

Differences in ESBL Genes between *E. coli*, *Klebsiella* spp. and *Enterobacter Cloacae* Strains

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Abstract

Background: Resistance to beta-Lactam antibiotics in Enterobacteriaceae is increasing worldwide; however, information about the different beta-Lactamase genes in diverse regions is scarce. We aimed to identify beta-lactamase genes in presumably ESBL positive isolates of Enterobacteriaceae.

Methods: A nonredundant collection of 757 strains of *E. coli*, *Klebsiella pneumoniae*, *Klebsiella oxytoca* and *Enterobacter cloacae* resistant to third generation cephalosporins and ESBL positive by VITEK II analysis was analyzed by PCR for presence of beta-lactamase genes of the TEM, SHV, CTX-M and ampC families.

Results: Distribution of patient age was bimodal, with peaks at <1 year of age and at 60-80 years of age. The mean number of beta-Lactamase genes detected per strain was 2.01 (range 0-6), with *E. cloacae* tending to have more beta-lactamase genes than *E. coli* or *Klebsiella* spp. CTX-M genes, in particular from subgroup I, were most frequently detected, followed by SHV and TEM genes. Only 10% of the strains examined were positive for ampC genes. *E. coli* and *K. pneumoniae* harbored ampC genes from several subgroups, while in *E. cloacae* and *K. oxytoca*, only EBC ampC genes were detected. Regarding overall beta-Lactamase diversity, *E. coli* and *K. pneumoniae* have fewer, more dominating strains than *K. oxytoca* and *E. cloacae*, where the contribution of the most frequent strains to overall diversity is much lower. In 47 strains from 23 patients, we found evidence of horizontal plasmid transfer occurring in vivo. Thus, among patients harboring >1 ESBL positive bacterial species, 71% may have been repeatedly colonized by different strains, while in 29%, plasmid transfer may have occurred after a single colonization event.

Conclusions: The molecular epidemiology of beta-Lactamase genes is complex even if analyzed in a single institution. CTX-M enzymes dominate, and co-occurrence of ampC enzymes is rare. Plasmid transfer between species of *Enterobacteriaceae* seems to occur in vivo in some patients.

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Case Report

The increase of antibiotic resistance in gram negative bacteria is of worldwide concern. Most prominently, resistance to oxyimino cephalosporins is mediated by Extended Spectrum beta-Lactamases (ESBL), which are often localized on mobile genetic elements like plasmids. Concomitantly with resistance against cephalosporins, these strains are often also resistant against non-beta-Lactam antimicrobials [1].

The molecular epidemiology of ESBLs is complex. Most the enzymes are from the Temoneira (TEM), sulfhydryl variable (SHV), and cefotaximinase (CTX-M) families, but several other ESBL families have also been described. Even within these families, heterogeneity is substantial, with 192 SHV genes, 231 TEM genes, and 166 CTX-M genes described to date (www.lahey.org/studies, accessed on Apr 10, 2015)

A proper understanding of ESBL epidemiology is important, since antibiotic resistant strains are of major concern especially, but not only, in hospitals. Recent studies showed that an increasing proportion of ESBL carriers, mostly carrying CTX-M enzymes, is community-associated [2]. Results from a check-up center in Paris, France, showed that the rate of community-associated ESBL carriage increased by a factor of 10 between 2006 and 2011 [3]. Recent data from Bavaria (Germany) demonstrate that 6.3% of healthy volunteers carry ESBL positive *Enterobacteriaceae*, most frequently CTX-M-15 and CTX-M-1 genes [4]. More and more data suggest that this increase is associated with increasing ESBL rates in livestock, and

significant genetic similarities between ESBL isolates in chicken meat and humans could be demonstrated [5]. ESBLs have also recently been described in wildlife animals, with genes which are also found in humans [6].

Usually, ESBLs can easily be identified in the microbiology lab by phenotypic assays based on inhibition of cephalosporin hydrolysis by clavulanic acid [1]. However, co-occurrence of inhibitor resistant beta-lactamases greatly reduces the accuracy of these assays. This occurs most often when plasmid-encoded ampC beta-lactamase genes are present additionally to the ESBL gene [7]. However, the precise rate of co-occurrence of ESBL and ampC genes is unknown. Moreover, to the best of our knowledge, there are no data derived from larger datasets comparing differences in ESBL genes occurring in *Escherichia coli*, *Klebsiella* species (spp.) and *Enterobacter cloacae*. Thus, we examined a large, non-redundant strain collection available at our institute for the occurrence and co-occurrence of the most frequently occurring ESBL and ampC genes.

