New Delhi metallo-β-lactamase-producing *Acinetobacter baumannii*: a novel paradigm for spreading antibiotic resistance genes

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ABSTRACT: The impact of carbapenemase production among clinically significant Gram-negative rods is becoming a major medical issue. To date, *Acinetobacter baumannii* has been considered as a final recipient of carbapenemase genes (imipenemase, Verona metallo-β-lactamase, Guiana extended-spectrum β-lactamase and *Klebsiella pneumonia* carbapenemase types) from Enterobacteriaceae and *Pseudomonas aeruginosa*. However, recent findings regarding the spread of the bla_{NDM} carbapenemase genes revealed that *A. baumannii* likely acts as a source of emerging antibiotic resistance genes. The analysis of genetic structure surrounding the bla_{NDM-1} gene revealed that the genetic structure (Tn*125*) responsible for its dissemination most probably originates from *Acinetobacter*. Moreover, analysis of the bla_{NDM-1} gene itself demonstrated that it might be constructed in *Acinetobacter* through a recombination event with another resistance gene found in *A. baumannii* (*aphA6*). This novel paradigm highlights a novel and unexpected role played by *A. baumannii*.

KEYWORDS
• β-lactamase • antibiotic resistance • carbapenemase • Gram-negative rods

Members of the genus *Acinetobacter* seem to have the ability to quite rapidly acquire resistance to new antibiotics [1]. The rise of resistance to broad-spectrum antibiotics such as aminoglycosides, expanded-spectrum cephalosporins, carbapenems and tigecycline in *Acinetobacter baumannii* has left few therapeutic options [2]. The main problem corresponds to the rise of carbapenem resistance in *A. baumannii*, which is almost always identified in multidrug-resistant clinical isolates [3]. Until now, *A. baumannii* has been considered a final reservoir of antibiotic resistance genes by acquisition of foreign DNA from different sources, particularly from other Gram-negative species. Many resistance genes have been identified in *A. baumannii* including those encoding extended-spectrum β-lactamases (PER-, Guiana extended-spectrum β-lactamase [GES] and VEB type) and aminoglycoside resistance genes [1,4]. The acquisition of those genes is often related to an integration of foreign DNA originating from other clinical species (Enterobacteriaceae and *Pseudomonas aeruginosa*) [1,2,4–7].

Carbapenem resistance in *A. baumannii*

Genes encoding metallo-β-lactamases of verona metallo-β-lactamase- and imipenemase types, as well as those encoding class A carbapenemases (*Klebsiella pneumonia* carbapenemase or GES), are other examples of acquisition of foreign resistance genes as a source of carbapenem resistance in *A. baumannii* [2]. Analysis of the genetic structures surrounding those carbapenemase genes reveals that they are not specific to *Acinetobacter*. The identified genetic structures argue for a transfer

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from Enterobacteriaceae and *P. aeruginosa* to *A. baumannii*.

The most important source of carbapenem resistance in *A. baumannii* is the production of carbapenem-hydrolyzing Ambler class D β-lactamases (CHDL) [7]. Five different subgroups of acquired CHDLs have been identified, namely oxacillinase, class D β-lactamase (OXA)-23, -40, -58, -143 and -235 [2,7,8]. These five CHDL groups are specific to the *Acinetobacter* species and are not usually identified in other clinically relevant species, such as in Enterobacteriaceae and in *P. aeruginosa* [2,7]. In contrast to what is observed for other types of carbapenemase genes (those encoding the carbapenemases *K. pneumonia* carbapenemase, GES, verona metallo-β-lactamase and imipenemase), CHDL-encoding genes are associated with insertion sequences (IS) identified in *Acinetobacter* species (e.g., IS*Aba1*). The natural progenitor of one of these carbapenemase subgroups (OXA-23) has been identified as being *Acinetobacter radioresistens* [9]. To date, this corresponds to one of the few examples of a resistance gene originating from an *Acinetobacter* species and targeting another *Acinetobacter* species.

