New *Klebsiella oxytoca* β -Lactamase Genes $bla_{\text{OXY-3}}$ and $bla_{\text{OXY-4}}$ and a Third Genetic Group of *K. oxytoca* Based on $bla_{\text{OXY-3}}$

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The two genetic groups (oxy-1 and oxy-2) previously identified in the *Klebsiella oxytoca* taxon are recognizable by four independent molecular markers: (i) ERIC-1R profiles, (ii) 16S ribosomal DNA (rDNA) signature sequences, (iii) singular nucleotides in a defined fragment of the *rpoB* gene, and (iv) the type of the strain's bla_{OXY} gene (i.e., $bla_{\text{OXY-1}}$ or $bla_{\text{OXY-2}}$). *K. oxytoca* strains SG266 and SG271 could not be classified into these genetic groups based on their ERIC-1R profile and bla_{OXY} gene sequence. With regard to the gene identity percentages between the $bla_{\text{OXY-1}}$ and $bla_{\text{OXY-2}}$ gene groups ($86.8\% \pm 0.4\%$) and within a bla_{OXY} gene group ($>99\%$), it was concluded that the bla_{OXY} gene of strain SG271 was representative of a new bla_{OXY} gene group ($bla_{\text{OXY-3}}$), since the mean identity percentages between it and the two bla_{OXY} gene groups were $85.5\% \pm 0.2\%$ and $84.4\% \pm 0.4\%$, respectively. Since the corresponding percentages were $95.0\% \pm 0.4\%$ and $86.2\% \pm 0.3\%$ for strain SG266, it was impossible to classify its bla_{OXY} gene, which was therefore named $bla_{\text{OXY-4}}$. The 16S rDNA signature sequences of the two strains could be determined only after cloning experiments. The SG266 clones displayed the same signature sequence as that of the genetic group oxy-1, whereas the SG271 clones displayed three different 16S rDNA signature sequences that also differed from those of the two genetic groups. Singular nucleotides were found within the *rpoB* sequence of the two strains, allowing for their distinction from the two genetic groups. All of these results, combined with those previously obtained by the ERIC-1R PCR method, indicate that strain SG271 is representative of a new *K. oxytoca* genetic group (oxy-3), whereas strain SG266 could not be classified.

In 1974, Jain et al. (15) were able to distinguish the *Oxytoca* group from the *Klebsiella* genus by DNA-DNA hybridization. In spite of this discovery and also the fact that *K. oxytoca* strain 497-2 (ATCC 13182^T) included in their analysis was a typical representative of this new taxon, these authors did not suggest a new genus of *Enterobacteriaceae*, concluding that such a suggestion might contribute to further confusion for bacteriologists, since *Oxytoca* organisms look like true *Klebsiella* with the exception of an indole reaction. This absence of a specific name is undeniably linked to the fact that true *Klebsiella oxytoca* strains had received multiple names (*Klebsiella aerogenes* and *Klebsiella pneumoniae*) until 1986, when *Klebsiella* nomenclature was changed (2, 12). Although less common than *K. pneumoniae* in human infectious diseases (23), *K. oxytoca* was the object of particular interest since some strains were found as early as 1980 with a singular β -lactam resistance phenotype in comparison with *K. pneumoniae* strains. In fact, these *K. oxytoca* strains were found to be resistant to aztreonam in the first assays performed with this molecule by the manufacturer (24). Fifteen years later, Fournier et al. (9) demonstrated that this aztreonam resistance was due to mutations located in the promoter region of the gene (bla_{OXY}) encoding the class A chromosomal β -lactamase (KOXY) of the resistant strains. Fournier et al. (11) also showed that the bla_{OXY} genes