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Materials and Methods

Between 2005 and 2009, strains of ESBL positive *Enterobacteriaceae* were collected from routine microbiology samples submitted to the Institut für Medizinische Mikrobiologie, Immunologie und Hygiene of the Technische Universität München. Samples were cultured on blood, chocolate and MacConkey agar plates according to standard microbiology techniques, and clinically relevant strains were identified (Vitek II, Bio-Merieux; or Bruker Biotyper) and their antibiotic susceptibility determined (Vitek II AST-N117 cards), including an automated ESBL confirmatory test. Strains detected as ESBL positive were collected and stored for further analysis.

Before molecular analysis, the ESBL phenotype was first confirmed by E-Test (AB Biodisk) or double disk synergy test using cefotaxim, ceftazidim and cefpodoxim disks with and without clavulanic acid (BD Biosciences) for *E. coli* and *Klebsiella spp.*, and cefepim disks with and without clavulanic acid (Mast Diagnostica) for *E. cloacae* isolates, according to Clinical and Laboratory Standards Institute (CLSI) standard procedures. Confirmed ESBL positive strains were submitted to PCR analysis using the following primers: broad range TEM as described in [8], broad range CTX-M as described in [9], CTX-M subgroups I and II as described in [10], group III as described in [11], and groups IV and V as described in [9]. Primers for plasmidic ampC genes were from [12]. Broad range SHV primers were 5'- TTC GCC TGT GTA TTA TCT CC -'3 and 5'- TCC GCT CTG CTT TGT TAT TC -'3, developed by Y. Pfeifer, Robert Koch Institut in Wernigerode, Germany. PCR products were visualized by agarose gelelectrophoresis with ethidium bromide staining according to standard procedures. Data were collected and analyzed in Microsoft Excel.

Results

Sources of ESBL positive samples: Between Dec 2005 and Dec 2009, a total of 767 non-redundant strains of *Enterobacteriaceae* resistant to 3rd generation cephalosporins and ESBL positive by Vitek II analysis

were collected at the Institut für Medizinische Mikrobiologie, Immunologie und Hygiene of the Technische Universität München. Two-hundred and forty-four (32%) strains were from intensive care units, 93 (12%) were from outpatient clinics. Most of the isolates were from urine (305 strains, 40%), 268 strains (35%) were from swab cultures, and 125 strains (16%) were from respiratory materials (tracheal secretions, bronchoalveolar lavage, or sputum). Twenty-one (2.7%) and eleven (1.4%) strains were from blood cultures or intravascular devices, hence unambiguously indicate invasive infections. Almost half of the strains were detected in 60 to 80 year old patients (378 strains, 49%), but a second peak occurred in infants less than one year of age (45 strains, 5.8%) (Figure 1).

Microbiology of ESBL producers: Among the 767 strains analyzed were 448 *E. coli*, 245 *K. pneumoniae*, 21 *K. oxytoca* and 53 *E. cloacae* strains. Genes for TEM- SHV-, CTX-M or ampC beta-lactamases could be detected by PCR in all but 9 strains. The mean number of detected beta-Lactamase genes per strain was 2.01. In *E. cloacae*, a mean of 3.25 beta-Lactamase genes could be detected per strain (range, 1 to 6), followed by *K. pneumoniae* (mean, 2.29, range 0-4), *K. oxytoca* (mean 1.86, range 1-4) and *E. coli* (mean 1.71, range 0-5) (Figure 2A).

Occurrence of TEM-, SHV-, CTX-M, and ampC beta-lactamases: The most frequent beta-Lactamase group was CTX-M, with a total of 645 strains positive. This was followed by SHV (418 strains) and TEM(294 strains) beta-lactamases (Figure 2B). Among *E. coli*, *E. cloacae* and *K. oxytoca* strains, the most frequent type was CTX-M with 87%, 83% and 100% of strains positive, respectively. By contrast, in *K. pneumoniae* strains, SHV genes were more frequent than CTX-M genes (95% vs. 78% of strains respectively). In *E. cloacae*, SHV and CTX-M genes occurred with similar frequency. Only 36% of *E. coli* strains carried a TEM gene.

