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Review

Molecular epidemiology of *Escherichia coli* producing CTX-M β-lactamases: the worldwide emergence of clone ST131 O25:H4

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ABSTRACT

Since 2000, *Escherichia coli* producing CTX-M enzymes have emerged worldwide as important causes of community-onset urinary tract and bloodstream infections owing to extended-spectrum β-lactamase (ESBL)-producing bacteria. Molecular epidemiological studies suggested that the sudden worldwide increase of CTX-M-15-producing *E. coli* was mainly due to a single clone (ST131) and that foreign travel to high-risk areas, such as the Indian subcontinent, might in part play a role in the spread of this clone across different continents. Empirical antibiotic coverage for these resistant organisms should be considered in community patients presenting with sepsis involving the urinary tract, especially if the patient recently travelled to a high-risk area. If this emerging public health threat is ignored, it is possible that the medical community may be forced, in the near future, to use carbapenems as the first choice for the empirical treatment of serious infections associated with urinary tract infections originating from the community.

1. Introduction

The extended-spectrum β-lactamases (ESBLs) are a group of enzymes with the ability to hydrolyse and cause resistance to the oxyimino-cephalosporins (i.e. cefotaxime, ceftazidime, ceftriaxone, cefuroxime and cefepime) and monobactams (i.e. aztreonam), but not the cephamycins (i.e. cefoxitin and cefotetan) or carbapenems (i.e. imipenem, meropenem, doripenem and ertapenem) [1]. These enzymes are inhibited by the so-called 'classical' β-lactamase inhibitors such as clavulanic acid, sulbactam and tazobactam. The majority of ESBLs belong to the class A Ambler classification and include the SHV or TEM types that have evolved from parent enzymes (e.g. TEM-1, -2 and SHV-1) due to point mutations around the active site of the β-lactamases [1]. ESBLs are often located on large plasmids that also harbour genes for resistance to other antimicrobial classes and therefore will often exhibit multidrug-resistant (MDR) phenotypes including resistance to aminoglycosides and co-trimoxazole.

Organisms, especially *Klebsiella* spp., producing SHV and TEM types of ESBLs have traditionally been responsible for serious nosocomial infections. Specific risk factors for acquisition of these bacteria identified previously include length of hospital stay, severity of illness, time in the Intensive Care Unit (ICU), intubations with mechanical ventilation, urinary or arterial catheterisation, and previous exposure to antibiotics [1]. The majority of patients infected with ESBL-producing organisms have been admitted to ICUs, but infection can also occur in almost any other area of the hospital.

ESBL-producing organisms are also isolated with increasing frequency from patients in extended-care facilities [2]. Infections caused by ESBL-producing bacteria are often associated with increased morbidity, mortality and healthcare-associated costs [3,4].

Organisms producing ESBLs are clinically relevant and have become important players among antimicrobial-resistant organisms. A report from the Infectious Diseases Society of America (IDSA) in 2006 listed ESBL-producing *Klebsiella* spp. and *Escherichia coli* as priority drug-resistant microbes to which new therapies are urgently required [5].

2. CTX-M β-lactamases

CTX-M β-lactamases (i.e. 'active on CefoTaXime, first isolated in Munich') were first reported from Japan in 1986 (the enzyme was initially named TOHO-1 and was later changed to CTX-M) [6]. During the 1990s, general dissemination and occasional nosocomial outbreak, mostly of CTX-M-2-producing Enterobacteriaceae, were reported from South America (especially Argentina) (Gabriel Gutkind, personal communication) [7,8]. However, since 2000, *E. coli* producing CTX-M β-lactamases have emerged worldwide as

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an important cause of community-onset urinary tract infections (UTIs) and this has been called 'the CTX-M pandemic' [9–11]. This phenomenon accelerated rapidly, especially during the past 5 years, and today these enzymes are the most common type of ESBL found in most areas of the world [12].

The CTX-M β-lactamases are encoded by genes that have been captured by mobile elements (such as insertion sequence IS*Ecp1*) from the chromosomes of the environmental bacteria called *Kluyvera* spp. [13]. Several studies have reported that dissemination of $bla_{\text{CTX-M}}$ genes is associated with highly efficient mobile genetic elements, including the IS*Ecp1*, IS*CR1* or phage-related sequences [14]. IS*Ecp1* plays an important role in the expression and continuous spread of these β-lactamases [15]. The genes responsible for CTX-M β-lactamases are encoded by plasmids belonging to the narrow host-range incompatibility types (i.e. IncFI, IncFII, IncHI2 and IncI) or the broad host-range incompatibility types (i.e. IncN, IncP-1-a, IncL/M and IncA/C) [16].