were made up of two groups ($bla_{\text{OXY-1}}$ and $bla_{\text{OXY-2}}$) on the basis of the gene nucleotide sequence, with an identity percentage between the two gene groups of 87%. The corresponding KOXY β -lactamases were subsequently classified into the β -lactamase groups OXY-1 and OXY-2. More recently, an *Enterobacteriaceae* taxonomic analysis based on the sequence of certain fragments of the 16S ribosomal DNA (rDNA) and *rpoB* genes showed that *K. oxytoca* constitutes a monophyletic species clearly separated from *K. pneumoniae* (7). However, through different molecular studies, Brisse et al. (3) and Granier et al. (14) have shown that the *K. oxytoca* taxon is divided into two clades. We also determined that each clade corresponds to a *K. oxytoca* genetic group (oxy-1 or oxy-2) recognizable by four independent molecular markers: (i) characteristic bands in the profiles generated by the ERIC-1R PCR method, (ii) a signature sequence in a 16S rDNA fragment located from position 435 to position 458 according to the numbering of the *Escherichia coli* 16S rDNA sequence, (iii) singular nucleotides dispersed over the entire length of the 512-bp fragment of the *rpoB* gene shown by Mollet et al. (18) to identify enterobacterial species, and (iv) the type of the strain's bla_{OXY} ($bla_{\text{OXY-1}}$ or $bla_{\text{OXY-2}}$) gene (14).

Using the ERIC-1R PCR method to classify a panel of *K. oxytoca* clinical isolates into these two genetic groups, we detected two particular *K. oxytoca* strains: SG266 and SG271. In fact, neither their ERIC-1R profiles nor the sequences of their bla_{OXY} genes allowed us to classify them into the two *K. oxytoca* genetic groups previously described (13). Thus, the present study was carried out to determine whether these two

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isolates might be representative of two new *K. oxytoca* genetic groups.

MATERIALS AND METHODS

Bacterial strains and plasmids. Strains SG266 and SG271, identified by the routine method (API System; bioMérieux, Marcy l'Étoile, France) as *K. oxytoca* were obtained, both in 2000, from two patients hospitalized in the Saint Joseph Hospital in Paris (13). Strain SG266 infected a diabetic foot wound, and strain SG271 was isolated from peritoneal fluid. *E. coli* strain Epicurian Coli XL10-Gold and plasmid pPCR-Script (Cam^R; Stratagene, La Jolla, Calif.) were used for cloning experiments.

K. oxytoca strains ATCC 13182^T and SC 10,436, which both harbored a *bla*_{OXY-2} gene controlled by a low and a strong promoter, respectively (12, 14), were used as control strains in β -lactam MIC tests.

Biochemical identification. Strains SG266 and SG271 were resubmitted to biochemical identification by using the BIOTYPE-100 carbon source strip system (bioMérieux). This test was performed by the French *Enterobacteriaceae* Reference Center (Institut Pasteur, Paris, France).

β -Lactam susceptibility. β -Lactam susceptibility was determined by the agar dilution method on Mueller-Hinton agar with a Steers multiple inoculator and 10⁴ CFU per spot. The following antibiotics were tested: amoxicillin (Glaxo-SmithKline, Marly-le-Roi, France); ticarcillin (GlaxoSmithKline), alone or combined with 2 μ g of clavulanate (GlaxoSmithKline)/ml; piperacillin (Wyeth-Lederle, Paris-La Defense, France), alone or combined with 4 μ g of tazobactam (Wyeth-Lederle)/ml; cephalothin (Lilly, Suresnes, France); cefotaxime (Aventis, Paris, France); ceftazidime (GlaxoSmithKline); cefepime (Bristol-Myers Squibb, Paris-La Defense, France); aztreonam (Sanofi Winthrop, Gentilly, France); ceftiofime (Panpharma, Fougères, France); and imipenem (Merck Sharp & Dohme-Chibret, Paris, France). The results were interpreted according to the French Antibigram Committee recommendations (1).