A total of 80 strains were positive for ampC genes (Figure 2B), all but one *E. coli* strains with a single gene. EBC was the most frequent

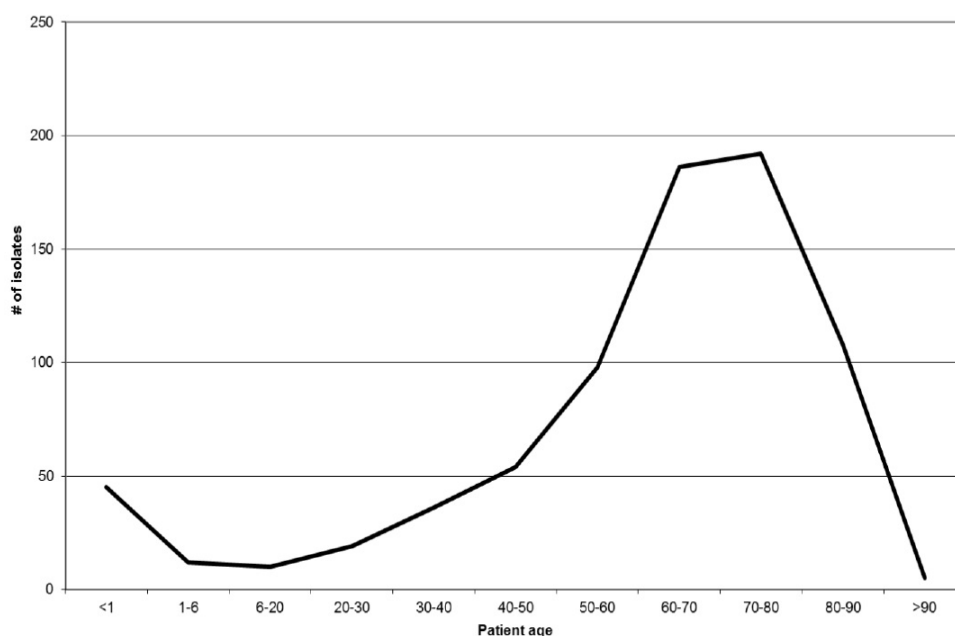


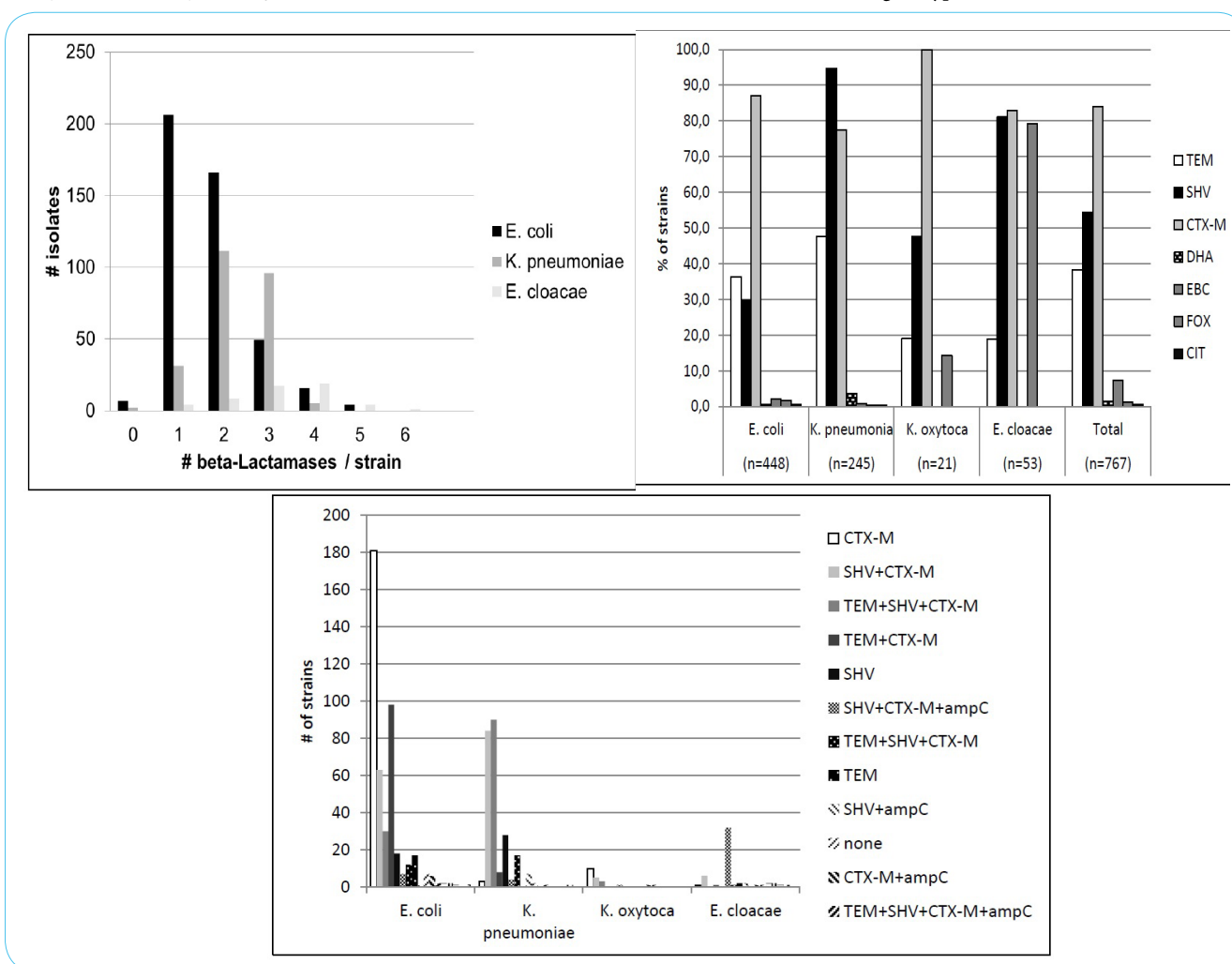
Figure 1: Age dependent incidence of ESBL positive isolates of Enterobacteriaceae. X-axis: patient age at time of sample receipt in the clinical microbiology laboratory. Y-axis: number of ESBL positive isolates.

