in most cases, piperacillin–tazobactam. However, they are resistant to ampicillin–sulbactam and intermediately resistant or resistant to amoxycillin–clavulanate [28] (Table 1). Amino-acid replacements at various positions in the original TEM enzymes are responsible for the resistance profiles of IRT producers (Table 2).

Initially found in *E. coli* [29], IRTs have also been reported in *Klebsiella* spp., *Enterobacter cloacae*, *Proteus mirabilis*, *Citrobacter freundii* and *Shigella sonnei* [28,30]. They were originally named TRC (TEM enzymes resistant to
clavulanic acid) [29], and later TRI (TEM resistant to β-lactamase inhibitors) [31], and were finally named IRT [10]. The presence of IRTs in *P. aeruginosa* or other non-fermenters has not been reported. The occurrence of an undetectable IRT phenotype in these genera, which cannot be completely ruled out, could be attributable to the presence of a superimposed resistance mechanism masking the IRT enzyme.

**Structure–function relationships**

The inhibition mechanisms exerted by clavulanate, sulbactam and tazobactam are quite similar Table 1. β-Lactam phenotypes due to the expression of different types of β-lactamase in *Escherichia coli*

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<th>β-Lactamase</th>
<th>AMX</th>
<th>AMC</th>
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<th>T/C</th>
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<th>1st-CEP</th>
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AMX, amoxycillin; AMC, amoxycillin–clavulanate; TIC, ticarcillin; T/C, ticarcillin–clavulanate; PIP, piperacillin; P/T, piperacillin–tazobactam; 1st-CEP, first-generation cephalosporins; FOX, cefoxitin; 3rd-CEP, third-generation cephalosporins; ESBL, extended-spectrum β-lactamase; S, susceptible; R, resistant; I, intermediate.

*Hyperproduction of the corresponding β-lactamase.

*Fourth-generation cephalosporins might be affected.

In some ESBL producing isolates, third-generation cephalosporins may be less affected.

**Table 2.** Amino-acid substitutions in inhibitor-resistant β-lactamases derived from TEM-1 and TEM-2 based on http://www.lahey.org/studies/temtable.asp

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</table>

NA, not available.
and involve a secondary covalent cross-linking reaction (between Ambler positions Ser70 and Ser130 of TEM β-lactamase) after the initial nucleophilic attack by the enzyme on the β-lactam ring [32]. Analysis of X-ray crystallographic structure and site-directed mutagenesis have been used to assess the effect of specific amino-acid substitutions in IRT enzymes and the corresponding diminution or even abolition of β-lactam inhibitor activity. The basis of resistance to inhibition consists of a set of subtle but crucial structural modifications due to amino-acid changes (confirmed by atomic resolution) that lead to the perturbation of the local environment of the cross-linking point (Ser130 in the parental enzyme), but have less effect on the hydrolytic mechanism. These amino-acid replacements, either alone or in combination, are usually found at positions Met69, Arg244, Asn276 and Leu275 of TEM-1 and -2 [6,9]. Although infrequently found among clinical isolates, some IRT variants (TEM-59, -76 and -89) have a simple amino-acid change, specifically, substitution of the highly conserved Ser130 itself (in all cases by a glycine residue), thereby preventing the mechanism-based inhibition [32,33]. Substitutions of other residues, such as Trp165, Met182 and Val261, have also been reported, as well as silent mutations that do not affect the amino-acid sequence from progenitor blaTEM genes [9,10,34] (Table 2). The traceability of blaIRT genes enables them to be grouped in three principal linkage groups: TEM-1A-like, TEM-1B-like and TEM-2-like. Further analysis of promoter and coding region sequences of blaIRT genes demonstrated that a given mutation could be associated with two different gene sequence frameworks, and two different mutations could reside in one and the same framework. A convergent evolution model is therefore accepted for IRT enzymes, as mutations have occurred independently in different gene frameworks [35]. Consumption of β-lactam–β-lactamase inhibitor formulations is considered to be a risk-factor for the selection of IRT enzymes. It has been confirmed that avoidance of strong exposure to these associations is clinically relevant in order to circumvent the risk of treatment failures [12].