The case of the *bla*$_{NDM}$ genes

The *bla*$_{NDM-1}$ gene is one of the latest carbapenemase genes to have been reported. They have now been extensively reported in Enterobacteriaceae (*Escherichia coli* and *K. pneumoniae*), first from India, Pakistan and Bangladesh, and then from the rest of the world [10–12]. The occurrence of the *bla*$_{NDM-1}$ gene in the environment has also been evidenced in India, particularly among environmental Gram-negative species [13]. Sequencing of a *bla*$_{NDM-1}$-bearing plasmid, as part of the analysis of the resistosome of a multidrug-resistant *E. coli*, led to the identification of a remnant of IS*Aba125*, located immediately upstream of the *bla*$_{NDM-1}$ gene [14]. The IS*Aba125* element provided the -35 sequence of the hybrid promoter responsible for the expression of the *bla*$_{NDM-1}$ gene [14]. Interestingly, this IS element had been originally identified from an *A. baumannii* isolate (hence its name) without any physical association with the *bla*$_{NDM}$ gene [15]. Following this, IS*Aba125* was identified in many other *A. baumannii* isolates [6,16]. By contrast, this IS has been identified in Enterobacteriaceae and *P. aeruginosa* only as a remnant of the Tn*125* originating from *Acinetobacter* species and has never been identified alone in these species [17]. This observation suggests that *A. baumannii* is a likely reservoir of IS*Aba125*.

While the first studies dealt with the *bla*$_{NDM}$-like genes from enterobacterial isolates, many *A. baumannii* isolates carrying the *bla*$_{NDM}$-like gene have been now identified **(Table 1)** [2,18]. The possible progenitor (still not identified) of the *bla*$_{NDM}$ gene is considered to be phylogenetically distant from *A. baumannii* owing to the guanine-cytosine content of this gene (62% for the *bla*$_{NDM}$ gene vs from 38 [*Acinetobacter calcoaceticus*] up to 42% [*Acinetobacter lwoffii*] for genomes of *Acinetobacter* species). Likewise, the guanine-cytosine content of IS*Aba125* is 37% and the fact that IS*Aba125* has been identified alone (not associated with the *bla*$_{NDM-1}$ gene) seems to indicate the occurence of independent acquisition of *bla*$_{NDM-1}$ and IS*Aba125*. Transfer of the *bla*$_{NDM-1}$ gene to *A. baumannii* is probably the result of a transfer from an unknown bacterial species to *A. baumannii,* with both donor and recipient probably being present concomitantly in a same environment. In *A. baumannii*, the *bla*$_{NDM-1}$ gene is part of a 10,099-bp composite transposon made of two copies of IS*Aba125* **(Figure 1)** [19]. Downstream of *bla*$_{NDM-1}$/*bla*$_{NDM-2}$, eight open reading frames have been identified **(Figure 1)**. The first corresponds to the *ble*$_{MBL}$ gene, encoding a 121-amino acid-long protein conferring resistance to bleomycin (anticancer/antibiotic drug), previously found associated with *bla*$_{NDM-1}$ in enterobacterial isolates [20]. Then, several genes encoding putative proteins sharing similarities with genes identified from the *Brevundimonas* and *Xanthomonas* genera were identified [19]. Downstream, two genes encoding the GroES and GroEL chaperonin proteins, respectively, were identified. Finally, a gene encoding the putative transposase of an IS common region (IS*CR*)-like element was identified sharing 93% protein sequence identity with IS*CR19* [21]. IS*CR* elements are IS able to mobilize DNA fragment located at their left-hand extremity by rolling-circle transposition [22]. At the right-hand extremity of IS*CR21* and before the second copy of IS*Aba125* of Tn*125*, a truncated gene encoding a putative phospholipid acetyltransferase was identified, with the corresponding protein sequence sharing 91% amino acid identity with that of *Acinetobacter junii* **(Figure 1)** [19]. This finding indicates a likely intermediate state that may have occurred in an *Acinetobacter* species other than *A. baumannii*. Our hypothesis is

Table 1. Epidemiologic features of NDM-producing *Acinetobacter* species.

