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OXA-14, Another Extended-Spectrum Variant of OXA-10 (PSE-2) β-Lactamase from *Pseudomonas aeruginosa*

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***Pseudomonas aeruginosa* 455, isolated in Ankara, Turkey, produced a pI 6.2 β-lactamase determined by plasmid pMLH53 and resisted all β-lactams except carbapenems. This β-lactamase, named OXA-14, corresponded to OXA-10 (PSE-2) except that aspartate replaced glycine at position 157 and thus is intermediate between OXA-10 and OXA-11, which has aspartate at position 157 and a further substitution at position 143.**

Most extended-spectrum β-lactamases are mutants of the class A enzymes, TEM-1, TEM-2, and SHV-1 (1, 6). Recently, however, we described an extended-spectrum mutant of the class D enzyme OXA-10 (PSE-2) (4) and named this enzyme OXA-11 (2). OXA-11 differed from OXA-10 by two mutations. We now describe a further OXA-10-derived extended-spectrum β-lactamase, from a *Pseudomonas aeruginosa* strain isolated at the hospital at which the original OXA-11 producer was isolated.

P. aeruginosa 455 was obtained in December 1991 from a patient treated at Hacettepe University Hospital, Ankara, Turkey, and was retained because of its considerable resistance to ceftazidime. Identification was confirmed with an API 20NE strip (Bio-Merieux, La Balme les Grottes, France). Other *P. aeruginosa* strains used included ABD, the original producer of OXA-11 enzyme (2); PU21 *ilv leu* Str^r Rif^r (5), used as a recipient; and PU21 transconjugants with plasmids pMLH51 and pMLH52, encoding OXA-10 and -11 enzymes, respectively (2). *Escherichia coli* NCTC 50192 (12) with plasmids of 154, 66, 38, and 7 kb was used in plasmid-sizing studies.

Antimicrobial agents tested were as follows: aztreonam and cefepime (Bristol-Myers Squibb, Syracuse, N.Y.); cefsulodin (Ciba-Geigy, Basel, Switzerland); ceftazidime and streptomycin (Glaxo, Greenford, Middlesex, United Kingdom); piperacillin sodium, tazobactam, and tetracycline (Lederle, Gosport, Hampshire, United Kingdom); cephalothin, moxalactam, and tobramycin (Lilly, Basingstoke, Hampshire, United Kingdom); cefoxitin and imipenem (Merck Sharp and Dohme, Hoddesdon, Hertfordshire, United Kingdom); ceftriaxone (Roche, Welwyn Garden City, Hertfordshire, United Kingdom); cefotaxime, cefpirome, and trimethoprim (Roussel, Uxbridge, Middlesex, United Kingdom); amikacin, benzylpenicillin, cephaloridine, chloramphenicol, ciprofloxacin, gentamicin, kanamycin, oxacillin, rifampin, and sulfamethoxazole (Sigma, St. Louis, Mo.); ampicillin sodium, carbenicillin disodium, clavulanate lithium, and cloxacillin (SmithKline Beecham, Brentford, Middlesex, United Kingdom); and meropenem (Zeneca, Macclesfield, Cheshire, United Kingdom).

MICs were determined on DST agar (Unipath, Basingstoke, Hampshire, United Kingdom) with inocula of 10^4 CFU per spot, as described previously (2). Resistance to sodium tellurite was tested by both methods described by Summers and Jacoby (11).

β-Lactamases were characterized by electrofocusing (8) of sonicates prepared from overnight nutrient agar cultures (2). Enzyme from strain 455 was extracted by sonication of log-phase cells, clarified by ultracentrifugation, and, in some cases, partially purified by anion-exchange chromatography on DEAE–Sephadex A-50, in 20 mM triethanolamine buffer (pH 7.6). Elution was with the same buffer containing a linear gradient of 0 to 0.5 M K_2SO_4. Extracts of OXA-10 enzyme were partially purified from PU21(pMLH51) by preparative isoelectrofocusing, as described previously (2). Hydrolysis assays were by UV spectrophotometry (2).