one, being the sole ampC gene detected in *E. cloacae* and *K. oxytoca* strains. In *E. cloacae*, EBC occurred almost as often as SHV or CTX-M. Detection of ampC in *E. coli* and *K. pneumoniae* was more diverse with DHA, EBC, FOX and CIT genes detected.

Figure 2C shows a detailed analysis of which genes occurred together in individual strains. In *E. coli*, most strains harbored only CTX-M gene(s), followed by co-occurrence of CTX-M with TEM and of CTX-M with SHV genes. By contrast, the combination of TEM with SHV and CTX-M genes was the most frequent genotype in *K. pneumoniae* strains, followed by SHV and CTX-M genes occurring together. Still differently, in *E. cloacae*, the combination of SHV with CTX-M and ampC was most frequent. Importantly, only two strains harbored ampC genes without concurrent TEM-, SHV-, or CTX-M genes, indicating that false positive phenotypic ESBL tests by VITEK II analysis mediated by ampC genes are rare.

combination of CTX-M-II with CTX-M-IV was most frequent in all four species (Figure 3B). However, this combination was followed by CTX-M-I with CTX-M-II and CTX-M-IV in *E. coli*, by CTX-M-I with CTX-M-V in *K. pneumoniae*, and by CTX-M-II with CTX-M-IV and CTX-M-V in *E. cloacae*.

Overall assessment of ESBL genotype diversity *E. coli*, *K. pneumoniae*, *K. oxytoca* and *E. cloacae*: So far, our data indicate that there may be important differences in ESBL genotype between the four bacterial species examined. Figure 4 compares how many different strains contribute to overall genotype diversity in *E. coli*, *Klebsiella spp.* and *E. cloacae*. Interestingly, in *E. coli* and *K. pneumoniae*, a much smaller proportion of all strains examined accounts for a given extend of genotypic variability than in *K. oxytoca* and *E. cloacae*. For example, in *E. coli* and *K. pneumoniae*, less than 20% of the strains examined cover 80% of the detected genotypes.



Occurrence of CTX-M subgroups: The most frequently detected CTX-M subgroup was CTX-M-I in *E. coli*, *K. pneumoniae* and *K. oxytoca* (Figure 3A). However, in *E. cloacae*, CTX-M-II and CTX-M-IV genes were more frequent, these groups also were the second and third most frequent group in *E. coli* (Figure 3A). Thus, the repertoire of CTX-M genes was much broader in *E. coli* and *E. cloacae* than in *Klebsiella spp.* Only in 28 (4.3%) of all CTX-M positive strains, all CTX-M subgroup PCRs remained negative. Examining which subgroups frequently occur together in individual strains, the

By contrast, in *K. oxytoca* and *E. cloacae*, approx. 60% of the strains examined are needed to cover for 80% of the genotypes. Thus, regarding overall beta-Lactamase diversity, *E. coli* and *K. pneumoniae* have fewer, more dominating strains than *K. oxytoca* and *E. cloacae*, where the contribution of the most frequent strains to overall number of genotypes is much lower.