Isolates or species	Isolates (n)	Acquired carbapenemase	Worldwide clone	MLST analysis[†]	Country of isolation	Probable origin	Ref.
Acinetobacter baumannii *isolates*							
A. baumanii JH	1	NDM-1, OXA-23	WWI	ST1[P]	Switzerland	Balkans region	[18]
A. baumannii ANC4097	1	NDM-1, OXA-23	WWI	ST1[P]	Czech Republic	Egypt	[40]
A. baumannii 161/07	1	NDM-1	None	ST25[P]	Germany	Serbia	[41]
A. baumannii Slo	1	NDM-1	None	ST25[P]	Slovenia	Balkans region	[18]
A. baumannii	8	NDM-1	None	ST85[P]	France	North Africa	[32]
A. baumannii Ab11314	1	NDM-1	WWII	ST92[B]	Belgium	Algeria	[42]
A. baumannii A28, A32 and A36	3	NDM-1, OXA-23	N/A	N/A	India	India	[26]
A. baumannii	1	NDM-1, OXA-23	N/A	N/A	Japan	India	[43]
A. baumannii ABCA207, ABC3229, ABC4289, ABWA7	4	NDM-1	N/A	N/A	China	China	[27]
A. baumannii ML	1	NDM-2	None	ST103[P]	Germany	Egypt	[33]
A. baumannii I1, I2, I15, I16, I17	5	NDM-2	None	ST103[P]	Israel	?	[34]
A. baumannii 124, 132	2	NDM-2	None	ST103[P]	United Arab Emirates	United Arab Emirates	[35]
Acinetobacter *non*-baumannii *isolates*							
A. lwoffii WJ10621	1	NDM-1	N/A	N/A	China	?	[44]
A. pittii D499	1	NDM-1	N/A	N/A	China	?	[45]
A. junii 1454	1	NDM-1	N/A	N/A	China	?	[46]
Acinetobacter spp.	9	NDM-1	N/A	N/A	China	China	[28]
A. lwoffii SGC-HZ9	1	NDM-1	N/A	N/A	China	China	[29]
A. pittii	27	NDM-1	N/A	ST63[P]	China	China	[47]
A. johnsonii	2	NDM-1	N/A	N/A	China	China	[30]

[†]Two different MLST schemes are currently available for the typing of *Acinetobacter* species. Here, ST[P] designed Pasteur's Institute MLST scheme and ST[B] designed Bartual and colleagues MLST scheme.
MLST: Multilocus sequence typing; N/A: Not applicable; NDM: New Delhi metallo-β-lactamase; OXA: Oxacillinase, class D β-lactamase; ST: Sequence type; WWI: Worldwide clonal lineage I; WWII: Worldwide clonal lineage II.

that an *Acinetobacter* species acquired the bla_{NDM} gene from an environmental species, probably via a natural transformation process (a physiological process encoded by a wide range of bacteria, permitting the uptake of exogneous DNA via the binding of dsDNA on a specific membrane receptor followed by the entry of ssDNA; finally, homologous recombination is necessary to incorporate the exogeneous DNA) **(Figure 2)**. In that species, the transposon Tn*125* was built. Subsequently, *A. baumannii* acquired the bla_{NDM} gene through the acquisition of this transposon. After its dissemination among *Acinetobacter* species, an interspecies transfer via a broad-host range plasmid permitted the acquisition of Tn*125* by Enterobacteriaceae or *P. aeruginosa*. This step was followed by a large dissemination of the bla_{NDM} in Enterobacteriaceae **(Figure 2)**.

Interestingly, a recent study suggested that the bla_{NDM-1} has been constructed in *Acinetobacter* [23]. Precise genetic analysis of the bla*NDM-1* gene itself revealed an interesting feature. It shared the 5′ end sequence of the aminoglycoside resistance gene *aphA6* (displaying 100% identity in the spacer sequence [260 bp] between IS*Aba125* and either the bla_{NDM-1} or *aphA6* genes and the first 20 nucleotides of the both genes) [23]. **Figure 1B** demonstrates the two hypotheses of the construction of the bla_{NDM-1} into *Acinetobacter* species:

- The first hypothesis is a homologous recombination process between, on the one hand, a composite transposon made of two copies of IS*Aba125* and carrying the *aphA6* gene, and,