***bla*_{OXY} gene and KOXY β -lactamase sequence comparison.** The *bla*_{OXY} genes of strains SG266 and SG271 (GenBank accession numbers AY077481 and AF491278, respectively) and the corresponding KOXY β -lactamases were aligned by using Align software (20), with the *bla*_{OXY} genes and the corresponding proteins registered under the following GenBank accession numbers: Z30177, Y17715, AY077486, AY077484, AY077483, AY077482, and M27459 for the *bla*_{OXY-1} genes and Z49084, AF473577, AY055205, AY077489, AY077488, AY077487, AY077485, D84548, and Y17714 for the *bla*_{OXY-2} genes. Three *bla*_{OXY-1} genes (strains 756, 563, and 1879-77) and three *bla*_{OXY-2} genes (strains K1794, 11V, and KER) that had been published (10, 22) but not submitted to GenBank were also included in the comparative analysis.

Preparation of crude extracts and isoelectrofocusing. This experiment was performed as previously described (17).

The pI values of the β -lactamases produced by strains SG266 and SG271 were determined by comparison with the pI values of known β -lactamases—OXY-2-1 (pI 5.2), TEM-1 (pI 5.4), TEM-2 (pI 5.6), PSE-2 (pI 5.7), SHV-3 (pI 7), OXY-1-1 (pI 7.5), ACC-1 (pI 7.75), and SHV-5 (pI 8.2)—and isoelectrofocusing standards (Bio-Rad Laboratories, Hercules, Calif.).

KOXY β -lactamase phylogenetic tree. The amino acid sequences of the KOXY β -lactamases previously published (2, 9–12, 14, 16, 22, 25) and those of the β -lactamases of strains SG266 and SG271 were aligned by using Dialign 2 software (19). The KOXY β -lactamase phylogenetic tree was built with TreeTop software from GeneBee services (4).

PCR, sequencing, and comparison of *rpoB* and 16S rDNA genes. The 512-bp fragment of the *rpoB* gene and the 387-bp fragment of the 16S rDNA gene, allowing for the identification of enterobacterial species (7) and *K. oxytoca* genetic groups, were amplified from strains SG266 and SG271 and sequenced as previously described (14). The *rpoB* sequences were then directly compared by using Align software (20) to the previously published *rpoB* consensus sequences characteristic of the *K. oxytoca* genetic groups oxy-1 and oxy-2 (14). For the 16S rDNA fragment, the sequences were compared to the 16S rDNA consensus sequences only after cloning experiments.

16S rDNA cloning experiments and analysis of recombinant plasmids. The 16S rDNA amplified product obtained from strains SG266 and SG271 was purified and then cloned into pPCR-Script cloning vector according to the manufacturer's recommendations (Stratagene, La Jolla, Calif.). The recombinant plasmids were transferred into *E. coli* XL10-Gold by using the heat shock method. Transformants were selected onto agar plates containing chloramphenicol (30 mg/liter), X-Gal (5-bromo-4-chloro-3-indolyl- β -D-galactopyranoside; 40 mg/liter), and IPTG (isopropyl- β -D-thiogalactopyranoside; 40 mg/liter), and those displaying a white color were analyzed. Thus, the fragment inserted into the

TABLE 1. Identity percentages between the nucleotide sequences of the *bla*_{OXY} and *rpoB* genes of *K. oxytoca* strains SG266 and SG271 and the previously published *bla*_{OXY} genes and the *rpoB* consensus sequences characteristic of the *K. oxytoca* genetic groups oxy-1 and oxy-2

Genetic group or strain (n)	% Identity $\pm$ SD					
	Genetic group oxy-2		Strain SG266		Strain SG271	
	<i>bla</i> _{OXY-2}	<i>rpoB</i> consensus	<i>bla</i> _{OXY}	<i>rpoB</i>	<i>bla</i> _{OXY}	<i>rpoB</i>
Genetic group oxy-1						
<i>bla</i> _{OXY-1} (10)	86.8 $\pm$ 0.4		95.0 $\pm$ 0.4		85.5 $\pm$ 0.2	
<i>rpoB</i> consensus		96.7		96.5		96.5
Genetic group oxy-2						
<i>bla</i> _{OXY-2} (12)			86.2 $\pm$ 0.3		84.4 $\pm$ 0.4	
<i>rpoB</i> consensus				95.3		97.3
Strain SG266						
<i>bla</i> _{OXY}					85.8	
<i>rpoB</i>						95.9

recombinant plasmids was amplified by using the plasmidic T3 and T7 primers; fragments with the expected size were then sequenced.