Although CTX-M enzymes also belong to the class A Ambler classification, they are not related to the TEM or SHV types of ESBLs. Presently, CTX-M β-lactamases include more than 80 different enzymes that are clustered into five groups based on their amino acid identities and include the CTX-M-1, -2, -8, -9 and -25 groups [17]. Members of these clusters exhibit >94% amino acid identity within each group and ≤90% amino acid identity between the different groups.

Risk factors for acquiring community-onset infections due to CTX-M-producing *E. coli* include repeat UTIs, underlying renal pathology, previous antibiotics including cephalosporins and fluoroquinolones, previous hospitalisation, nursing home residents, diabetes mellitus, underlying liver pathology and international travel [18,19].

3. CTX-M-15-producing *Escherichia coli*

3.1. Introduction

Currently, the most widely distributed CTX-M enzyme on a worldwide basis is CTX-M-15, which was first detected in *E. coli* isolated from India during 2001 [20]. CTX-M-15 belongs to the CTX-M-1 cluster and is derived from CTX-M-3 by one amino acid substitution at position 240 (Asp → Gly); however, the flanking sequences of the β-lactamases can be very different. This substitution confers an increased catalytic activity against ceftazidime, and bacteria producing these enzymes often test resistant to this agent [21]. Mobilisation and production of CTX-M-15 is also associated with the insertion element IS*Ecp1* located 49 bp upstream of $bla_{\text{CTX-M-15}}$ [20,22].

The CTX-M-15 β-lactamase has often been associated with co-production of other β-lactamases such as TEM-1 and OXA-1 as well as the aminoglycoside-modifying enzyme *aac(6′)-Ib-cr* [23]. *aac(6′)-Ib-cr* has the additional ability to acetylate fluoroquinolones with an unprotected amino nitrogen on the piperazine ring, including norfloxacin and ciprofloxacin but not levofloxacin [24]. Production of CTX-M-15, TEM-1, OXA-1 and *aac(6′)-Ib-cr* has been linked to epidemic narrow host-range IncFII plasmids [16].

3.2. Distribution of CTX-M-15-producing Escherichia coli

MDR CTX-M-15-producing *E. coli* are emerging worldwide, especially since 2003, as an important pathogen causing community-onset and hospital-acquired infections [10] and have been reported from most countries in Europe [25], some countries in Asia [26], Africa [12], North America [27,28], South America [29] and Australia [30].

CTX-M-15 β-lactamases are the most common type of ESBLs identified in Europe and have been increasingly described in community isolates, particularly associated with infections in healthcare-associated patients [31]. Widespread dispersion of CTX-M-15 across Western and Eastern Europe (including the UK) has been associated with specific clones as well as the transfer of specific epidemic IncFII plasmids harbouring the $bla_{\text{CTXM-15}}$ gene [22,25,31].

CTX-M enzymes (most often CTX-M-14 and -27) have been described in Asia especially since the late 1990s and early 2000s. Reports on the presence of CTX-M-15 in Asia remains relatively scarce outside of those studies from the subcontinent (i.e. India and Pakistan) [26]. Reports from India indicate that *E. coli* producing CTX-M-15 is very common in the community as well as hospital settings [32,33]. It is therefore possible that India represents a significant reservoir and source of *E. coli* producing CTX-M-15 β-lactamases. CTX-M-15 β-lactamases have also been reported from community and hospital isolates in the Middle East [34].

In Africa, *E. coli* producing CTX-M-15 β-lactamases have been identified in several Saharan (i.e. Algeria, Tunisia) and sub-Saharan countries including Cameroon, Tanzania and the Central African Republic [35–38].