Figure 1. Diversity of genetic structures surrounding the *bla*$_{NDM-1}$ gene (facing page). (A) Genetic analysis comparison of the structures identified in *Acinetobacter baumannii* and other Gram-negative rods: Tn*125* (Genbank accession number JN872329) from *A. baumannii* NRZ [19], pKP-NDM (Genbank accession number JN157804) from *Klebsiella pneumoniae* Kp7 [48], pNDM-GUE (Genbank accession number JQ364967) from *Escherichia coli* GUE [17] and p271A (Genbank accession number JF785549) from *E. coli* 271 [14]. Gene name abbreviations are deduced from their corresponding proteins. Insertion sequence elements are IS*Aba125*, ISCR*21*, IS*Kpn7*, IS*26*, IS*EC33* and IS*Sen4*, whereas transposon is Tn*5403*. *aadA2* and *drfA17* encode aminoglycoside and trimethoprim resistance, respectively. Base pair duplications are indicated by 3 and 8 bp. The *ori*IS of IS*CR21* is indicated by a circle. The conserved *bla*$_{NDM-1}$ locus is indicated by vertical black lines. **(B)** Possible hypotheses of the *bla*$_{NDM-1}$ construction: hypothesis 1: the *bla*$_{NDM-1}$ gene could have been constructed by a recombination event removing the sequence between the arrows, including the *aphA6* gene, and giving rise to the *bla*$_{NDM-1}$ gene; hypothesis 2: the *bla*$_{NDM-1}$ could have been constructed by rolling-circle transposition mediated by IS*CR21* into the *aphA6* gene. The yellow box into the *bla*$_{NDM-1}$ gene indicates the shared sequence between the *bla*$_{NDM-1}$ and the *aphA6* genes.
IRR: Inverted repeat right; IRL: Inverted repeat left; IS: Insertion sequence; IS*CR*: Insertion sequence common region.

on the other hand, the *bla*$_{NDM-1}$ gene, giving rise to the neoformed *bla*$_{NDM-1}$ gene identified in the Tn*125*;

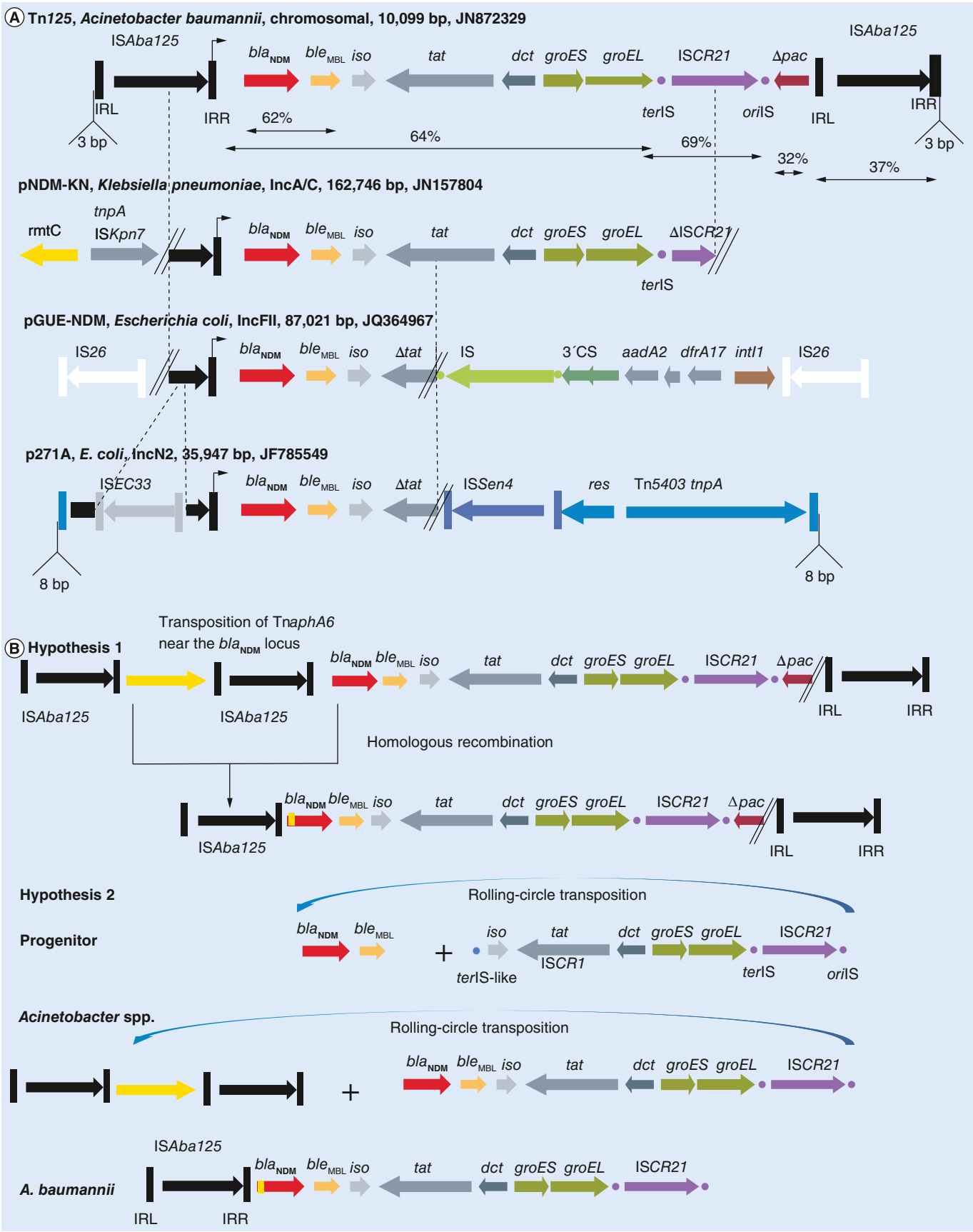
- The construction was due to two steps of the rolling-circle transposition event, which inserted the fragment containing the *bla*$_{NDM-1}$ gene from the progenitor into the *aphA6* gene **(Figure 1B)**.