GenBank accession numbers. The *rpoB* gene partial sequences of strains SG266 and SG271 have been registered in GenBank database under accession numbers AF491282 and AF491280, respectively.

RESULTS

***bla*_{OXY} gene and KOXY β -lactamase comparison.** As indicated in Table 1, pairwise comparative analysis of the *bla*_{OXY} gene of strains SG266 and SG271 and the *bla*_{OXY} genes previously published, i.e., 10 *bla*_{OXY-1} and 12 *bla*_{OXY-2} genes showed that the mean percentages of *bla*_{OXY} gene identity were 95.0% $\pm$ 0.4% and 86.2% $\pm$ 0.3% for strain SG266 and *bla*_{OXY-1} and *bla*_{OXY-2} genes, respectively, and 85.5% $\pm$ 0.2% and 84.4% $\pm$ 0.4%, respectively, for strain SG271. These percentages were 86.8% $\pm$ 0.4% between the *bla*_{OXY-1} and the *bla*_{OXY-2} genes and only 85.8% between the *bla*_{OXY} genes of strains SG266 and SG271. The comparison of the KOXY β -lactamases encoded by all of these *bla*_{OXY} genes allowed us to determine that certain amino acids ($n = 34$) located at positions not previously defined as being involved in the enzymatic active site (8) were singular for one of the four β -lactamase types: OXY-1 group, OXY-2 group, the KOXY β -lactamase of strain SG266, and the KOXY β -lactamase of strain SG271 (Table 2). A particular amino acid was more often observed in the KOXY β -lactamase of strain SG271 ($n = 15$) and the OXY-2 group ($n = 14$) than in the OXY-1 group ($n = 3$ or 5) or the KOXY β -lactamase of strain SG266 ($n = 3$). As indicated in Table 2, the pI values of the β -lactamases of strains SG266 and SG271 were 7.7 and 6.7, respectively.

According to the phylogenetic tree constructed from the amino acid sequence of all of these KOXY β -lactamases, we observed three phylogenetically distant branches (Fig. 1). The first branch included all of the OXY-1 β -lactamases tested and the KOXY β -lactamase of strain SG266 which was, however, the most distant β -lactamase in this β -lactamase group. The second branch included only the KOXY β -lactamase of strain SG271, and the third branch included exclusively the OXY-2 β -lactamases tested. This subdivision was obtained with bootstrap values of 100%.

TABLE 2. pI values and discriminant amino acids for OXY-1 and OXY-2 β -lactamase groups and the β -lactamases of *K. oxytoca* strains SG266 and SG271

β -Lactamase group or strain (<i>n</i>)	pI value(s)	Amino acid ^a at position (Ambler numbering):												
		4 (-)	9 (-)	11 (-)	12 (8)	15 (11)	17 (13)	22 (18)	25 (21)	29 (25)	30 (26)	31 (27)	32 (28)	33 (29)
OXY-1 group (8) ^b	7.1, 7.5, 8.2, 8.8	S	T	L	M	A	V	A	S	S	A/V	D	A	I
OXY-2 group (9) ^c	5.2, 5.7, 6.4, 6.5, 6.8	S	I	M	L	del	V	A	A	S	T	D	A	I
SG266	7.7	S	S	L	M	del	V	A	S	S	A	D	T	L
SG271	6.7	T	S	L	I	del	L	C	S	N	A	del	del	I

^a Amino acid numbering is based on the sequence of the OXY-1 β -lactamases.
^b Strains SL781 (10), KH66 (25), SG344 (14), 756 (10), SG49 (14), 563 (10), 1879-77 (10), and SG337 (14).
^c Strains SC 10,436 (12), KoKER (22), 11V (10), D488 (10), SL911 (10), ATCC 13182 (14), SG4 (14), SG77 (14), and SG176 (14). Amino acids in boldface are specific for genetic groups or strains.

