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Association between *Pseudomonas aeruginosa* O-antigen serotypes, resistance profiles and high-risk clones: results from a Spanish nationwide survey

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Objectives: To evaluate the correlation of O-antigen serotypes with resistance profiles and high-risk clones in a Spanish nationwide survey.

Methods: Up to 30 consecutive healthcare-associated *Pseudomonas aeruginosa* isolates were collected during October 2017 from each of 51 hospitals (covering all Spanish regions) with a total of 1445 isolates studied. MICs of 13 antipseudomonal agents and MDR/XDR profiles had been previously determined, as well as whole-genome sequences of 185 representative XDR isolates. O-antigen serotypes (O1–O16) were determined by agglutination using serotype-specific antisera (BioRad). The *Pseudomonas aeruginosa* serotyper (PAst) program was used for *in silico* serotyping.

Results: The most frequent serotypes were O6 (17.8%), O1 (15.4%) and O11 (13.3%). In contrast, the most frequent serotype among XDR isolates (17.3%) was O4 (34.1%), distantly followed by O11 (15.9%). Within serotypes, XDR phenotypes were more frequent for O12 (60.0%) and O4 (57.3%). The most frequent clone among the XDR isolates was ST175 (40.9%), followed by CC235 (10.7%), ST308 (5.2%) and CC111 (3.6%). Up to 81.6% of XDR ST175 isolates typed O4, whereas 18.4% were non-typeable. O4 genotype was detected in all sequenced (n=55) ST175 isolates. On the other hand, CC235 and ST308 were associated with O11, whereas CC111 was linked to serotype O12.

Conclusions: O4 serotype is linked to the MDR/XDR profile of widespread ST175 (typically only susceptible to colistin, amikacin and the novel combinations ceftolozane/tazobactam and ceftazidime/avibactam) and therefore, after local validation, its detection in the microbiology laboratory might be useful for guiding semi-empirical antipseudomonal therapies and infection control measures in Spanish hospitals.

Introduction

The growing prevalence of nosocomial infections produced by MDR and particularly XDR *Pseudomonas aeruginosa* strains is associated with significantly increased morbidity and mortality.[1] This increasing threat results from the extraordinary capacity of *P. aeruginosa* for developing resistance to nearly all available antibiotics by the selection of mutations in chromosomal genes and from the growing prevalence of transferable resistance determinants, particularly those encoding carbapenemases or ESBLs.[2] The dissemination of MDR/XDR global strains, the high-risk clones, in multiple hospitals worldwide adds further concern.[3] Since high-risk clones are associated with defined MDR/XDR profiles, diagnostic approaches to their early detection would be useful for guiding antipseudomonal therapies. The most widely accepted gold-standard technique for the definition of such epidemic clones is MLST.[3,4] Moreover, WGS provides further relevant information for understanding the dissemination and the resistome of MDR/XDR *P. aeruginosa* high-risk clones.[5–7] However, MLST and WGS, and other classical molecular typing techniques such as PFGE, are still time consuming and thus not practical for implementation in the

current diagnostic microbiology routine for guiding antipseudomonal therapies. On the other hand, O-antigen serotyping is a rapid, simple and cheap procedure that, despite not being as discriminatory as those mentioned above, might be useful for the presumptive detection of at least some MDR/XDR high-risk clones.[3,8,9] Moreover, a program [*Pseudomonas aeruginosa* serotyper (PAst)] for *in silico* serotyping of *P. aeruginosa* isolates from WGS data has recently been developed.[10] However, large-scale surveys of O-antigen serotypes are very scarce and/or old, and none has been performed so far in Spain. Thus, the objective of this work was to determine the association between *P. aeruginosa* O-antigen serotypes, resistance profiles and high-risk clones, taking advantage of a recent large-scale Spanish nationwide survey of *P. aeruginosa* infections.[11]

Material and methods

P. aeruginosa strain collection

The collection studied included up to 30 consecutive healthcare-associated non-duplicated (one per patient) *P. aeruginosa* clinical isolates collected during October 2017 from each of the 51 participating hospitals, covering all 17 Spanish regions.[11] A total of 1445 isolates were studied. The distribution of sample types was as follows: respiratory (32.8%), urine (23.7%), soft tissue and osteoarticular (23.1%), blood culture (5.7%) and others (14.9%). MICs of ticarcillin, piperacillin/tazobactam, ceftazidime, cefepime, ceftolozane/tazobactam (4 mg/L), ceftazidime/avibactam (4 mg/L), aztreonam, imipenem, meropenem, ciprofloxacin, tobramycin, amikacin and colistin had been determined by broth microdilution according to EUCAST guidelines (www.eucast.org). EUCAST v 8.1 clinical breakpoints were used for interpretation. Up to 252 (17.4%) of the isolates met the XDR criteria.[12] In the previous study,[11] clonal relatedness among XDR isolates had been initially evaluated by PFGE and one representative XDR isolate from each unique macrorestriction pattern and hospital (n=185) was further analysed through WGS, including MLST and resistome analysis.

