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SHORT REPORT
In*99*, an In*100*-related integron, its occurrence and prevalence in clinical *Pseudomonas aeruginosa* strains from a central region of Portugal

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SUMMARY

In*99*, a possible ancestor of In*100*, is a class 1 integron associated with carbenicillinase (bla_{PSE}) and aminoglycoside resistance genes [*aac*(6′)-*Ib* and *aadA2*]. In*99* was present in 8 of 81 clinical isolates of *Pseudomonas aeruginosa* from unrelated patients collected in different years. The strains fell into two clonal groups and exhibited resistance to β-lactams and aminoglycosides.

Integrons are a site-specific recombination system that is able to acquire one or more gene cassettes and convert them into functional genes by providing a promoter for their expression [1, 2]. Several classes of integrons, based on the integrase gene are known but class 1 is the most frequently described in clinical isolates and is often associated with multiple antibiotic resistance [3]. Class 1 integrons are comprised of two elements: the 5′ conserved segment (5′-CS), containing an *intI1* gene coding for an integrase and a recombination site, *attI1*, and the 3′ conserved segment (3′-CS) containing antimicrobial and antiseptic resistance genes and an open reading frame (ORF) of unknown function [1]. A variable region is present between the two conserved regions where the gene cassettes are inserted. These genes are the mobile elements that can be excised or integrated by the integrase immediately after the 5′-CS, constituting an array of genes ordered from the newest to the oldest inserted cassette [4, 5].

Class 1 integrons have been described in nosocomial species such as *Pseudomonas aeruginosa* and *Acinetobacter baumannii* and the Enterobacteriaceae worldwide [4] and carry genes determining resistance to β-lactams and sometimes to aminoglycosides. There have been two previous reports of the presence of integrons in carbapenem-resistant isolates of *P. aeruginosa* from Portugal [5, 6] but no survey of the frequency of these elements among clinical isolates of this species in the community has been reported from this country.

A total of 81 non-duplicate isolates of *P. aeruginosa* were obtained from clinical specimens collected from patients within a 48-h period after admission to a hospital in the central region of Portugal during March–October 2003, and the same period in 2005. Class 1 integrons were detected by PCR with specific primers for the *intI1* gene [7]. Eight strains (10%) were positive for the *intI1* gene. The inserted gene cassette regions were amplified and sequenced with primers for 5′-CS and 3′-CS from class 1 integrons as previously described [7]. A 3000-bp fragment was obtained for five strains (4, 5, 24, 25, 29) isolated in 2003 and three strains (54, 55, 80) from 2005.

Nucleotide sequence of the fragments corresponding to the variable regions revealed a class 1 integron containing an array of three distinct gene cassettes (Fig. 1), which was designated as In*99* (GenBank accession no. DQ219465). The integron has in the first position an *aac*(6′)-*Ib* gene cassette, encoding an aminoglycoside 6′-*N*-acetyltransferase of type II conferring resistance to gentamicin, tobramycin and netilmicin [5]. A bla_{PSE} gene encoding resistance to

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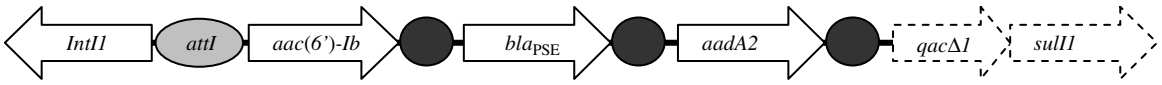

Fig. 1. Schematic structure of In*99*. The gene cassettes are represented by boxed arrows indicating the transcriptional orientation. A grey circle represents the *attI1* site and the black circles represent the gene cassettes' recombination site, *attC* or 59-bp element.

carbenicillin and ampicillin is present in the second position [8] and immediately downstream is located a streptomycin and spectinomycin resistance gene, *aadA2* [5].

The eight strains exhibited different antimicrobial resistance profiles but all were resistant to ticarcillin and piperacillin, gentamicin, tobramycin and netilmicin suggesting that the In*99* is being expressed. No plasmids were detected in the strains by a modification of alkaline lysis extraction [9] suggesting that In*99* is located in the chromosome.