β -Lactam MICs. As indicated in Table 3 *K. oxytoca* strains SG266 and SG271 were resistant to amoxicillin and ticarcillin but susceptible to these two molecules when they were combined with 2 μ g of clavulanate/ml and were also susceptible also to all other β -lactam molecules tested.

***rpoB* and 16S rDNA sequence comparison.** The comparison (Table 1) of the 512-bp fragment of the *rpoB* gene of strains SG266 and SG271 with the two *rpoB* consensus sequences previously defined for the strains belonging to the *K. oxytoca* genetic groups oxy-1 and oxy-2, respectively, showed that the percentage of identity was higher between strain SG266 and the oxy-1 genetic group (96.5%) than between strain SG266 and the oxy-2 genetic group (95.3%) and, inversely, higher

between strain SG271 and the oxy-2 genetic group (97.3%) than between strain SG271 and the oxy-1 genetic group (96.5%). The percentage of the *rpoB* gene identity between strains SG266 and SG271 was only 95.9%, whereas this percentage was 96.7% between the two *rpoB* consensus sequences (Table 1). The nucleotide differences observed all along the 512-bp *rpoB* fragment between strain SG266, strain SG271, the consensus sequence of the genetic group oxy-1, and that of the genetic group oxy-2 resulted in several singular nucleotides at given positions according to the two genetic groups and the two strains (Table 4) Thus, C162, T258, C264, C270, C276, and G285 were only observed in the *rpoB* consensus sequence of the genetic group oxy-1, whereas A3, C114, C123, and T339

FIG. 1. KOXY β -lactamase phylogram. The tree was built from the KOXY β -lactamase sequences of 19 strains (as indicated on the right side of the figure). Eight β -lactamases belong to the OXY-1 group, and nine belong to the OXY-2 group. Numbers within the phylogram indicate the bootstrap values of branching. The distance ladder is indicated under the tree.

TABLE 2—Continued

Amino acid ^a at position (Ambler numbering):																				
38 (34)	43 (39)	44 (40)	54 (50)	56 (52)	75 (72)	90 (—)	101 (98)	103 (100)	111 (108)	129 (128)	140 (137)	143 (140)	161 (158)	168 (165)	171 (168)	134 (181)	194 (191)	230 (227)	270 (269)	278 (277)
A	R	S	N	A	G	N	K	S	I	A	K	S	V	T	A	T	R	A	Q	E/K
T	R	S	N	A	S	N	N	A	I	T	L	G	A	T	T	S	R	E	Q	E
A	R	S	N	A	G	H	K	S	I	A	K	G	V	M	A	T	R	A	Q	E
A	S	T	D	T	G	N	K	S	V	A	K	G	V	T	A	T	H	A	L	D

were only observed in that of the genetic group oxy-2. Six (T2, T3, A36, G84, A108, and G141) and nine (C87, T108, T168, C192, T255, G270, C372, T468, and T489) other particular amino acids were observed in the 512-bp *rpoB* fragments of strains SG266 and SG271, respectively.

Concerning the sequence of the 387-bp 16S rDNA fragments of strains SG266 and SG271, we observed (Table 5) different nucleotide possibilities on the chromatograph, exactly at the nucleotide positions that allowed us to distinguish (signature sequences) the two 16S rDNA consensus sequences characteristic of the two *K. oxytoca* genetic groups (14). After we cloned the 387-bp fragment, which was amplified from strains SG266 and SG271, we analyzed the sequence of the 387-bp fragment from five clones derived from strain SG266 and four clones derived from strain SG271. The five clones derived from strain SG266 displayed an identical signature sequence corresponding to that previously defined for the *K. oxytoca* genetic group oxy-1 (Table 5), whereas the four clones derived from strain SG271 displayed three different signature sequences that were, moreover, different from those previously defined for the two genetic groups of *K. oxytoca* (Table 5). Pairwise analysis of the sequences of the 16S rDNA fragment of the three different clones obtained from strain SG271 and the 16S rDNA consensus sequence of each genetic group showed (Table 5) that clone C had a high percentage of identity with the 16S rDNA consensus sequence of the genetic group oxy-1, whereas clone D displayed a similarly high percentage of identity with the 16S rDNA consensus sequence of the genetic group oxy-2. Clones A and B, which showed the same 16S rDNA signature sequence, displayed a lower identity percentages with each 16S rDNA consensus sequence, a percentage that was similar to that observed between the two 16S rDNA consensus sequences (Table 5).