Transfer of resistance to *P. aeruginosa* PU21 was by overnight plate mating of logarithmic-phase cells on drug-free DST agar. Transconjugant selection was on the same medium containing ceftazidime at 25 or 50 μg/ml plus rifampin at 100 μg/ml. Presumptive transconjugants were confirmed as β-lactamase-producing auxotrophs. For size estimations, plasmids were extracted by the method of Hansen and Olsen (3) and electrophoresed at 100 V for 4 h in 0.7% agarose gels at 4°C. For restriction analysis, these extracts were precipitated twice with ethanol, dissolved in TE buffer (10 mM Tris HCl, 1 mM EDTA [pH 8.0]), digested with *Eco*RI, and electrophoresed overnight at 40 V in 0.9% agarose gels.

Total DNA for gene-probing studies was extracted, digested with *Bam*HI, and then electrophoresed, Southern blotted, and hybridized with probes exactly as described previously for strain ABD (2). The probe for $bla_{OXA-10/11}$ was constructed by PCR amplification of the OXA-11 coding region of pMLH52 with primers ABD1 and ABD4 (Fig. 1) and was labeled with digoxigenin (DIG DNA Labeling and Detection Kit; Boehringer, Lewes, East Sussex, United Kingdom). The sequence of the OXA-10-like gene from isolate 455 was determined from a DNA fragment amplified by PCR with primers ABD1 and 5′-biotin-labeled ABD4 (Fig. 1). The strands were separated by using paramagnetic beads conjugated with streptavidin (Dynabeads M-280 Streptavidin; Dynal, New Ferry, Wirral, United Kingdom) and used as a template for sequencing by chain termination with ABD1, ABD2, and ABD3 (Fig. 1) as primers.

Strain 455 resembled *P. aeruginosa* ABD, the original OXA-11 producer, in being highly resistant to aminoglyco-

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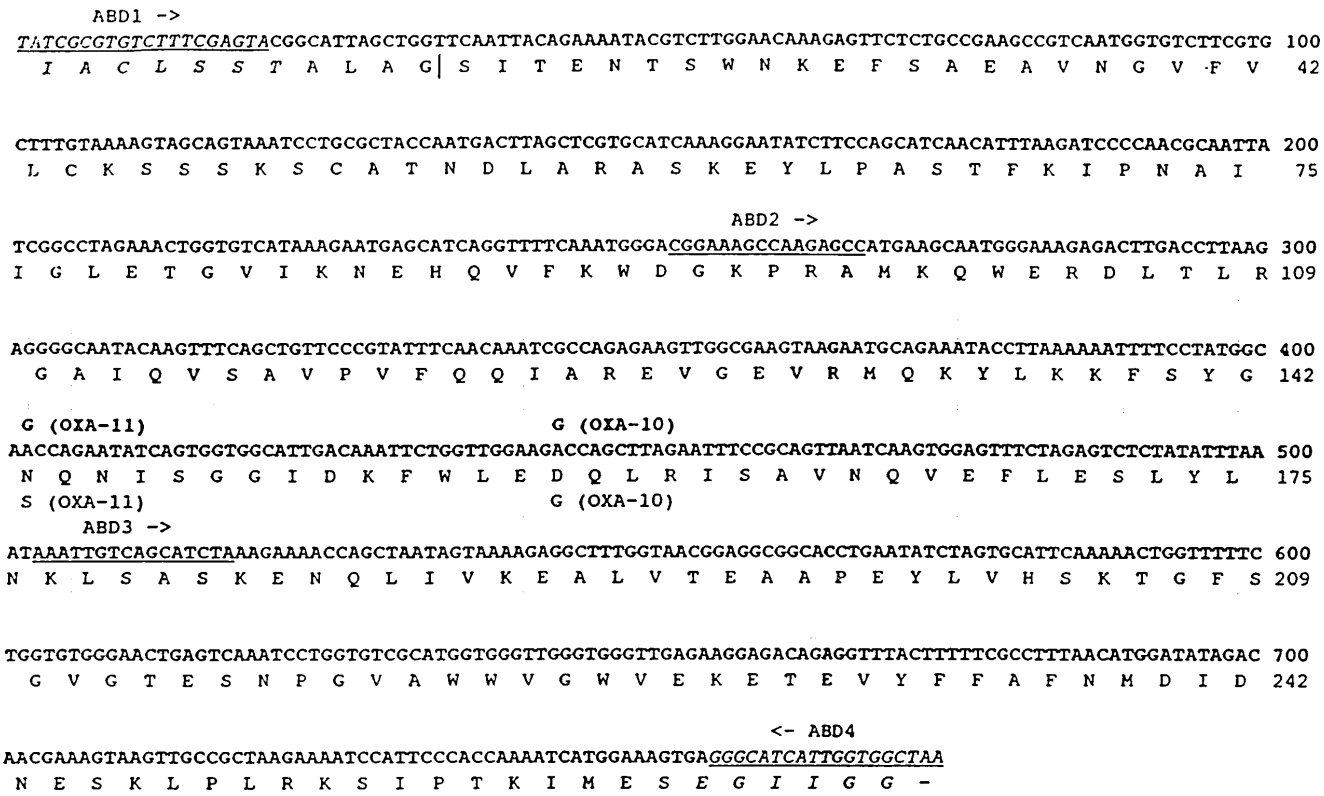

