

β-LACTAMASES ENCODED BY *bla*_{CTX-M} GROUP I GENES AS DETERMINANTS OF RESISTANCE OF ESBL-POSITIVE ENTEROBACTERIACEAE IN EUROPEAN SOLDIERS IN TROPICAL MALI

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ESBL (extended-spectrum-β-lactamase)-positive Enterobacteriaceae, which colonized European soldiers in tropical Western African Mali, were subjected to a molecular assessment of their resistance determinants. By doing so, a better insight into the locally endemic pattern of ESBL-associated β-lactamase genes was aspired.

From a previous study on diarrhea in European soldiers on deployment in tropical Mali, 15 ESBL-positive *Escherichia coli* with demonstrated high clonal diversity and one positive *Klebsiella pneumoniae* were assessed. Polymerase chain reactions (PCRs) for *bla*_{TEM} and *bla*_{SHV} β-lactamase genes with subsequent sequencing for the discrimination of ESBL- and non-ESBL variants were performed, followed by four group-specific PCRs for *bla*_{CTX-M} genes.

Non-ESBL-associated *bla*_{TEM-1} was identified in six out of 15 (40%) *E. coli* strains, while 100% of the assessed strains were positive for group I *bla*_{CTX-M}.

Considering the known clonal diversity of the assessed strains, the striking restriction to one group of *bla*_{CTX-M} genes accounting for the ESBL phenotypes of the isolates suggests little genetic exchange in the local setting. Under such circumstances of restricted numbers of locally endemic target genes, PCR-based screening approaches for ESBL colonization might be promising.

Keywords: extended-spectrum β-lactamase, resistance, colonization, Enterobacteriaceae, Mali, deployment

Introduction

Atypically resistant or even multidrug resistant Enterobacteriaceae show a world-wide distribution which does not spare tropical and subtropical settings. Studies with civilian returnees from journeys to the tropics identified frequent intestinal colonization with such bacteria. In particular, Enterobacteriaceae with extended-spectrum β-lactamases (ESBLs) quantitatively dominate [1–4]. Antibiotic therapy, e.g., for the treatment of travellers' diarrhea, facilitates colonization with ESBL-positive strains [1]. Resistant bacteria are selected under antibiotic pressure while the natural gut flora is depleted and thus deprived from its protective potential.

African tropical settings are frequent military deployment sites of European soldiers. The European Union Training Mission (EUTM) in Mali, providing military

training for local soldiers, is just one example. During a recent surveillance of EUTM soldiers with diarrhea in Koulikoro, Mali, from the 49th calendar week 2013 to the 34th calendar week 2014, a total of 16 ESBL-positive Enterobacteriaceae, comprising 15 *Escherichia coli* and one *Klebsiella pneumoniae*, had been isolated from the stool sample of 13 out of 48 patients (27.1%) as recently described [5]. As further demonstrated during this surveillance, there was a high degree of clonal diversity of the *E. coli* strains, suggesting multiple transmission events rather than ongoing nosocomial transmission within the field camp [5].

Considering this high colonization pressure with ESBL-positive Enterobacteriaceae in Mali, a molecular assessment of the 16 isolates from Mali was performed to find out whether the pattern of genetic resistance determinants is similarly diverse.

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Materials and methods

Assessed strains

Altogether, 15 *E. coli* isolates and one *K. pneumoniae* isolate from stool samples of EUTM soldiers in Koulikoro, Mali, with diarrhea were included in the assessment. Details on the mode of isolation of the strains, the observed resistance patterns, and the clonal distribution have been reported elsewhere [5].

β -Lactamase polymerase chain reactions (PCRs)

A duplex PCR was performed targeting *bla*_{TEM} and *bla*_{SHV} β -lactamases as described [6, 7], followed by four simplex PCRs targeting the *bla*_{CTX-M} groups I–IV (Table 1), respectively, also exactly as described [7, 8].

Amplicons were visualized using a Lonza FlashGel System (Lonza Rockland, Inc., Rockland, ME, USA) according to the manufacturer's instructions.

Sequencing

Because not all *bla*_{TEM} and *bla*_{SHV} β -lactamases are associated with ESBL phenotypes, respective amplicons were subjected to sequencing. Amplicons were purified using the NAT Clean-up/Nucleospin Extract II kit (Macherey & Nagel, Düren, Germany) according to the manufacturer's instructions. After purification, sequencing in both directions was performed by SeqLab – Sequence Laboratories Göttingen GmbH (Göttingen, Germany). Sequence alignment was performed using the software BioNumerics 7.1 software (Applied Maths, Sint-Martens-Latem, Belgium). The alignment settings were open gap penalty 100%, unit gap penalty 0%, match score 100%, and fast algorithm (= minimum match sequence: 2, maximum number of 98). All sequences were analyzed using the nucleotide BLAST algorithm (www.ncbi.nlm.nih.gov/BLAST/). The analyses included sequences of enterobacteria (taxid: 91347) only and excluded models as well as uncultured/environmental sample sequences.