In light of these recent findings, a new role for *A. baumannii* may be considered. Although usually considered as a final recipient of resistance genes, *A. baumannii* may transfer them to Enterobacteriaceae, and *P. aeruginosa* after a probable acquisition from an environmental resistome **(Figure 2)**. To date, the role of *A. baumannii* as a source of resistance genes for other clinically significant bacterial species has not been underlined. There is only one single report of the *bla*$_{OXA-23}$ gene outside *A. baumannii*, being identified in *Proteus mirabilis*, as well as another single report of acquisition of the natural cephalosporinase *bla*$_{ADC}$ gene in *Oligella urethralis* (a Gram-negative rod belonging to Moraxellaceae [24,25]. However, in these two cases, *A. baumannii* has played the role of progenitor, whereas it played the role of an intermediate reservoir for the *bla*$_{NDM}$ genes.

Epidemiology of NDM-producing *Acinetobacter* species

NDM-1-producing *A. baumannii* were first identified in India [26] and then in China [27]. NDM-1-producing non-*baumannii Acinetobacter*, including *A. lwoffii*, *A. junii*, *Acinetobacter pittii*, *Acinetobacter haemolyticus* and *Acinetobacter* genomospecies 10 and 15, have been reported from nosocomial settings and environmental sources in China [28–30]. In these studies, the *bla*$_{NDM-1}$ gene is carried by a plasmid with a size ranging from approximately 30 to 55 kb, which may be transferred to *E. coli* by conjugation. Although these experiments are *in vitro* analyses, we might hypothesize that the dissemination of the *bla*$_{NDM}$ genes from *Acinetobacter* species to Enterobacteriaceae could be due to the interspecies transfer of this plasmid. In contrast to what is observed in China, a report highlighting the scattered diffusion of NDM-1-producing *A. baumannii* in Europe demonstrated that this dissemination was neither due to a single clone nor to plasmid diffusion, but rather to a spread of different clones carrying a same transposon Tn*125* [18]. Multilocus sequence typing (MLST) analysis using Pasteur's Institute scheme [31] revealed that the clones circulating in Europe belonged to sequence type (ST)1 (also named worldwide clone I) and ST25 with a probable link with the Balkans region. Recently, several *A. baumannii* clinical isolates possessing the *bla*$_{NDM-1}$ gene and belonging to ST85 according to Pasteur's Institute MLST scheme have been identified. These isolates were recovered from patients originating from North Africa, with no obvious link to the Indian subcontinent. This finding strongly suggests that a single NDM-producing *A. baumannii* clone is likely widespread in North Africa and that it may act as a secondary reservoir of spread of the NDM-1 resistance trait [32]. These genome comparison analyses indicated that several reservoirs of NDM-1-producing *A. baumannii* have been established at least in Asia (China and India), the Balkans region and North Africa.