Evidence for plasmid transfer between bacterial species in vivo in clinical settings: Our dataset contains 80 patients in which more than

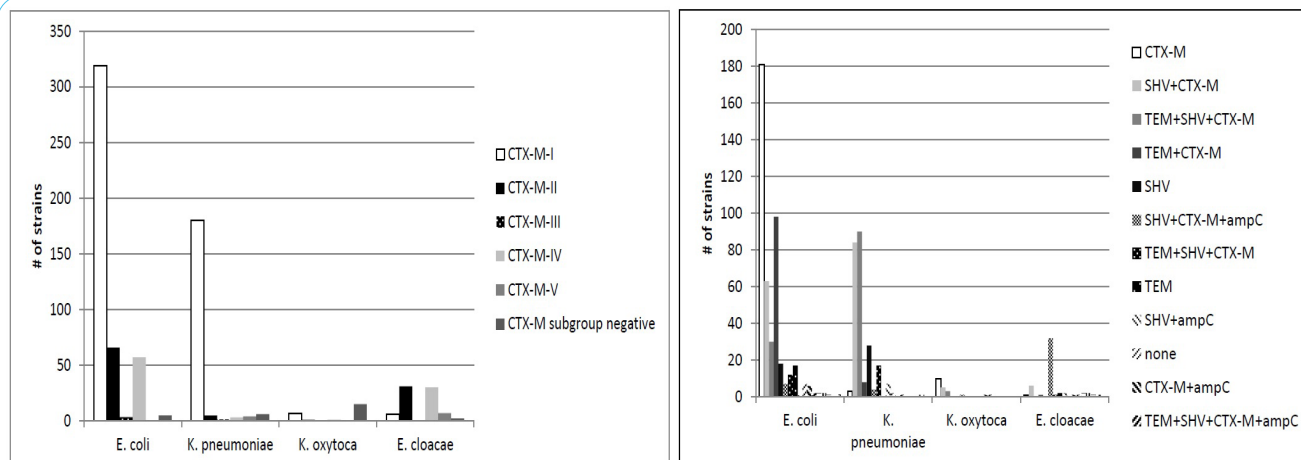


Figure 3: (A) Distribution of CTX-M subgroups in *E. coli*, *Klebsiella spp.* and *E. cloacae*. (B) Co-occurrence of CTX-M subgroups in strains of *E. coli*, *Klebsiella spp.* and *E. cloacae*. y-axis gives the number of strains, while beta-lactamase genes or combinations thereof are indicated in the legend.

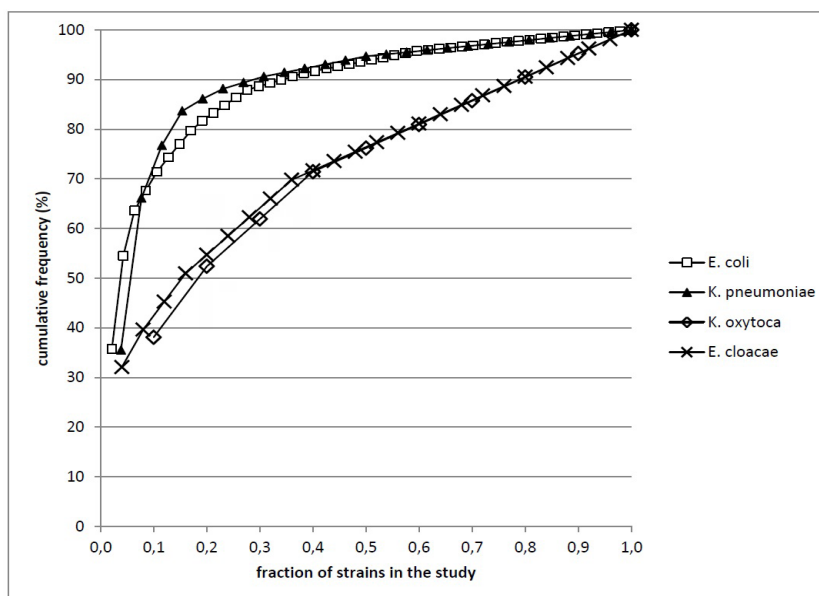


Figure 4: Cumulative frequency distribution of genotype diversity in *E. coli*, *Klebsiella spp.* and *E. cloacae*. x-axis: fraction of bacterial strains of the respective species in the study; y-axis: cumulative frequency of different beta-lactamase genotypes.