IRT kinetic parameters and inhibitor profiles

$K_{cat}$, $K_m$ and catalytic efficiency ($K_{cat}/K_m$) data reveal that most IRT enzymes have lower catalytic efficiency for all substrates than does TEM-1, due to decreased $K_{cat}$ and high $K_m$ values [9]. IC$_{50}$s of inhibitors are higher than those of TEM-1, and sulbactam is the least effective inhibitor against these enzymes (highest IC$_{50}$ and $K_m$ values) [7,36]. The piperacillin–tazobactam combination retains inhibitory activity against most isolates with IRT enzymes, probably owing to the increased activity of piperacillin as compared with that of amoxycillin and the strong inhibitory activity of tazobactam, except when mutations at position 69 are present [37]. Nevertheless, it is of note that this combination appears, at least in vitro, to be bacteriostatic, with bacterial regrowth consistently detected at 24 h [9].

Geographical distribution and epidemiology of IRT-harbouring isolates

Despite the overuse of β-lactam–β-lactamase inhibitor formulations in most countries, IRTs have been more frequently found in Europe than in the USA, where they have been rarely reported [38]. Their presence has also been detected in Malaysian E. coli isolates [39]. However, there is a clear dearth of information about IRT geographical distribution, probably due to insufficient identification using standard laboratory susceptibility tests.

IRT β-lactamases occur among both community- and hospital-acquired isolates. In general, the pattern of the appearance of IRTs in both settings cannot be ascribed to an epidemic phenomenon. Conversely, the independent emergence of these variants in non-related strains may be explained by the strong selective pressure exerted by overuse of β-lactam–β-lactamase inhibitor combinations [34].

Reports on the prevalence of clinical E. coli isolates with IRT enzymes among those with reduced susceptibility or resistance to amoxycillin–clavulanate are scarce. To date, two main studies have been published in Spain, reporting percentages of 5.4% [40] and 9.5% [41]. In a recent follow-up in our hospital, the prevalence of E. coli isolates resistant to clavulanate was lower than 5%, with fewer than 3% of isolates displaying an IRT phenotype (unpublished data). In France, the reported frequency in 1993 of resistance to amoxycillin–clavulanate (MIC >16/2 mg/L) was 25.0% in hospital isolates and 10.0% in community urinary tract infection.
is a geriatric facility in France. The IRT-2-producing K. pneumoniae is the result of the dissemination of clonally related enzyme has been described. This outbreak was observed [39]. Isolates from hospitalised patients in Malaysia of IRTs in clinical E. coli isolates has been reported [43]. Contributing to these data, an incidence of 16.1% of IRTs in clinical E. coli isolates from hospitalised patients in Malaysia has been observed [39].

At present, only one outbreak involving an IRT enzyme has been described. This outbreak was the result of the dissemination of clonally related IRT-2-producing K. pneumoniae isolates recovered in a geriatric facility in France. The \texttt{bla}_{\text{IRT-2}} gene was harboured in a non-conjugative plasmid [44].

**Detection of IRTs**

Standard in-vitro susceptibility tests are not sufficiently reliable for identification of IRTs, and discrepancies have been observed when comparing disk-diffusion and MIC results, particularly in those isolates with intermediate resistance values. Differences between breakpoint values of international guidelines and inhibitor concentration make comparisons even more complicated, particularly comparison of results reported from various surveillance studies [38]. Disk inhibition diameters of cefepime, ceftazidime and mecillinam have been suggested to improve IRT detection [45]. Additional determinations of pI and kinetic parameters are useful tools, although final confirmation by molecular techniques, comprising PCR of the coding gene and subsequent sequencing of the product, are crucial for definite identification.

Another conflicting aspect of susceptibility testing relates to the discrepant values obtained when using a fixed \(\beta\)-lactam/\(\beta\)-lactamase inhibitor ratio (e.g., 2:1 amoxycillin to clavulanate) as opposed to a fixed concentration of inhibitor (2 mg/L or 4 mg/L for clavulanate) [28,41,46]. It is generally accepted that these different approaches do not produce comparable results. In general, the use of a fixed concentration of 2 mg/L of clavulanate (still not standardised worldwide) is preferable for detection of the presence of IRTs, as isolates containing these enzymes appear to be resistant at the breakpoint of \(\geq 32\) mg/L, while they are often categorised as susceptible or intermediate when using the fixed 2:1 ratio. A fixed concentration of clavulanate with amoxycillin is advocated as the best predictor of likely clinical efficacy [47]. Another factor to take into account is that the level of resistance to inhibitor combinations correlates with the amount of IRT enzyme synthesised, again meaning that low-level production could be undetectable when using the fixed 2:1 ratio [47,48].