In North America, ESBL profiles differ considerably between the USA and Canada. Until 2007, reports of isolates producing CTX-M β-lactamases were rare in the USA, whilst TEM and SHV types were the dominant ESBLs in this country [39,40]. Lewis et al. [27] reported the first emergence of CTX-M-15 in Texas as the most common enzyme among CTX-M groups. Castanheira et al. [41] performed a surveillance study of β-lactam resistance in Enterobacteriaceae recovered from US medical centres during the MYSTIC program of 2007. CTX-M-encoding genes were detected in 38.8% of ESBL-positive isolates and were observed in 80.0% of the participating hospitals. In Canada, the largest outbreak involving CTX-M-15 occurred in multiple long-term care facilities in Toronto between 2000 and 2002 [23] and later its emergence was also reported in several studies from the Calgary Health Region [42,43].

In South America, CTX-M-15 was first reported in 2004 among faecal *E. coli* isolates from Peru and Bolivia [44] and later in Colombia [45], although South America is particularly dominated by Enterobacteriaceae that produce CTX-M-2 and CTX-M-9 [29].

A recent report from Sydney, Australia, has described CTX-M-15 as the dominant ESBL among clinical isolates of *E. coli* and *Klebsiella pneumoniae*, and CTX-M-15 was present in a wide range of community isolates [30].

3.3. Molecular epidemiology of CTX-M-15-producing Escherichia coli

The molecular epidemiology of clinical CTX-M-15-producing *E. coli* on a countrywide or regional scale has been described from various continents and countries, including Russia [46], the UK [47], India [32], Spain [48], Austria [49], Italy [50], Portugal [51], France [52], Canada [42], the USA [27] and Sweden [53]. These studies included *E. coli* isolates collected from different parts of the respective countries either as part of prospective surveillance studies over a specific period of time or acting as a reference laboratory for resistance isolates. Some of the reports describe some clonal similarity among the CTX-M-15-producers, especially in studies from Russia, Italy, Spain, Portugal, France, Sweden, the UK and Canada. However, typing of *E. coli* producing CTX-M-15 from India, Austria and the USA demonstrated great diversity among the different isolates.

Interestingly, in the studies that specifically used pulsed-field gel electrophoresis (PFGE) for typing, the clonal relatedness among the different isolates often did not meet the 'possibly related (or four to six bands difference)' criteria of Tenover et al. [54]. Some of the *E. coli* producing CTX-M-15 formed separate clones with

>80% similar PFGE profiles and, for example in Calgary, Canada, this clone was named 15A [42] whilst in the UK this clone was named clone A [47]. However, the clonal relatedness among some of the other CTX-M-15-producing isolates in the same studies was often <80% (ca. 60–65% similar PFGE profiles being reported, and in Calgary the related isolates were named 15AR, i.e. related to clone 15A). Subsequent studies from Calgary and the UK using multilocus sequencing typing (MLST) have shown that clones A, 15A and the related isolates belong to a single clone named sequence type (ST) 131 [55,56]. Results from these two studies showed that PFGE, as a typing technique, had the propensity to over-split *E. coli* clone ST131 that produce CTX-M-15. The possible reason for this phenomenon is probably due to the fact that the interpretation of PFGE results for dissimilar groups of isolates (i.e. isolates that had been acquired over a period of time or have been isolated from different geographical areas) is complicated by the lack of obvious epidemiological connections [57]. PFGE as a typing technique is comparative in nature and not really definitive. PFGE patterns among clonally related strains can change through the processes of mutation, DNA transfer and rearrangement events. These types of events can hide fundamental relatedness among clones. MLST is a definitive typing technique and offers a more fundamental perspective of the population biology of a species, defining STs based on polymorphisms within strongly conserved 'housekeeping' genes.

Molecular characterisation of plasmids encoding CTX-M-15 from *E. coli* strains involved in outbreaks in different countries showed that they carried additional antibiotic resistance genes such as bla_{OXA-1}, bla_{TEM-1}, *tetA*, *aac(6′)-Ib-cr* and *aac(3)-II*, and sometimes these genes are contained within a class 1 integron [16,23,58]. $bla_{CTX-M-15}$ was most often located on closely related IncFII plasmids of various sizes (85–200 kb), transferability properties and replicon contents (FII or FII-FIA) [16]. However, the association with IncFI plasmids had also been noted [59]. Marcadé et al. [60] reported that $bla_{CTX-M-15}$ was carried by FIA-FIB, FIA-FIB-FII and FIB-FII multireplicons. The diversity of such plasmids may be explained by recombination events between IncFII plasmids with different variations in *copA*, which may alter their compatibility properties [16].