Discussion

to a maximum of three different strains per patient (151 strains in total). We hypothesized that if inter-species plasmid transfer occurred in vivo in these patients, strains from different species should have the same beta-Lactamase genotype, except for beta-Lactamases which may be chromosomally encoded. We thus looked specifically for strains of different species detected in the same patient with identical beta-lactamase genotype, but accepting additional occurrence of potentially chromosomally encoded TEM in *E. coli*, SHV in *Klebsiella spp.* and EBC in *E. cloacae*. In fact, we could identify 23 patients with 47 strains where plasmid transfer from one species to another may have occurred (Table 1). Thus, among patients harboring >1 ESBL positive bacterial species, 71% may have been repeatedly colonized by different strains, while in 29%, plasmid transfer may have occurred after a single colonization event.

With a total of 767 nonredundant clinical strains analyzed, our study constitutes – to the best of our knowledge – the largest body of information about molecular epidemiology of broad spectrum beta-lactamases to date. The vast majority of our strains (726 of 767) has been collected between 2007 and 2009 and comprises all different ESBL strains from our institution isolated in this timeframe. Like many other recent studies from Europe, we find a strong dominance of CTX-M enzymes in our dataset (84% of all strains tested were positive for CTX-M genes). Earlier, albeit much smaller, studies from Germany detected CTX-M enzymes in up to 98% of examined strains [13-15]. In 195 hospital-acquired strains of the U.S. SENTRY antimicrobial surveillance program, only 44% carried CTX-M genes [16]. However, another study detected CTX-M enzymes in 91% of community-acquired *E. coli* strains in the US [17]. Whether this constitutes a real difference, sampling bias, or changes in molecular

Patient number	Bacterial species	TEM	SHV	CTX-M	CTX-M subtype					ampC subtype					
					I	II	III	IV	V	MOX	DHA	EBC	ACC	FOX	CIT
1	<i>E. coli</i>	0	1	1	0	1	0	1	0	0	0	0	0	0	0
1	<i>E. cloacae</i>	0	1	1	0	1	0	1	0	0	0	1	0	0	0
2	<i>K. pneumoniae</i>	0	1	1	1	0	0	0	0	0	0	0	0	0	0
2	<i>E. coli</i>	1	1	1	1	0	0	0	0	0	0	0	0	0	0
2	<i>K. pneumoniae</i>	1	1	1	1	0	0	0	0	0	0	0	0	0	0
3	<i>E. cloacae</i>	0	1	1	0	1	0	1	0	0	0	1	0	0	0
3	<i>E. coli</i>	1	1	1	0	1	0	1	0	0	0	0	0	0	0
4	<i>E. coli</i>	1	1	1	0	1	0	1	0	0	0	0	0	0	0
4	<i>E. cloacae</i>	0	1	1	0	1	0	1	0	0	0	1	0	0	0
5	<i>K. pneumoniae</i>	1	1	1	1	0	0	0	0	0	0	0	0	0	0
5	<i>E. coli</i>	0	1	1	1	0	0	0	0	0	0	0	0	0	0
6	<i>E. coli</i>	0	1	1	0	1	0	1	0	0	0	0	0	0	0
6	<i>E. cloacae</i>	0	1	1	0	1	0	1	0	0	0	1	0	0	0
7	<i>K. pneumoniae</i>	0	1	1	1	0	0	0	0	0	0	0	0	0	0
7	<i>E. coli</i>	1	0	1	1	0	0	0	0	0	0	0	0	0	0
8	<i>E. cloacae</i>	0	1	1	0	1	0	1	0	0	0	1	0	0	0
8	<i>E. coli</i>	0	1	1	0	1	0	1	0	0	0	1	0	0	0
9	<i>K. pneumoniae</i>	0	1	1	1	0	0	0	0	0	0	0	0	0	0
9	<i>E. coli</i>	0	0	1	1	0	0	0	0	0	0	0	0	0	0
10	<i>K. pneumoniae</i>	0	1	1	1	0	0	0	0	0	0	0	0	0	0
10	<i>E. coli</i>	0	0	1	1	0	0	0	0	0	0	0	0	0	0
11	<i>K. pneumoniae</i>	0	1	1	1	0	0	0	0	0	0	0	0	0	0
11	<i>E. coli</i>	1	0	1	1	0	0	0	0	0	0	0	0	0	0
12	<i>E. coli</i>	1	1	1	0	1	0	1	0	0	0	0	0	0	0
12	<i>E. cloacae</i>	0	1	1	0	1	0	1	0	0	0	1	0	0	0
13	<i>K. pneumoniae</i>	0	0	1	1	0	0	0	0	0	0	0	0	0	0
13	<i>E. coli</i>	0	0	1	1	0	0	0	0	0	0	0	0	0	0
14	<i>K. pneumoniae</i>	1	1	1	1	0	0	0	0	0	0	0	0	0	0
14	<i>E. coli</i>	1	0	1	1	0	0	0	0	0	0	0	0	0	0
15	<i>E. coli</i>	1	1	1	0	1	0	1	0	0	0	0	0	0	0
15	<i>E. cloacae</i>	0	1	1	0	1	0	1	0	0	0	1	0	0	0
16	<i>E. coli</i>	0	0	1	1	0	0	0	0	0	0	0	0	0	0
16	<i>K. pneumoniae</i>	0	1	1	1	0	0	0	0	0	0	0	0	0	0
17	<i>E. coli</i>	1	1	1	0	1	0	1	0	0	0	0	0	0	0
17	<i>E. cloacae</i>	0	1	1	0	1	0	1	0	0	0	1	0	0	0
18	<i>K. pneumoniae</i>	0	1	1	1	0	0	0	0	0	0	0	0	0	0
18	<i>E. coli</i>	1	0	1	1	0	0	0	0	0	0	0	0	0	0
19	<i>K. pneumoniae</i>	1	0	1	1	0	0	0	0	0	0	0	0	0	0
19	<i>E. coli</i>	1	0	1	1	0	0	0	0	0	0	0	0	0	0
20	<i>K. pneumoniae</i>	1	1	0	x	x	x	x	x	0	0	0	0	0	0
20	<i>E. coli</i>	1	1	0	x	x	x	x	x	0	0	0	0	0	0
21	<i>E. coli</i>	0	1	0	x	x	x	x	x	0	0	0	0	0	0
21	<i>K. pneumoniae</i>	0	1	0	x	x	x	x	x	0	0	0	0	0	0
22	<i>K. pneumoniae</i>	1	1	1	1	0	0	0	0	0	0	0	0	0	0
22	<i>K. oxytoca</i>	1	1	1	1	0	0	0	0	0	0	0	0	0	0