As a consequence of these challenges to detection, the prevalence of IRT enzymes is probably underestimated. Moreover, and particularly among E. coli isolates, other resistance mechanisms, such as the hyperproduction of parental TEM \(\beta\)-lactamases or the presence of OXA-type enzymes, may mask or confound a first-glance assessment of an IRT profile. Although more discernable, the concomitant presence of porin-mediated loss of permeability, a low level of chromosomal AmpC production or a low-level-expressed, plasmid-mediated AmpC (generally of the CMY type) may make the detection of an IRT enzyme even more difficult [48].

**EVOLUTION OF IRT ENZYMES AND IRT-PRODUCING ISOLATES**

As previously stated, IRT enzymes have been mainly described in E. coli isolates and also in isolates that are naturally resistant to \(\beta\)-lactam–\(\beta\)-lactamase inhibitor combinations due to chromosomal AmpC production. In the early descriptions, IRT enzymes were found in E. coli and C. freundii isolates from calf faeces [49]. More recently, TEM-80 (or IRT-24) \(\beta\)-lactamase has been characterised in an E. cloacae isolate that was simultaneously recovered with an E. coli isolate displaying an IRT phenotype from the urine of a patient living in a nursing home [50].
isolates. In addition, TEM-30 (or IRT-2) has been detected in a *K. pneumoniae* epidemic strain in New York, harbouring an acquired carbapenemase (KPC-2) [43]. Phenotypic detection of the expression of IRT enzymes in these isolates is difficult, and this enzyme was recognised only after molecular studies.

From an evolutionary point of view, these epidemiological findings raise speculations about why a given bacterium harbours different β-lactamase genes that affect the same antimicrobials. In a natural AmpC producer, this can be explained by a mutational event in a previously acquired *bla*<sub>TEM</sub>-1 or *bla*<sub>TEM</sub>-2 gene, whereas in a carbapenemase-producing isolate, it might be a consequence of the secondary acquisition of a *bla*<sub>IRT</sub> gene. This could also be the case in *E. coli* or *K. pneumoniae* isolates expressing inhibitor-susceptible β-lactamases such as the TEM-1, TEM-2 or SHV-1 broad-spectrum enzymes [51], in chromosomal OXY-2-producing *Klebsiella oxytoca* isolates [52] or even in CTX-M-producing enterobacterial isolates [53].

Different substitutions in the promoter region of the *bla*<sub>IRT</sub> gene coding region can also affect expression of IRT enzymes, thus affecting potential selection of IRT-producing isolates. This has been noted not only in clinical isolates [35] but also in laboratory-derived mutants [21]. The introduction of the four promoters known to control *bla*<sub>TEM</sub> gene expression in *E. coli* isolates harbouring *bla*<sub>TEM-30</sub> gradually increased IRT activity and thus amoxycillin–clavulanate MIC values in relation to the presence of promoters P3, PPa/Pb and P4 upstream of the corresponding gene. Promoter P5, only found upstream of the *bla*<sub>TEM-1B</sub> gene, was related to the highest expression [21].

### Complex mutant TEM β-lactamases

One of the most interesting aspects of recent evolution of IRT enzymes is that, in some cases, mutations affecting *bla*<sub>TEM</sub> may affect the activity of both β-lactamase inhibitors and the extended-spectrum cephalosporins. These variants have been named CMT enzymes (or complex mutant TEM β-lactamases) and require concurrence of mutations in the β-lactamase genes of the ESBL and IRT variants (Table 3). They have been identified in different Enterobacteriaceae, including *E. coli*, *K. pneumoniae*, *P. mirabilis* and Enterobacteriaceae.