Hitherto, only a single NDM-1 variant has been described in *A. baumannii*, NDM-2 [33]. This variant has been identified in a German patient (with a travel history in Egypt), from Israel and from the United Arab Emirates [33–35]. MLST analysis revealed that these isolates belong to the same clone, ST103 (or ST253 according to MLST scheme developed by Bartual and



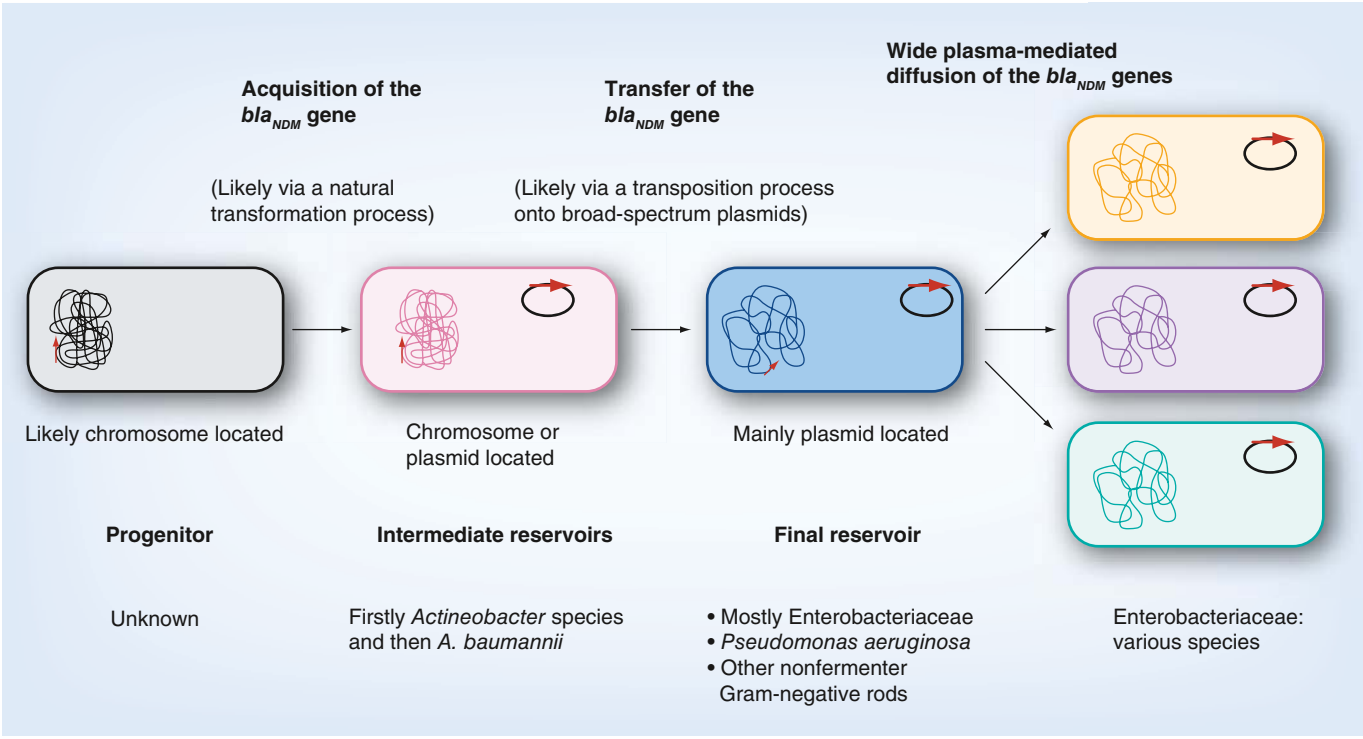


Figure 2. Hypothesis of the diffusion of the bla_{NDM} genes from the progenitor to clinical isolates. The different species are indicated by different colors.

colleagues [36]), corresponding to a new clone with no link to sequence types found elsewhere. In contrast to NDM-1-producing *A. baumannii*, the reservoir of the bla_{NDM-2} gene seems to be present at least in the Middle East.