As the *bla*_{CTX-M} β -lactamase groups that are identified by the above mentioned PCRs are generally associated with ESBL phenotypes, no sequence-based discrimination was aspired here for economic reasons. Instead, discrimination on group level as allowed by the used PCRs [8] was considered as sufficient.

Results and discussion

From the analyzed strains, six out of 15 *E. coli* (40%) were positive for *bla*_{TEM} in PCR. Sequence analyses identified *bla*_{TEM-1}, a β -lactamase which is not associated with an ESBL phenotype, in all instances. All strains including both the 15 *E. coli* isolates and the single *K. pneumoniae* strain were positive for β -lactamases of the ESBL-associated *bla*_{CTX-M} group I (Table 1).

Considering the high clonal diversity of the strains as previously described [5], the close homogeneity with only one detected *bla*_{CTX-M} group and a complete absence of all other analyzed genetic ESBL mechanisms is surprising. Obviously, genetic exchange due to international travel from and to terror- and war-haunted Mali is low. This situation may allow for a wide spread of a restricted spectrum of resistance mechanisms under the selective pressure of antibiotic use and misuse.

Broad studies on ESBL distribution among the local population of Mali are widely missing. However, a French study with samples from adopted children from an orphanage in Bamako, Mali [10], allowed for the isolation of 52 ESBL-positive Enterobacteriaceae during a three-year-interval from 2002 till 2005 from 24 out of 25 adoptees after their arrival in France. ESBL-positive *E. coli* dominated quantitatively; the most frequent resistance determinant was the β -lactamase encoded by *bla*_{CTX-M-15}, a member of the *bla*_{CTX-M} group I, which was identified in 93% of the *E. coli* strains [10].

After 10 years, again all 16 isolated ESBL-positive Enterobacteriaceae were positive for *bla*_{CTX-M} group I, suggesting a locally very stable distribution (Table 2).

While data on ESBL distribution in Western African Mali are virtually absent, more respective data are avail-

Table 1. *bla*_{CTX-M} β -lactamase genes of the groups I–IV according to Refs. [8, 9]

<i>bla</i> _{CTX-M} groups	I	II	III	IV
Comprised β -lactamase genes	<i>bla</i> _{CTX-M-1, -3, -10, -11, -12, -15, -22, -23, -28, -29, -30}	<i>bla</i> _{CTX-M-2, -4, -5, -6, -7, -20}	<i>bla</i> _{CTX-M-8}	<i>bla</i> _{CTX-M-9, -13, -14, -16 to -19, -21, -27}

Table 2. Distribution of positive β -lactamase PCRs

Species/PCR	<i>bla</i> _{TEM}	<i>bla</i> _{SHV}	<i>bla</i> _{CTX-M} group I	<i>bla</i> _{CTX-M} group II	<i>bla</i> _{CTX-M} group III	<i>bla</i> _{CTX-M} group IV
<i>E. coli</i> (n = 15)	6 × <i>bla</i> _{TEM-1} * (40%)	–	15 × (100%)	–	–	–
<i>K. pneumoniae</i> (n = 1)	–	–	1 × (100%)	–	–	–

*Not associated with an ESBL phenotype

able for Western African Ghana. In large hospitals of the Ghanaian towns Accra [11] and Kumasi [12], the proportion of ESBL-positive strains among isolated Enterobacteriaceae was reported to be about 50%. Of note, a broader distribution of *bla*_{TEM}, *bla*_{SHV}, and *bla*_{CTX-M} genes was reported for the strains from Kumasi [12], suggesting free regional and international exchange of resistance determinants.

The introduction of ESBL-positive Enterobacteriaceae by European soldiers returning from deployments in Sub-Saharan Africa to their home countries is bothersome, because it bears the risk of transmission to their families. This phenomenon is well-documented for adopted children from Mali, who have spread their colonizing ESBL-positive strains among their French adoptive families in a percentage as high as 23% [10]. It seems advisable to offer screening options for Gram-negative pathogens for European soldiers who return from deployments in tropical high-endemicity settings with consecutive counselling regarding hand and sanitary hygiene to reduce the risk of transmission.

Molecular screenings for ESBL-positive strains are not broadly applied in diagnostic routine. One reason is the fact that there are numerous different molecular mechanisms, exceeding the potentials even of multiplex PCR approaches. However, if only singular or few resistance determinants circulate within a local population as suggested for Mali, PCR-based screening might be successfully applied as recently demonstrated for a setting in Madagascar [13].

However, such interpretations are limited by the fact that only small numbers of ESBL-positive strains from Mali were assessed in this study as well as in previous assessments [10]. A broader surveillance seems useful to more reliably estimate the true distribution of ESBL-associated genes in colonizing Enterobacteriaceae in Mali.

Conclusions

In line with previous results from 10 years ago [10], *bla*_{CTX-M} group I was confirmed as the major resistance mechanism of ESBL-positive Enterobacteriaceae in Mali. European soldiers deployed in Mali are at considerable risk of introducing such resistant strains to their home countries and their families. The obviously lacking diversity of locally distributed ESBL-associated resistance genes in Mali suggests successful use of PCR-based ESBL screening approaches for this region.

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Declaration of Interests

The authors declare that there are no conflicts of interest.

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