Extensive biochemical analysis of strains SG266 and SG271.

Although all of the genetic analyses performed indicated that strains SG266 and SG271 belong to the *K. oxytoca* taxon, we decided to submit them to an extensive biochemical analysis since they displayed particular features regarding the genes studied.

Although strain SG266 displayed negative tests for three biochemical reactions normally positive for *K. oxytoca* strains (*meso*-tartrate, *trans*-aconitate, and DL-glycerate), it was confirmed as belonging to the *K. oxytoca* taxon. Strain SG271, which displayed numerous negative reactions (notably for *meso*-tartrate, D-malate, *trans*-aconitate, tricarballoylate, putrescine, succinate, fumarate, DL-glycerate, D-glucosamine, and L-aspartate), could only be identified as *Klebsiella* but without species definition.

DISCUSSION

We have previously determined that the *K. oxytoca* taxon is divided into two genetic groups (oxy-1 or oxy-2) recognizable by four independent molecular markers (14) and have proved that the genetic group classification made on the basis of the analysis of one of these four markers was confirmed by the secondary analysis of another marker. Thus, we have classified *K. oxytoca* strain ATCC 13182^T into the oxy-2 genetic group on the basis of its 16S rDNA and *rpoB* sequences available in GenBank and then confirmed this classification by sequencing its *bla*_{OXY} gene (14). We have also classified 15 clinical isolates identified as *K. oxytoca* through routine biochemical analysis into either of the two genetic groups on the basis of their ERIC-1R profile and then confirmed this classification by sequencing their *bla*_{OXY} genes (13). However, we were unable to classify strains SG266 and SG271 into the two *K. oxytoca* genetic groups on the basis of their ERIC-1R profiles and the sequences of their *bla*_{OXY} genes (13). Subsequently, the present study was carried out to determine whether these two strains could be representative of two new *K. oxytoca* genetic groups.

Taking into consideration the fact that the *bla*_{OXY} gene of strain SG271 displayed a lower identity percentage with the *bla*_{OXY-1} gene group (85.5% $\pm$ 0.2%) and with the *bla*_{OXY-2} gene group (84.4% $\pm$ 0.4%) than that observed between these

TABLE 3. β -Lactam MICs for *K. oxytoca* strains ATCC 13182^T, SC 10,436, SG266, and SG271

β -Lactam	MIC (μ g/ml)			
	ATCC 13182 ^{Ta}	SC 10,436 ^b	SG266	SG271
Amoxicillin	128	>1,024	64	16
+ 2 μ g of clavulanate/ml	1	128	0.5	0.25
Ticarcillin	32	>1,024	64	16
+ 2 μ g of clavulanate/ml	1	256	2	0.5
Piperacillin	2	512	2	1
+ 4 μ g of tazobactam/ml	0.5	256	0.5	0.12
Cephalothin	2	64	2	2
Cefoxitin	0.5	1	1	0.5
Cefotaxime	$\leq$ 0.06	2	$\leq$ 0.06	$\leq$ 0.06
Ceftazidime	0.12	1	$\leq$ 0.06	$\leq$ 0.06
Cefepime	$\leq$ 0.06	2	$\leq$ 0.06	$\leq$ 0.06
Aztreonam	$\leq$ 0.06	32	$\leq$ 0.06	$\leq$ 0.06
Imipenem	0.5	0.5	0.5	0.5

^a Strain ATCC 13182^T (14) harboring a *bla*_{OXY-2} gene under the control of a wild type promoter.