23	<i>K. pneumoniae</i>	0	1	0	x	x	x	x	x	0	0	0	0	0	0
23	<i>E. coli</i>	1	1	0	x	x	x	x	x	0	0	0	0	0	0
20	<i>K. pneumoniae</i>	1	1	0	x	x	x	x	x	0	0	0	0	0	0

Table 1: Evidence for horizontal plasmid transfer in individual patients. Shown are beta-lactamase genotypes of patients where identical plasmid encoded beta-lactamase genes occur in different bacterial species, indicating that horizontal gene transfer may have occurred. 0: PCR negative, 1: PCR positive, x: not tested

epidemiology over time remains to be determined: In suburban New York, the prevalence of CTX-M positive *Klebsiella pneumoniae* isolates strongly increased in recent years, while the increase in CTX-M bearing *E. coli* was much less pronounced [18]. Similarly, an increase in CTX-M-bearing *E. coli* and *Klebsiella spp.* was described in Texas between 2000 and 2006 [11]. By 2012, CTX-M group I became the most prevalent group of ESBL throughout the United States, with 43% of 701 strains collected [19]. Recent data from Canadian hospitals show a similar dominance of CTX-M enzymes, particularly CTX-M15, as in Germany [20]. Thus, we conclude that our data are in accordance with the global spread of CTX-M enzymes currently described in several regions worldwide.

In the German studies, CTX-M15 was the most frequently isolated gene [13-15]; as this belongs to the CTX-M-I group, this is also in concordance with our data. It should be noted, however, that the predominance of certain CTX-M alleles shows substantial geographic variation: in Spain, the most common CTX-M allele is CTX-M14 [21, 22]. In the US SENTRY dataset, CTX-M15 is the most frequently isolated CTX-M gene [16], as it is in Canada [16]. However, we would like to point out that the distribution of CTX-M alleles is different in different bacterial species: While the CTX-M-I group is the predominant group in *E. coli* and *Klebsiella spp.*, this is not the case in *E. cloacae*, where subgroups CTX-M-II and CTX-M-IV dominate. The reasons for this difference are currently unclear, since *E. cloacae* is only infrequently examined for ESBL production and molecular studies are rare. Qureshi et al reported that among 31 isolates from blood stream infections, 29 harbored an SHV-like ESBL, while only two strains carried a CTX-M enzyme [23]. While we also find a high proportion of our *E. cloacae* strains to carry SHV genes, most of them also have CTX-M genes. In Brazil, more than 50% of ESBL positive *E. cloacae* strains have been shown to carry CTX-M15, a result quite different from ours [24]. In China, most *E. cloacae* strains resistant to carbapenems have been shown to co-express TEM and CTX-M ESBLs, with a smaller proportion being positive for SHV enzymes [25]. Thus, in line with our data, the molecular epidemiology of ESBL producing *E. cloacae* may be more diverse worldwide than that of other *Enterobacteriaceae*.

