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CASE REPORTS

Immediate Appearance of Plasmid-Mediated Resistance to Multiple Antibiotics upon Antibiotic Selection: an Argument for Systematic Resistance Epidemiology▽

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We describe a conjugative plasmid appearing in a bacteremic clone of *Escherichia coli* immediately upon exposure to the antibiotics for which it encoded resistance. Effective antibiotic choice was made possible by prior screening for this plasmid. Surveillance for transmissible resistance plasmids may be clinically important.

CASE REPORT

A 64-year-old local nursing home resident was admitted to the intensive care unit (ICU) of Westmead Hospital in Sydney, Australia, with brain stem infarction complicated by aspiration pneumonia and multisystem organ failure. Ticarcillin-clavulanate and gentamicin were commenced with good effect. He was discharged to the surgical ward on ticarcillin-clavulanate on day 4 and underwent a below-the-knee amputation for persistent ischemia on day 17. Ticarcillin-clavulanate was converted to oral amoxicillin (amoxicilline)-clavulanate on day 23.

A routine ICU admission perineal surveillance swab was identified as positive for the locally endemic metallo-β-lactamase (bla_{IMP-4}) gene by PCR (3). Ticarcillin/clavulanate-resistant colonies obtained on subculture proved to be a *Serratia* sp. strain (JIE515) containing the bla_{IMP-4} gene. No other ticarcillin/clavulanate-resistant colonies were identified.

On day 30 of his admission, the patient developed fever and pneumonia after an aspiration event. Intravenous ticarcillin-clavulanate and gentamicin were restarted. Upon notification of a gram-negative rod in the blood culture the next day and in light of the ICU surveillance isolate, it was advised that amikacin be substituted for gentamicin and a second blood culture drawn. The patient defervesced after 72 h and made a full recovery following 10 days of intravenous amikacin.

The first blood culture (day 30) grew a fully sensitive *Escherichia coli* strain (JIE527). However, blood drawn only ~20 h later (day 31), after two doses of ticarcillin-clavulanate and one dose of gentamicin, grew a highly resistant *E. coli* strain (JIE528) which was resistant to both of these antibiotics and to ceftriaxone, cefotaxime, and tobramycin but susceptible to amikacin (Table 1). DNA fingerprints derived by pulsed-field gel electrophoresis of whole genomic DNA after XbaI digestion (Fig. 1), as well as after EcoRI digestion (not shown), revealed that the two *E. coli* isolates (JIE527 [sensitive] and JIE528 [resistant]) were identical.

The day 3 *Serratia* sp. strain (JIE515) and the resistant (day 31) *E. coli* strain (JIE528) readily transferred a plasmid of incompatibility (Inc) group L/M (Table 2) to (laboratory strain) *E. coli* DH5α, along with resistance to gentamicin, cefoxitin, ceftriaxone, and ticarcillin-clavulanate, when ampicillin selection was used as previously described (3) (Table 1). The bla_{IMP-4} gene and the IncL/M replicon were present in all of the DH5α transconjugants obtained from strains JIE515 and JIE528 but was absent from strain JIE527, although three other transferable plasmid replicons were present in both strains JIE527 and JIE528 (Table 2). PCR mapping of the *E. coli* DH5α transconjugants JIE515 and JIE528 (Table 1) with combinations of overlapping primers, including hep58/hep59 (8) and IS26-F2/IMP-R1, indicated a genetic context identical to that of the locally dominant bla_{IMP-4}-carrying IncL/M plasmid, pJIBE401 (3).

An understanding of the resistance potential of the microflora is essential for good antibiotic policy. However, much of the most important emerging antibiotic resistance in the *Enterobacteriaceae* moves through the microflora on highly transmissible multiresistance plasmids. In our local *Enterobacteriaceae*, this includes carbapenem or extended-spectrum β-lactamase resistance in combination with aminoglycoside resistance, and the epidemiology of resistance is determined by the transmission characteristics of these plasmids (3, 9).

Surveillance for antibiotic-resistant bacteria is routinely performed by screening for the problem phenotype. However, an

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TABLE 1. Bacterial strains used in this study

Isolate	Detail	MIC (μg/ml)[a]			
		TIM	CTX	IPM	GEN
JIE515	*Serratia* sp. strain	>64/2	>32	4	>8
JIE527	*E. coli* blood culture isolate from day 30	16/2	<2	<1	<2
JIE528	*E. coli* blood culture isolate from day 31	>64/2	>32	<1	>8
DH5α	*E. coli* DH5αRif (laboratory strain, no plasmid)	8/2	4	<1	<2
pJIBE401	*E. coli* DH5αRif with pJIBE401 (IMP-4 plasmid)	>64/2	32	2	>8

[a] Abbreviations: TIM, ticarcillin-clavulanic acid; CTX, ceftriaxone; IPM, imipenem; GEN, gentamicin. For all of the strains, the tobramycin MIC equaled the gentamicin MIC, the amikacin MIC was <8 μg/ml, and the ciprofloxacin MIC was ≤1 μg/ml.

important characteristic of plasmid-borne resistance is the minor strain-to-strain phenotypic variation which occurs due to differences between host bacteria (3, 9). We have previously argued that surveillance and containment efforts should be focused on the plasmid transmitting the resistance rather than the organism hosting it (7). Here, we highlight the rapidity with which a pathogenic organism can acquire multiple antibiotic resistance by conjugal transfer, describing a case in which it was clinically important, and discuss the implications for infection control screening strategies.