FIG. 1. Nucleotide sequence of the OXA-14 gene (Genome Sequence Data Base accession number L38523) from *P. aeruginosa* 455 and its deduced amino acid sequence. Nucleotides marked above, and amino acids marked below, the OXA-14 sequences indicate differences in the OXA-10 and OXA-11 genes. Amino acid numbering corresponds to the full-length precursor protein, as deduced by homology to OXA-10 (4). The vertical bar indicates the start of the mature protein. The locations of primers ABD-1, -2, -3, and -4 are underlined. Sequences corresponding to the amplification primers have not been independently determined for OXA-14 and are shown in italics.

sides, ciprofloxacin, and all antipseudomonal β-lactams except carbapenems. Strains ABD and 455 also were more resistant than were typical *P. aeruginosa* isolates (e.g., PU21) to chloramphenicol, though not to tetracycline (Table 1) or to sodium tellurite (MIC, 0.4 mM). Resistance to ceftazidime, piperacillin, or carbenicillin was not reversed by addition of clavulanate or tazobactam. Isolate 455 produced a pI 6.2 β-lactamase, which focused marginally below OXA-11 (pI 6.4). A pI 7.7 β-lactamase was also produced and was assumed to be the chromosomal *ampC* type, found in all *P. aeruginosa* isolates. The pI 6.2 enzyme and all the resistances, except that to ciprofloxacin, transferred to *P. aeruginosa* PU21 at a frequency of 10^{-7}. The original strain and its transconjugants contained a plasmid larger than the largest marker used (154 kb). This element was indistinguishable from pMLH52 from strain ABD in size, in the resistances that it conferred, and in its *Eco*RI digestion profile. Likewise, total DNA restriction patterns of strains ABD and 455 were indistinguishable. Probing of Southern blots of DNA digests revealed that both the plasmid described above and pMLH52 carried an OXA-11-like β-lactamase gene on a 3.5-kb *Bam*HI restriction fragment.

These data indicated close similarity between both plasmids and the β-lactamase genes of strains ABD and 455, and between the organisms themselves. However, whether their enzymes were identical remained to be determined. This was investigated by sequencing a PCR product, amplified from isolate 455, which comprised the entire β-lactamase coding region except for the first 46 nucleotides (within the signal peptide) and the last 19 nucleotides. The sole base difference compared with bla_{OXA-10} was that guanine replaced adenine at position 444, giving aspartate instead of a glycine at position 157 of the protein (Fig. 1). The present enzyme thus is an intermediate between OXA-10 and OXA-11, which not only has aspartate at position 157 but also has serine instead of asparagine at position 143. Since the designations OXA-12 (10) and -13 (1a) have already been allocated, we suggest OXA-14 as a designation for the enzyme of strain 455, and we designate its encoding plasmid pMLH53. It seems that aspartate instead of glycine at position 157 is critical for extended-spectrum activity in OXA-10-related class D β-lactamases. The same replacement occurred also in an extended-spectrum laboratory mutant of OXA-13 (9), which itself shares 96% amino acid homology with OXA-10.