O-antigen serotyping

O-antigen serotypes (O1–O16) were determined in the 1445 isolates by agglutination using commercially available serotype-specific antisera (BioRad ref. 58901–58916, from O1 to O16). The PAst program[10] (https://cge.cbs.dtu.dk/services/PAst-1.0/) was used for *in silico* serotyping of the 185 XDR isolates sequenced. The χ^2 test was used for the analysis of the prevalence of serotypes in different subgroups. A P value <0.05 was considered statistically significant.

Results

As shown in Table 1, the most frequent serotypes among the 1445 isolates tested were O6 (17.8%), O1 (15.4%) and O11 (13.3%). Up to 14.5% of the isolates were non-typeable (no agglutination or polyagglutination with the 16 antigens tested). Significant differences in serotype distribution according to the sample type were not detected (not shown). However, the distribution of serotypes was very different among XDR isolates. Interestingly, the most frequent serotype among XDR isolates (17.4%) was by far O4, detected in 34.1% of the isolates (versus 10.4% of all isolates P<0.0001), distantly followed by O11 (15.9%) (Table 1). Although globally not frequent, O12 serotype was also significantly associated with the XDR phenotype (4.8% versus 1.4%, P<0.0001). Finally, non-typeability was also significantly associated with XDR isolates (24.2% versus 15.5%, P=0.0007).

Table 1. Distribution of O-antigen serotypes among the complete collection of *P. aeruginosa* isolates as well as those showing an XDR phenotype

Serotype	Total isolates, n (%) (n=1445)	XDR isolates, n (%) (n=252)[a]
O1	222 (15.4)	13 (5.2)*
O2	58 (4.0)	4 (1.6)*
O3	100 (6.9)	8 (3.2)*
O4	150 (10.4)	86 (34.1)**
O5	75 (5.2)	1 (0.4)*
O6	257 (17.8)	18 (7.1)*
O7	39 (2.7)	0 (0.0)*
O8	17 (1.2)	0 (0.0)
O9	20 (1.4)	1 (0.4)
O10	49 (3.4)	3 (1.2)*
O11	193 (13.3)	40 (15.9)
O12	20 (1.4)	12 (4.8)**
O13	3 (0.2)	0 (0.0)
O14	6 (0.4)	0 (0.0)
O15	7 (0.5)	2 (0.8)
O16	20 (1.4)	3 (1.2)
Non-typeable	209 (14.5)	61 (24.2)**

[a]Statistically significant (χ^2, P<0.05) lower (*) or higher (**) prevalence of each serotype among XDR isolates compared with non-XDR isolates.

The distribution of non-MDR/MDR/XDR phenotypes among the main serotypes is shown in Figure S1 (available as Supplementary data at *JAC* Online). As shown, the serotype more strongly associated with XDR phenotypes was O12 (60.0% of XDR isolates), closely followed by O4 (57.3%). The next serotypes were O11 and O16, with only 20.7% and 15% of XDR isolates, respectively. Moreover, all other serotypes yielded XDR rates <10%. On the other hand, the prevalence of XDR phenotype among non-typeable isolates was 29.2%.

In silico O-antigen serotyping was analysed in the 185 sequenced XDR isolates. Table S1 shows the *in vitro* and *in silico* O-antigen serotypes for these strains, together with their STs, susceptibility profiles and resistomes. Agreement between *in vitro* and *in silico* serotyping was documented in 125 (67.6%) of the isolates. Lack of coincidence was mostly caused by isolates non-typeable by conventional serotyping (30.8%) rather than by serotype discrepancies (1.6%). Table 2 shows the comparative analysis of *in vitro* and *in silico* serotyping in the most frequent XDR clones detected in the study (ST175, CC235, CC111 and ST308). As can be observed, ST175 was clearly associated with O4 serotype; 81.6% of all ST175 isolates analysed (n=103) were O4, and the remaining 18.4% were non-typeable, by *in vitro* serotyping, whereas all 55 sequenced ST175 isolates showed an O4 *in silico* serotype. Likewise, CC111 isolates were associated with O12 serotype and CC235 and ST308 with O11. However, some exceptions were documented, such as one ST111 showing an O4 *in silico* serotype (non-typeable by *in vitro* serotyping) and a few CC235 isolates showing an O1 serotype by *in vitro* serotyping. Association of serotypes with susceptibility profiles and resistance mechanisms was not evidenced beyond the link with STs, although the percentage of non-typeable strains was particularly high (40%) among those producing carbapenemases or ESBLs (Table S1).