<table>
<thead>
<tr>
<th>Designation</th>
<th>Isolate of first description</th>
<th>Country, year</th>
<th>Source</th>
<th>Isolate</th>
<th>Mutations</th>
<th>PI</th>
<th>AMC</th>
<th>AMC</th>
<th>CEF</th>
<th>CAZ</th>
<th>CTX</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>CMT-1</td>
<td><em>Escherichia coli</em></td>
<td>France, 1997 (R)</td>
<td>Faeces</td>
<td>TEM-35</td>
<td>(IRT-4)</td>
<td>5.6</td>
<td>64&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8</td>
<td>8</td>
<td>64&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1</td>
<td>[54]</td>
</tr>
<tr>
<td>CMT-2</td>
<td><em>Klebsiella pneumoniae</em></td>
<td>Poland, 1996</td>
<td>Various</td>
<td>TEM-35</td>
<td>(IRT-9, 14)</td>
<td>5.7</td>
<td>ND</td>
<td>8</td>
<td>0.25</td>
<td>&lt;0.06</td>
<td>&lt;0.06</td>
<td>[55]</td>
</tr>
<tr>
<td>CMT-3</td>
<td><em>Proteus mirabilis</em></td>
<td>France, 2001</td>
<td>Urine</td>
<td>TEM-35</td>
<td>(IRT-17)</td>
<td>6.3</td>
<td>16</td>
<td>256</td>
<td>256</td>
<td>0.06</td>
<td>0.06</td>
<td>[56]</td>
</tr>
<tr>
<td>CMT-4</td>
<td><em>Enterobacter aerogenes</em></td>
<td>France, 2002</td>
<td>Faeces</td>
<td>TEM-35</td>
<td>(IRT-2)</td>
<td>5.9</td>
<td>32&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.25</td>
<td>&lt;1024&lt;sup&gt;a&lt;/sup&gt;</td>
<td>64</td>
<td>0.06</td>
<td>[57]</td>
</tr>
<tr>
<td>CMT-5</td>
<td><em>Escherichia coli</em></td>
<td>France, 2001</td>
<td>Faeces</td>
<td>TEM-35</td>
<td>(IRT-10)</td>
<td>5.3</td>
<td>32</td>
<td>0.125</td>
<td>1024&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8</td>
<td>0.06</td>
<td>[58]</td>
</tr>
<tr>
<td>CMT-6</td>
<td><em>Klebsiella pneumoniae</em></td>
<td>France, 2004</td>
<td>Urine</td>
<td>TEM-35</td>
<td>(IRT-17)</td>
<td>5.7</td>
<td>16</td>
<td>512</td>
<td>256</td>
<td>0.06</td>
<td>0.06</td>
<td>[60]</td>
</tr>
<tr>
<td>CMT-7</td>
<td><em>Escherichia coli</em></td>
<td>France, 2006</td>
<td>Faeces</td>
<td>TEM-35</td>
<td>(IRT-14)</td>
<td>5.3</td>
<td>32</td>
<td>1024&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.25</td>
<td>0.06</td>
<td>[61]</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>2m g/L fixed clavulanate concentration.
<sup>b</sup>Phenotype resembling IRT enzymes.

Table 3. Complex mutant TEM (CMT) β-lactamases

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robacter aerogenes [54–60]. They variably affect oxyimino-cephalosporin and β-lactamase inhibitor activities. The corresponding phenotype, due to the expression of CMT β-lactamases in E. coli in comparison with other β-lactamases, is presented in Table 1. The expression is not homogeneous and can affect different cephalosporins and penicillin-β-lactamase inhibitor combinations to different extents. Recently, a new CMT-type β-lactamase, TEM-125, has been characterised in a urinary E. coli isolate; this combines mutations previously described in the ESBL TEM-12 and in the IRT TEM-39 (IRT-10) [34,59], conferring high-level resistance to ceftazidime (MICs of 16 mg/L) but not to cefotaxime (0.06 mg/L), and to penicillin-clavulanate combinations (MIC of amoxycillin–clavulanate, 1024/2 mg/L). This phenotype is similar to that conferred by the last described CMT enzymes, TEM-151 and TEM-152 [60].

Detection of isolates harbouring CTM enzymes, because of their clinical implications, could represent an emerging microbiology problem. The efficiency of ancillary tests based on the synergy of oxyimino-cephalosporins and clavulanate, including the double-disk synergy test, can be reduced in these isolates as a result of high-level resistance to clavulanate and the variable effect on cephalosporins.

As with other mutational events, the emergence of CMT β-lactamase variants might represent a selection process within a selection compartment [61]. Most IRT enzymes, as well as CMT enzymes, have been recovered from urinary isolates, where amoxycillin–clavulanate and other penicillin-β-lactamase inhibitor combinations reach high concentrations. Potential selection of producers might also occur in the bowel, where these antibiotics can produce different selective concentrations over time and, indeed, some of the new CMT variants have been characterised in isolates recovered from faeces [55,58,60]. In addition, a higher prevalence of faecal carriage with amoxycillin–clavulanate-resistant Gram-negative bacilli, including E. coli isolates with IRT enzymes, was demonstrated in patients treated with this combination than in those treated with third-generation cephalosporins or fluoroquinolones [42]. These faecal isolates can later produce urinary tract infections or episodes of bacteremia.