The genetic relation of these strains was investigated by pulsed-field gel electrophoresis (PFGE) of *Spe*I chromosomal DNA digests. Electrophoresis was performed in a CHEF-DRII (Bio-Rad, Portugal) apparatus with the following conditions: 6 V/cm for 20 h at 12 °C in 1% agarose with 0·5× Tris-borate-EDTA (TBE), containing 50 μM of thiourea (Sigma, Spain), with switch time intervals from 2 to 60 s. Clonal groupings were based on less than three DNA band differences in profile corresponding to 85% or greater similarity. Two clonal groups (A and B) were identified (Fig. 2). The presence of In*99* in these genetically distinct groups in strains from unrelated patients in different years is indicative of the persistence of the integron in the local community and suggests horizontal gene transfer by a mobile element such as a transposon. Indeed, the correspondence of DNA profiles between In*99*-positive strains in each of the clonal groups does also suggest that some dissemination of the integron into specific *P. aeruginosa* strain populations has already occurred.

The nucleotide sequence of In*99* sequence is similar to In*100*, previously reported in a clinical *P. aeruginosa* strain from a patient in Coimbra (Portugal) in 2000 [5]. However, In*100* possesses an extra gene cassette (bla_{VIM-2}), between the 5′-CS and the *aac*(*6′*)-*Ib* gene. The observed array of genes suggests that In*99* could be the precursor of the In*100*, supporting the evolution through the acquisition of new gene cassettes [10]. Both integrons have not been described in other parts of the world apart from Portugal underlying the finding that some

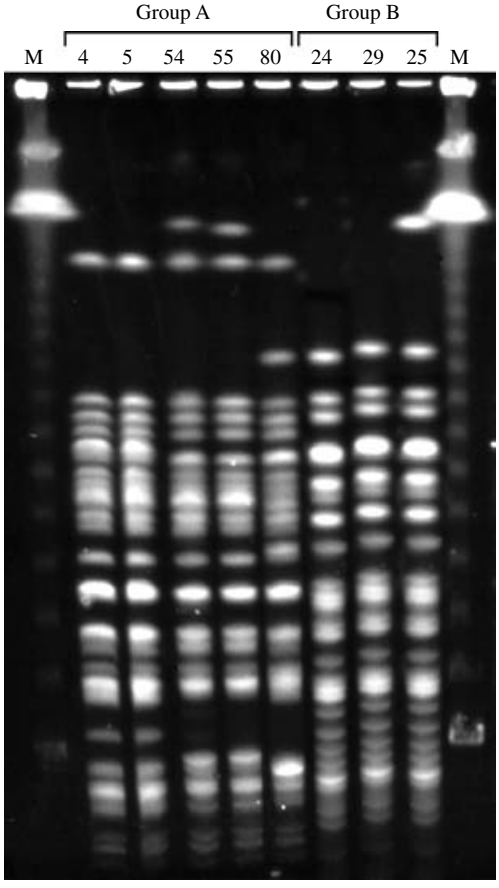

Fig. 2. PFGE profile of *Spe*I-digested DNA of *P. aeruginosa* clinical strains containing In*99*. Lanes 1 and 10 (M), λ ladder (BioRad); lanes 2–9, strains.

combinations of gene cassettes have a local distribution in contrast to the widespread distribution of others [4]. Moreover, Coimbra and Aveiro are both cities in the central region of Portugal, justifying the possible exchange of genetic material between strains in the community and consequently in hospital-admitted patients.

Quinteira *et al.* [5] found In*99* in a *P. aeruginosa* isolate from a pig and suggested that other ecological niches where antibiotics are widely used such as in animals for food production may act as reservoirs for integrons carrying resistance genes. Our findings confirm the presence of *P. aeruginosa* strains from

different clinical niches carrying In*99* and this may be consistent with spread probably through food production or man/animal contact. Molecular epidemiological studies of wider clinical and environmental strains are called for to elucidate their genetic relation.

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DECLARATION OF INTEREST

None.

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