3.4. Emergence of clone ST131 O25:H4 producing CTX-M-15

A clone named ST131 has been identified using MLST among *E. coli* that produce CTX-M-15 enzyme isolated during 2000–2006 from several countries including Spain, France, Canada, Portugal, Switzerland, Lebanon, India, Kuwait and Korea [61,62]. Serogroup O25 is associated with clone ST131 and belongs to the highly virulent phylogenetic group B2 whilst harbouring MDR IncFII plasmids. Historically, *E. coli* serotype O25 formed part of enterotoxigenic *E. coli* (also known as ETEC), and ETEC is not considered to be part of extraintestinal pathogenic *E. coli* (ExPEC) [63]. It does not seem that serotype O25 was ever a major enterotoxigenic clone. These two initial studies showed that clone ST131 had emerged independently in different parts of the world spanning three continents at the same time [61,62]. Their findings suggested that the emergence of clone ST131 could either be due to the ingestion of contaminated food/water sources and/or is being imported into various countries via returning travellers.

MLST is the most reliable method for identification of clone ST131. This technique is the most suitable typing method for comparing data generated independently from different laboratories and is therefore ideal for tracking antimicrobial-resistant bacteria on a worldwide basis [64]. Unfortunately, MLST is expensive, time consuming and is not really suitable to track resistant clones in a rapid real-time fashion. Methods for rapid and easy identification of clone ST131 have recently been published and include repetitive-element polymerase chain reaction (rep-PCR) typing schemes [55,56], PCR for the *pabB* allele [65], PCR for ST131-associated single nucleotide polymorphisms in *mdh* and *gyrB* combined with the O25b *rfb* allele [66] and a triplex PCR that targeted the operon afa FM955459 and part of the CTX-M-15 gene [67].

Clone ST131 producing CTX-M-15 has also recently been described in the UK [68], Italy [69], Turkey [70], Croatia [71], Japan [72], the USA [73] and Norway [74]. *Escherichia coli* belonging to clone ST131 but without CTX-M β-lactamases have been isolated from stools of healthy volunteers in Paris, France [75] and among isolates causing UTIs in Canada [66]. *Escherichia coli* producing CTX-M-15 enzyme belonging to clone ST131 have also been identified in isolates recovered from the community [76], hospital [77] and nursing homes settings [78] and, interestingly, in a companion animal [79].

We can now explore some new avenues, such as: where did clone ST131 originate from and what makes this clone so successful compared with other isolates that produce CTX-M enzymes? Is the success of clone ST131 due to the inherent pathogenicity and virulence associated with this clone or did plasmids that ST131 acquired over a period of time play an essential role in its global spread or dissemination? Is it perhaps a combination of both factors?

Johnson et al. [66] recently gave some insight into the origin of clone ST131. They studied 199 trimethoprim/sulfamethoxazole- and fluoroquinolone (FQ)-resistant *E. coli* isolated from urine during 2002–2004. Clone ST131 was identified in 23% of isolates and nearly all were FQ-resistant (i.e. 99%) but, notably, remained susceptible to the cephalosporin (i.e. only 2% of clone ST131 in that study were resistant to the cephalosporins!). Therefore, it is possible that clone ST131 is common among FQ-resistant *E. coli* and it seems that ST131 does not necessarily have to produce an ESBL [66]. This issue should be investigated by searching for clone ST131 among FQ-resistant *E. coli* that were isolated in the mid-to-late 1990s.

Plasmids carrying CTX-M-15 enzymes were most likely introduced at a later stage and it is possible that ST131 was an established successful FQ-resistant clone before it acquired plasmids encoding for CTX-M-15. However, did the acquisition of the CTX-M plasmids help to make this *E. coli* lineage an even more successful pathogen? A recent publication from the UK addressed this important question and found that the acquisition of IncFII plasmids probably exasperated the spread of clone ST131. Woodford et al. [80] determined the complete sequences of three plasmids that encode CTX-M ESBLs within three different lineages of clone ST131 and showed that IncFII plasmids harbouring $bla_{CTX-M-15}$, bla_{OXA-1}, bla_{TEM-1}, *tetA*, *aac(6′)-Ib-cr* and *aac(3)-II* have played a crucial role in the rapid global spread of CTX-M-15 β-lactamases in *E. coli*.