Medical implications

Identification of *A. baumannii* as a source of multidrug resistance is now well established. We suggest that identification of resistance genes in *A. baumannii* worldwide is important, not only for preventing the spread of multidrug resistance traits in *A. baumannii,* but also for preventing their potential transfer to Enterobacteriaceae, which are by far the most important sources of infection in humans. *A. baumannii,* which had been mostly considered a weak pathogen responsible for infections in immunocompromized patients, may become a Trojan horse for spreading antibiotic resistance genes in unrelated and clinically significant Gram-negative species.

More specifically, identification of NDM producers in *A. baumannii* appears to be crucial considering that these isolates have already disseminated at least in some areas (North Africa, Europe, China and India). Another important concern is that *A. baumannii* is well-known to persist much longer than Enterobacteriaceae in the environment [37]. Consequently, outbreaks involving multidrug-resistant *A. baumannii* are difficult to eradicate [1,2,38], as exemplified by a study demonstrating the re-emergence of the same *A. baumannii* clone 6 months later in a burns unit owing to environmental contamination [39]. The closure of this burns unit was necessary to eradicate the contamination [39]. Early identification is therefore very important.

Future perspective

The current diffusion of carbapenemase genes in Gram-negative rods is now a serious public health issue and, in the near future, could lead back to the preantibiotic era. The screening of multidrug-resistant *A. baumannii* and the monitoring, in particular, of the carbapenem resistance in that species has to be considered a crucial issue. The NDM saga demonstrates that the diffusion of the bla_{NDM-1} gene in Enterobacteriaceae, probably originating from *Acinetobacter* species, shows that diffusion of resistance genes is more complex than expected.

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EXECUTIVE SUMMARY

Background

- Members of the genus *Acinetobacter* have the ability to rapidly develop resistance to new antibiotics.
- The main problem concerning antibiotic resistance in *Acinetobacter baumannii* corresponds to the rise of carbapenem resistance, which is always associated with multidrug resistance.
- *A. baumannii* has been considered as a final recipient of antibiotic resistance genes originating from other clinically relevant Gram-negative species.

Carbapenem resistance in *A. baumannii*

- Carbapenem-hydrolyzing Ambler class D β-lactamases are the main source of carbapenem resistance in *A. baumannii*.
- The acquisition of most Ambler class B and A carbapenemases has resulted from the integration of foreign DNA in *A. baumannii* from Enterobacteriaceae or *Pseudomonas aeruginosa*.

The case of the *bla*$_{NDM}$ genes

- The *bla*$_{NDM}$ genes are associated with the insertion sequence *Aba125* (often truncated in Enterobacteriaceae), which plays a role in *bla*$_{NDM}$ expression.
- In *A. baumannii*, the *bla*$_{NDM-1}$ gene is part of a 10,099-bp composite transposon made of two copies of IS*Aba125*.
- A new role may be played by *A. baumannii* involving the acquisition of the *bla*$_{NDM-1}$ resistance gene and then transferring it to Enterobacteriaceae and *P. aeruginosa*.

Epidemiology of NDM-producing *Acinetobacter* species

- NDM-1-producing *A. baumannii* has been widely identified in India and China from nosocomial settings and environmental sources.
- Whereas the diffusion of NDM-1-producing *A. baumannii* in Asia seems to be due to a plasmid diffusion, in Europe, it was neither due to a single clone nor any plasmid diffusion, but rather to different clones carrying the transposon Tn*125*.
- Multilocus sequence typing analyses indicated several reservoirs of NDM-1-producing *A. baumannii* including Asia (mainly China and India), the Balkans region and North Africa.

Medical implication

- The identification of resistance genes in *A. baumannii* worldwide is important not only for preventing the spread of multidrug resistance in *A. baumannii*, but also for preventing their extension to Enterobacteriaceae.
- Outbreaks involving multidrug-resistant *A. baumannii* are known to be very difficult to eradicate owing to the ability of this bacterial species to persist in a nosocomial environment.

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