A characteristic of OXA-14 enzyme was that both crude extracts and the semipurified enzyme gave strongly biphasic hydrolysis curves for all the β-lactams tested (ampicillin, benzylpenicillin, cephalothin, ceftazidime, cephaloridine, and cefotaxime). In each case, initial hydrolysis was rapid under the conditions used but the rate declined during the first 3 min, thereafter stabilizing at steady-state velocity. Such kinetics suggest partitioning of the enzyme, or of an enzyme-substrate complex, between two isomeric forms. They occur also—though much less dramatically—for several antibiotics with OXA-10 (7) but only for oxacillin with OXA-11 enzyme (2). The further substitution at position 143 may allow OXA-11 to maintain more stable hydrolytic activity.

Relative hydrolysis rates for various substrates during the slow phase of hydrolysis are shown in Table 2, in comparison

TABLE 1. MICs[a] for *P. aeruginosa* isolates, transconjugants, and reference strains

Antibiotic	MIC (μg/ml) for:					
	Isolates		Transconjugants			Recipient PU21
	455 (OXA-14)	ABD (OXA-11)	PU21 (OXA-14)[b]	PU21 (OXA-11)[b]	PU21 (OXA-10)[b]	
Carbenicillin	512	512	512	256	512	64
Carbenicillin + clavulanate[c]	512	512	256	ND[d]	ND	64
Carbenicillin + tazobactam[c]	512	512	256	ND	ND	64
Piperacillin	64	32	64	64	64	2
Piperacillin + clavulanate[c]	64	32	32	ND	ND	4
Piperacillin + tazobactam[c]	64	32	32	ND	ND	4
Cefepime	128	32	64	128	2	2
Cefotaxime	32	128	32	32	16	16
Cefpirome	128	64	128	128	8	2
Cefsulodin	32	32	32	128	32	2
Ceftazidime	512	512	512	1,024	4	4
Ceftazidime + clavulanate[c]	1024	512	256	512	4	2
Ceftazidime + tazobactam[c]	512	512	256	512	4	2
Ceftriaxone	64	128	128	128	64	16
Moxalactam	64	64	64	128	16	8
Aztreonam	16	64	16	32	16	4
Impenem	2	1	2	2	2	1
Meropenem	2	8	4	2	2	2
Amikacin	128	32	32	32	8	8
Gentamicin	>128	>128	>128	256	ND	4
Tobramycin	256	128	128	128	1	1
Chloramphenicol	>512	>512	>512	>512	128	128
Ciprofloxacin	4	8	0.25	0.25	0.25	0.25
Tetracycline	64	128	64	64	64	64

[a] The MICs of kanamycin, streptomycin, sulfamethoxazole, and trimethoprim were at least 512 μg/ml for all the organisms.
[b] OXA-10 enzyme was encoded by plasmid pMLH51 (2), OXA-11 was encoded by pMLH52 (2), and OXA-14 was encoded by pMLH53.
[c] Inhibitors were used at a concentration of 4 μg/ml.
[d] ND, not determined.

with kinetic data for OXA-10. Like other class D (Bush group 2d) enzymes, OXA-14 had a strong activity against oxacillin and cloxacillin and was predominantly a penicillinase rather than a cephalosporinase. Hydrolysis of aminothiazolyl cephalosporins was slow, and the ability of the enzyme to cause resistance may depend on high affinity, as with OXA-11 (2). Further studies of kinetic aspects are in progress.

The emergence of extended-spectrum activity in class D β-lactamases is disturbing, as these, unlike most class A mutants, are resistant to clavulanate and sulfones. Nevertheless, OXA-10 is rare and its mutants pose considerably less risk than do the TEM and SHV derivatives.

TABLE 2. Rates of hydrolysis of 0.5 mM β-lactams at 37°C in 0.1 M phosphate buffer by semipurified OXA-14 and OXA-10 β-lactamases

Substrate	Steady-state rate (%)	
	OXA-14	OXA-10
Benzylpenicillin	100	100
Ampicillin	76	190
Carbenicillin	4	17
Cloxacillin	150	700
Oxacillin	120	460
Cephaloridine	3	7
Cephalothin	8	5
Cefoxitin	<0.5	<0.5
Cefotaxime	<0.5	0.5
Ceftazidime	1	<0.5
Aztreonam	<0.5	<0.5

Nucleotide sequence accession numbers. The Genome Sequence Data Base accession number L38523 has been allocated to bla_{OXA-14}.

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