^b Strain SC 10,436 (12) harboring a *bla*_{OXY-2} gene under the control of a mutated promoter responsible for the β -lactamase overproduction.

TABLE 4. Nucleotide variations within the 512-bp fragment of the *rpoB* gene of *K. oxytoca* belonging to the oxy-1 and oxy-2 genetic groups and of strains SG271 and SG266

Genetic group or strain	Nucleotide at position ^a :																																			
	2	3	6	24	36	84	87	96	108	114	120	123	141	162	168	192	225	246	255	258	264	270	276	285	288	294	300	339	351	372	447	468	486	489	504	
oxy-1	C	G	A	C	G	A	T	C/T	G	T	C	T	A	C	C	G	C/T	G	C	T	C	C	C	G	T	T	A	C	T	G	C	A	A	C	G/A	
oxy-2	C	A	G/A	C	G	A	T	T	G	C	T	C	A	T	C	G	C	G	C	C	T	T	T	T	C	C	G	T	T	G	C/T	A	T	C	G	
SG266	T	T	A	T	A	G	T	C	A	T	T	T	G	T	C	G	C	T	C	C	T	T	T	T	C	C	G	C	C	G	C	C	A	A	C	G
SG271	C	G	A	T	G	A	C	C	T	T	C	T	A	T	T	C	C	T	T	C	T	G	T	T	T	T	A	C	C	C	T	T	T	T	G	

^a Numbering starts at codon 500 of the *RpoB* protein of *E. coli* (18). Nucleotides in boldface are characteristic of genetic groups or strains. A still indicates the variability of the sequence within a genetic group, with the first nucleotide being the more frequent (consensus sequence).

two *bla*_{OXY} gene groups (86.8% ± 0.4%), we can reasonably conclude that the *bla*_{OXY} gene of strain SG271 is representative of a new *bla*_{OXY} gene group that we therefore suggest naming *bla*_{OXY-3}. This lower gene identity percentage resulted in the creation of a new branch of the KOXY β-lactamase phylogenetic tree, to which only the chromosomal β-lactamase of strain SG271 was attached. Thus, the OXY-3 β-lactamase of strain SG271 further enhances the aspect of the phylogenetic tree of KOXY β-lactamases. Regarding the sequence of the 512-bp fragment of the *rpoB* gene of strain SG271, we found six positions for which there were nucleotides specific for strain SG271 allowing us to distinguish this strain from the two genetic groups previously described (13). The third marker previously defined as being characteristic of the *K. oxytoca* genetic groups, namely, the 16S rDNA signature sequence, displayed new genetic features in strain SG271. In fact, by cloning the 387-bp 16S rDNA fragment from strain SG271, we were able to find three different signature sequences out of the four sequenced clones. This finding suggests the presence of at least three 16S rRNA operons in *K. oxytoca* and confirmed the existence of variations within the 16S rRNA operons of a same strain, as previously described for a very few bacterial species (6, 21, 26). Moreover, we found that the three different signature sequences also differed from either of the two 16S rDNA signature sequences characteristic of the two *K. oxytoca* genetic groups. All of these findings, combined with the fact that the ERIC-1R profile of strain SG271 did not display the oxy-1 or oxy-2 genetic group-specific bands (13), led us to conclude that we have discovered in strain SG271 a strain representative of a third genetic group in the *K. oxytoca* taxon. We suggest that this third genetic group be called oxy-3. The last point that we can emphasize about strain SG271 is the fact that the biochemical analysis performed by using 100 molecules as carbon source was able to identify it at only the genus level, *Klebsiella*, whereas all of the molecular studies that we carried out allowed for a species identification.