The emergence of mutations conferring both oxyimino-cephalosporin and clavulanate resistance in CMT variants is also matter for speculation. We can hypothesise that the selection of ESBL mutations, which do not affect clavulanate, took place first, and then the selection of IRT mutations; however, the opposite sequence cannot be excluded. Moreover, although its frequency should be lower, the simultaneous selection of both variants in the same context cannot be excluded. An in-vitro model using a hyper-mutable E. coli strain and a recombinant plasmid containing the class A β-lactamase ROB-1 gene was used in selection experiments [62]. Serial passages in tubes containing increasing concentrations of cefotaxime or amoxycillin–clavulanate revealed the difficulty in obtaining resistance to oxyimino-cephalosporins and clavulanate in the same enzyme. Other research has shown that there is frequent incompatibility between both resistance phenotypes, which may explain the low prevalence of CMT enzymes, as compared with that of the IRT or ESBL types [63,64].

Complex mutants of SHV variants with ESBL mutations and those resembling IRT enzymes have only very rarely been described, but the corresponding phenotypes are closely related to those conferred by the IRT enzymes [65,66]. The first example is the SHV-10 variant, which was identified in a urinary E. coli strain resistant to amoxycillin–clavulanate from a patient previously treated with this combination. The second case is the SHV-49 enzyme from a K. pneumoniae isolate resistant to amoxycillin–clavulanate recovered from a patient treated for over 50 days with this combination. Both isolates were susceptible to expanded-spectrum cephalosporins, despite mutations in both enzymes affecting these β-lactams.

Inhibitor resistance in CTX-M-producing isolates

The concomitant presence of IRT enzymes in producers of CTX-M enzymes has been rarely described [53]. Moreover, to date, IRT- or CMT-like enzymes derived from CTX-M types have not been detected. Rather, the frequent absence of susceptibility to amoxycillin–clavulanate in isolates harbouring CTX-M enzymes is due to the simultaneous presence of other β-lactamases, including OXA-1 and/or TEM-1, in the same genetic context. The best example is that of
CTX-M-15, which is commonly associated with both OXA-1 and TEM-1, conferring reduced susceptibility to the amoxicillin–clavulanate combination [25,67].

**THERAPEUTIC OPTIONS IN INFECTIONS DUE TO PATHOGENS WITH IRT ENZYMES**

As previously stated, the occurrence of IRT enzymes has been associated with the clinical failure of β-lactam–β-lactamase inhibitor combinations, particularly in urinary tract infections [10–12]. Therapeutic options for due to ESBL or carbapenemase producing strains are much less limited than those due to ESBL or carbapenemase producers, unless further narrowed by the presence of other resistance mechanisms affecting β-lactam or non-β-lactam antibiotics.

In outpatients with non-complicated urinary tract infections, the use of second-and third-generation oral cephalosporins, e.g., cefuroxime or cefixime, would be advised if *E. coli*—and not AmpC-inducible species—are involved. If susceptibility is demonstrated, the alternatives include fluoroquinolones, fosfomycin, nitrofurantoin, co-trimoxazole or even intramuscular amingoglycoside, in the case of complicated urinary infections. For hospitalised patients who require intravenous therapy, second-generation and expanded-spectrum cephalosporins, aztreonam and carbapenems, including ertapenem, can be recommended, as well as fluoroquinolones and aminoglycosides. As previously stated, piperacillin–tazobactam retains inhibitory activity against most isolates with IRT enzymes, but no clinical information regarding its use in this situation has been published.

**CONCLUSION AND FUTURE DIRECTIONS**

IRT enzymes emerged via mutations of broad-spectrum β-lactamases, mainly from TEM-1. Most of the information concerning these enzymes originated during the 1990s, and the number of subsequent studies updating this knowledge, or surveys including the prevalence of these enzymes, is small. Population structure analysis of isolates producing these enzymes has not been undertaken, and the potential clonal spread or the association of IRT enzymes with specific *E. coli* clonal complexes or virulence traits have not been studied. Moreover, no information is available concerning the role of natural or transient mutators in the evolution of IRT enzymes. Likewise, studies on the mobilisation of *bla*<sub>IRT</sub> genes and their association with plasmids, similar to those performed with ESBL and carbapenemase genes, have not been undertaken. From an epidemiological and clinical point of view, it is necessary to generate recommendations for better detection of isolates producing these enzymes and to enhance our knowledge of risk-factors for patients.

**REFERENCES**