Are there certain virulence factors (VFs) that make clone ST131 such a successful pathogen? Two studies have investigated the presence of different VFs in clone ST131, and the following VFs have been shown to be specific to clone ST131 [43,62]: uropathogenic-specific protein (*usp*); outer membrane protein (*ompT*); secreted autotransporter toxin (*sat*); aerobactin receptor (*iutA*); and pathogenicity island marker (*malX*). A study from Johnson et al. [66] compared the phylogenetic groups and virulence characteristics of clone ST131 (mostly non-ESBL-producing strains) with other *E. coli* clones such as O15:K52:H1 and clonal group A. They found that VFs *malX*, *ompT* and *usp* were more common in ST131 compared with the clones O15:K52:H1 and clonal group A [66]. A study by Pitout et al. has shown that the combination of phylogenetic group B2 and the presence of virulence factors *malX*, *ompT* and *usp* is more common among clone ST131 than in other *E. coli* that produce CTX-M β-lactamases and suggested that these factors might be important in the worldwide dissemination of clone ST131 (manuscript in review).

There is no doubt that several critical questions relating to the biology of *E. coli* clone ST131 remain unanswered, yet these issues

remain very important and will have a huge bearing on public health [57].

4. Summary

Why did *E. coli* producing CTX-M-15 enzyme emerge simultaneously in different continents as a cause of community-onset infections? Recent studies from Calgary, Canada and Auckland, New Zealand, shed some light on this intriguing question. The publication from New Zealand describes a series of patients who presented to an Auckland hospital with community-onset genitourinary tract infection due to *E. coli* producing CTX-M-15 enzyme with a history of travel to or recent emigration from the Indian subcontinent [81]. All the patients lacked the traditional risk factors associated with UTIs.

A Canadian study demonstrated that travel to the Indian subcontinent (i.e. India and Pakistan), Africa and the Middle East was associated with a high risk of UTI (including urosepsis) with an ESBL-producing *E. coli* in returning travellers [18]. A follow-up study showed that this high risk of infection was mostly due to the acquisition of clone ST131 producing CTX-M-15 [82].

A different study from Calgary over an 8-year period (2000–2007) showed that *E. coli* clone ST131 that produces CTX-M-15 had emerged as an important cause of community-onset bacteraemia during the later part of the study period [i.e. 1/18 (6%) of ESBL-producing *E. coli* isolated from blood between 2000 and 2003 were ST131 compared with 20/49 (41%) isolated between 2004 and 2007 [83]]. In this study, clone ST131 (compared with other *E. coli* that produce ESBLs) was more likely to be resistant to several antibiotics, more likely to produce the aminoglycoside-modifying enzyme *aac(6′)-Ib-cr* and more likely to cause community-acquired infections and urosepsis.

These studies suggest that the sudden worldwide increase of *E. coli* producing CTX-M-15 enzymes is at least due to clone ST131 and that foreign travel to high-risk areas such as the Indian subcontinent potentially plays an important role in spread across different continents. The latest data regarding the prevalence of ESBLs in isolates collected during 2007 from the SMART study showed some alarmingly high rates of ESBL-producing *E. coli* and *Klebsiella* spp. in certain areas of Asia. Rates as high as 55% were reported from China, whilst a staggering 79% of *E. coli* collected in India were positive for ESBLs [84,85]. An interesting aspect of the data from India was that the ESBL prevalence was equally high among *E. coli* collected from the hospital and community settings [85]. Empirical antibiotic coverage for these resistant organisms should be considered in community patients presenting with sepsis involving the urinary and biliary tracts, especially in areas with a high prevalence of ESBL-producing *E. coli*.

The successful spread of *E. coli* producing CTX-M-15 is due to the following mechanisms: the spread of an epidemic clone (such as ST131) with selective advantages (such as multiple antibiotic resistance and enhanced virulence factors) between different hospitals, long-term care facilities and the community; and the horizontal transfer of plasmids or genes that carry $bla_{\text{CTX-M-15}}$ alleles. The literature suggests that the spread of *E. coli* producing CTX-M-15 is mostly due to the dissemination of clone ST131, but the acquisition of IncFII plasmids harbouring $bla_{\text{CTX-M-15}}$ has accelerated the global spread.

There is a serious need to monitor the spread of this MDR clone throughout the world and there are methods available for rapid and easy identification of clone ST131. If this emerging public health threat is ignored, it is possible that the medical community may be forced to use the carbapenems as the first choice for empirical treatment of serious infections associated with UTIs originating from the community.

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