We can confirm earlier data that most of the ESBL positive strains carry more than one beta-lactamase gene. In the SENTRY antimicrobial surveillance program, two to nine beta-lactamase genes were detected in individual strains [16]. In the US 2012 strain collection [19], 63% of the isolates carried more than one ESBL gene. Similarly, the Canadian CANWARD surveillance reported multiple beta-Lactamase production in 74% and 83% of ESBL-positive *E. coli* and *Klebsiella spp.*, with a maximum of 4 genes detected [20].

The detection of chromosomally encoded EBC and SHV genes in *E. cloacae* and *Klebsiella spp.*, respectively, indicates that the chosen PCR strategy works well. However, since we did not sequence individual amplicons, we were not able to differentiate non-ESBL from ESBL enzymes in strains positive for TEM- or SHV genes.

While the molecular epidemiology of ESBL genes has been extensively characterized, much less is known about co-occurrence with ampC genes. We found 80 strains positive for ampC genes, constituting approx. 10% of all strains analyzed. Remarkably similar numbers are reported from the SENTRY surveillance program (11%) [20], from an international strain collection acquired during the tigecycline phase 3 trials (8,5%) [8], and from the US 2012 strain collection [19]. However, another study from Singapore reported only one isolate among 54 tested co-expressing ESBL and ampC genes [26]. One limitation of our study is that our strain collection is biased against ampC producers: We only analyzed strains which were positive for ESBL confirmatory tests, and strains concurrently producing an ampC enzyme could be expected to be negative in the phenotypic ESBL confirmatory tests. However, only very few strains were cefotaxim resistant and negative in ESBL confirmatory tests (data not shown), so this should not significantly affect our results and precludes inclusion of these strains as a control group. It would, however, be possible to extend on our observations by analyzing with PCR all cefotaxim resistant isolates irrespective from the result of the ESBL confirmatory tests. While the possibility of horizontal gene transfer *in vivo* in patients seems obvious, only very limited data are available. Fernandez et al. [27] report a patient with recurrent urinary tract infections being colonized by *E. coli* and *Proteus mirabilis* harboring the same CTX-M-32 bearing plasmid, demonstrating that horizontal gene transfer may indeed be possible *in vivo*. Similarly, Göttig et al report the occurrence of horizontal gene transfer of an OXA-48 bearing plasmid from *K. pneumoniae* to *E. coli* during a nosocomial outbreak in 1 of 6 patients [28]. More importantly, Doi et al. [29] try to quantify the extent of horizontal gene transfer and cross transmission, respectively, in long-term care facility patients. They found 6 occurrences of horizontal gene transfer events in 25 patients colonized with SHV-5 or SHV-12 bearing *E. coli* and *K. pneumoniae* strains (24%). This figure is quite similar to our analysis, where we found evidence of horizontal gene transfer in 29% of patients. We conclude that plasmid transfer *in vivo* in individual patients is not a rare, but rather a common, event.

According to our data, the molecular epidemiology of ESBL and ampC enzymes is complex. In our collection of 767 bacterial strains, we could detect 73 different genotypes. Castanheira et al reported 55 different gene combinations among 157 isolates [16]. Jones reported 50 genotypes from 272 isolates [8]. Thus, if a sufficiently large strain collection is analyzed, molecular epidemiology of ESBLs is as complex in a single institution as it is in national or even international strain collections.

Conclusion

Molecular epidemiology of beta-lactamase genes in enterobacterial strains positive in phenotypic ESBL assays is as complex in a single institution as it is on a national or international level. As in other parts of Europe, CTX-M enzymes dominate in our study. Co-occurrence of ESBL and plasmid-encoded ampC enzymes in species without

chromosomally encoded ampC seems to be a rare event. By contrast, co-occurrence of different genes potentially encoding ESBL enzymes is frequent. Transfer of resistance plasmids between bacterial species seems to occur in a certain percentage of patients in vivo.

Author Contributions

Thomas Miethke and Reinhard Hoffmann designed the study. Charlotte Bogner performed the experiments- Reinhard Hoffmann analyzed the data and wrote the manuscript. Nina Wantia, Friedemann Gebhard and Dirk Busch were involved in critically revising the manuscript for important intellectual content.

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