This anecdote illustrates the appearance of a multiresistance plasmid in a bacteremic *E. coli* strain after brief exposure to ticarcillin-clavulanate and gentamicin, the two isolates, JIE527 and JIE528, being distinguishable only by the presence of the multiresistance plasmid encoding resistance to these drugs. The two *E. coli* bloodstream isolates have identical DNA fingerprints, and both contain identical plasmid replicons except for IncL/M. We show here that the IncL/M plasmid in both JIE515 and JIE528 is closely related to the local IncL/M plasmid bearing bla_{IMP-4} and showed previously that this is stable with multiple passages and does not significantly disadvantage growth under these exact culture conditions (1). Dropout of this plasmid in blood culture broth is therefore not a plausible explanation for the differences between consecutive *E. coli* isolates JIE527 and JIE528.

It is possible that a polymicrobial source or sources (e.g., gut, wound, chest) generated bacteremias by two identical *E. coli* populations, one of which was highly resistant due to the presence of the plasmid. The long previous antibiotic exposure and the experimentally proven propensity of this particular plasmid to be transferred freely between *E. coli* and *Serratia* bacteria (3) mean that plasmid-carrying *E. coli* populations are likely to have been present in the microflora at the time of the bacteremias.

The gut is a likely source of the bacteremia, given the natural preponderance of the species *E. coli* there, and although the human gut typically harbors more than half a dozen different clones of *E. coli*, only a few of these normally dominate (2, 6).

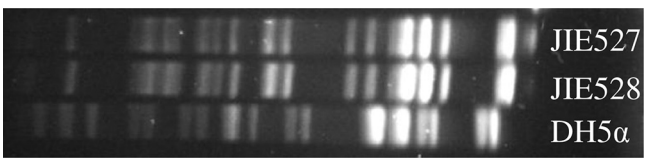

FIG. 1. Pulsed-field gel electrophoresis (XbaI) of whole genomic DNA.

It is therefore theoretically possible that a sensitive member of the same *E. coli* population invaded the bloodstream prior to the emergence of a plasmid-carrying isolate.

Much more credible is the possibility that a dominant *E. coli* population (JIE527) acquired the plasmid at the bacteremic source from a reservoir in another species, perhaps one with a lesser propensity to invade and survive in the bloodstream. It seems highly unlikely that clonally identical bacteremias on consecutive days came from different sources. This plasmid has been proven to be easily transmissible between many other members of the family *Enterobacteriaceae*, including *Serratia* spp. The simplest and most logical explanation is that a population of *E. coli* has been transformed by the acquisition of an antibiotic resistance plasmid under potent antibiotic selection pressure.

Irrespective of the mechanistics of acquisition, a highly transmissible multiresistance plasmid was evident in the microflora long in advance of its appearance in an invading *E. coli* clone. This plasmid appeared in that clone immediately when it was required to overcome the antibiotics to which the clone was exposed.

Foreknowledge of the presence of transmissible resistance is essential for control of infections due to multiresistant organisms and may be life saving in the critically ill (4, 5, 7). Unlike gram-positive bacteria, where resistance genotypes and phenotypes are predictably related (e.g., methicillin-resistant *Staphylococcus aureus*), the epidemiology and characteristics of important resistance plasmids in gram-negative bacteria may be more informative than those of the bacteria themselves (7).

We do not advocate surveillance for every multiresistance plasmid, but certainly for those which completely defeat standard antibiotic protocols in the critically ill and are known to have broad host ranges and high transmissibility. This is one

TABLE 2. Conjugative transfer of plasmids

Plasmid marker	Conjugative transfer[a] from:		
	Serratia JIE515	*E. coli* JIE527	*E. coli* JIE528
bla_{IMP-4}	+		+
IncL/M	+		+
IncHI2	±		
IncN		(+)	(+)
IncFIA		(+)	(+)
IncFII		(+)	(+)

[a] Conjugative transfer of bla_{IMP-4} and plasmids of Inc groups to *E. coli* DH5α was determined as previously described (1). Symbols: +, always; ±, sometimes; (+), present in wild-type strain but not transferred.

such example and is unlikely to be the last. Plasmid characterization is not routine in descriptions of transmissible antibiotic resistance, but it should have a prominent place both in academic reports and in infection control and management. The application of modern genomic tools to clinical microbiology is highly feasible, long overdue, and essential to our understanding and management of the rise of resistance in gram-negative bacteria.

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