With regard to β-lactam susceptibility, strain SG271 displayed a β-lactam resistance pattern identical to that displayed by *K. oxytoca* strains normally producing β-lactamase. This was also the case of strain SG266, which was confirmed to be *K. oxytoca* via extensive biochemical analysis. However, the results obtained for this strain with regard to the four molecular markers characteristic of the *K. oxytoca* genetic groups did not allow us to classify it. Indeed, even if its *bla*_{OXY} gene displayed a high identity percentage with the genes of the *bla*_{OXY-1} group (95.0% ± 0.4%) and a low percentage with the genes of the *bla*_{OXY-2} group (86.2% ± 0.3%), this did not allow us to classify the SG266 strain's *bla*_{OXY} gene in the *bla*_{OXY-1} group, since we had previously shown that the identity percentage within a *bla*_{OXY} group was >99% (14). We therefore suggest that this gene be called *bla*_{OXY-4}. Cloning experiments of the 387-bp 16S rDNA fragment of strain SG266 resulted in five clones displaying identical signature sequences that perfectly matched those of the strains belonging to the oxy-1 genetic group. Among the clones tested, we did not find any that were responsible for the variations observed in the sequence of the fragment amplified and directly sequenced from strain SG266. This result suggests that the sequences found in the five clones are probably dominant among the 16S rRNA operons of strain SG266. The two remaining oxy genetic group markers studied

TABLE 5. 16S rDNA signature sequences and percentages of the 16S rDNA fragment homology between *K. oxytoca* genetic groups, strains SG266 and SG271, and their 16S rDNA clones

Genetic group or strain	16S rDNA signature sequence between positions 435 and 458 ^a	% Identity between the 16S rDNA fragment and:				
		oxy-2	SG266 clone A	SG271 clone A	SG271 clone C	SG271 clone D
oxy-1	CGATA-(14n)-GTCG	98.1	100	98.6	99.5	98.4
oxy-2	GAGTG-(14n)-ATTC		98.1	98.9	97.8	99.7
SG266	G/C A/G A/G T A-(14n)-G/A T T/C C/G					
SG266 clone A ^b	CGATA-(14n)-GTCG			98.6	99.5	98.4
SG271	C/GG/A A/T/G T A-(14n)-A/G T/G T/C C/G					
SG271 clone A ^c	GGATA-(14n)-ATTC				98.1	99.2
SG271 clone C	CGTTA-(14n)-GGCG					98.1
SG271 clone D	GAGTA-(14n)-ATTC					

^a According to the numbering of the *E. coli* 16S rDNA sequence (5).

^b Clones B, C, D, and E from strain SG266 displayed the same sequence as clone A.

^c Clone B from strain SG271 displayed the same sequence as clone A.

for strain SG266, ERIC-1R profile and singular nucleotides in the 512-bp fragment of the *rpoB* gene, not only confirmed that it was not possible to classify strain SG266 into the oxy-1 genetic group but also suggested that strain SG266 could belong to a subgenetic group. Indeed, on the basis of these two molecular markers, strain SG266 can clearly be distinguished from those belonging to either of the three oxy genetic groups that have been defined in the *K. oxytoca* taxon up to now. Finally, the possibility that strain SG266 might belong to a subgenetic group was also suggested by the position of its β -lactamase in the β -lactamase phylogenetic tree.

Interestingly, by discovering the two strains SG266 and SG271, we observed that the number of singular nucleotides, by which the two *rpoB* consensus sequences could have been differentiated (14), decreased. In fact, instead of the 17 nucleotides found to be specific for the recognition of the two genetic groups oxy-1 and oxy-2, only 6 remained specific for the oxy-1 genetic group and only 4 remained specific for the oxy-2 genetic group. However, six and nine became specific for strains SG266 and SG271, respectively. Such a discriminatory power cannot be attributed to the 16S rDNA signature sequence since the 16S rDNA fragment studied did not allow for the differentiation of strain SG266 from those of the oxy-1 genetic group.

Overall, the present study confirms that the ERIC-1R PCR method is a powerful tool to detect particular strains in the *K. oxytoca* taxon (13) and that the chromosomal *bla* gene of *K. oxytoca*, although it is not classified within the housekeeping gene family, is able, like housekeeping genes, to trace the bacterial diversification and evolution in the *K. oxytoca* taxon (14).

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