Table 2. *In vitro* and *in silico* O-antigen serotypes among the most frequent high-risk clones detected in the study

	In vitro O-antigen serotype			*In silico* O-antigen serotype		
ST	total isolates tested, *n*	serotype	isolates, *n* (%)	total isolates tested, *n*	serotype	isolates, *n* (%)
ST175	103	O4	84 (81.6)	55	O4	55 (100)
		non-typeable	19 (18.4)			
CC235	29	O11	19 (65.5)	22	O11	22 (100)
		O1	3 (10.3)			
		non-typeable	7 (24.1)			
CC111	10	O12	6 (60)	10	O12	9 (90)
		non-typeable	4 (40)		O4	1 (10)
ST308	13	O11	11 (84.6)	9	O11	9 (100)
		non-typeable	2 (15.4)			

Discussion

To our knowledge, this is the first large-scale (1445 isolates) nationwide (51 hospitals covering all Spanish regions) survey on the distribution of *P. aeruginosa* O-antigen serotypes among healthcare-associated clinical isolates ever performed worldwide. Using commercially available antisera (BioRad) covering 16 (O1–O16) of the 20 O-specific antigens defined by the International Antigenic Typing Scheme (IATS),[9] 14.5% of the isolates were non-typeable and thus represented a relatively minor issue for the application of this typing scheme. This proportion of non-typeable isolates was indeed lower than that documented in another previous large study (35%),[8] likely owing to the overrepresentation of cystic fibrosis (CF) isolates, which are mostly non-typeable owing to the loss of the O-antigen as part of the adaptive process for long-term persistence in chronic infections. Although CF isolates were not excluded in our study, they represented <1% of the consecutive isolates included.

Serotypes O6, O1 and O11 were the most frequent among *P. aeruginosa* clinical isolates from Spanish hospitals in our study. Although a comparison with other previous surveys is not straightforward owing to the small number of isolates included or strain selection bias (such as MDR or CF), these three serotypes appear to be those most frequent globally.[8,13,14]

Beyond a typing procedure with low discriminatory capacity in outbreak characterization, O-antigen serotypes have been used as a (likely indirect) marker of strain virulence or clinical outcome.[13,15,16] However, the likely most prominent feature is the association of certain serotypes with MDR/XDR profiles. While O11 and O12 have been extensively linked to MDR/XDR strains worldwide,[3] our work shows that the most frequent serotype among Spanish XDR isolates is by far O4, linked to the widespread ST175 high-risk clone. Serotype O12 was also strongly linked to XDR profiles in our study, but the overall prevalence of this serotype, linked to ST111, was very low. On the other hand, despite the fact that our results showed that O11 was frequent among *P. aeruginosa* isolates from Spanish hospitals, it was not so strongly linked to XDR profiles, even if documented in ST235 and ST308 high-risk clones.

Overall, a high degree of concordance between *in vitro* and *in silico* serotyping was documented, but, as previously described,[10] the latter approach enabled nearly 100% typeability. Moreover, *in silico* serotyping detected one O4 ST111 isolate (non-typeable *in vitro*), whereas all other sequenced isolates from this clone were, as expected, O12. Interestingly, a recent study suggested that in fact ST111 was originally O4 and that it became an epidemic MDR clone following acquisition of O12 determinants and a quinolone resistance mutation in *gyrA* [C248T (T83I)].[17] Indeed, the resistome analysis performed indicated that the single O4 ST111 isolate was the only isolate studied from this clone not showing the GyrA T83I mutation (Table S1). Therefore, this isolate, despite showing an XDR profile, appears to have diverged prior to the emergence of the O12 ST111 epidemic strain. In relation to the potential association between O-antigen serotypes and GyrA mutations, it is noteworthy that the widespread O4 ST175 clone invariably shows a combination of two GyrA mutations (T83I and D87N) (Table S1); the role of this association in the epidemic dissemination of this clone needs to be further explored.

In summary, this large-scale nationwide survey on the distribution of *P. aeruginosa* O-antigen serotypes among healthcare-associated clinical isolates revealed that O4 serotype is very strongly linked to the MDR/XDR profile of widespread ST175 (typically only susceptible to colistin, amikacin and the novel combinations ceftolozane/tazobactam and ceftazidime/avibactam) and therefore its detection in the microbiology laboratory might be useful for guiding semi-empirical antipseudomonal therapies and infection control measures in Spanish hospitals. However, its implementation needs to be adapted to the local epidemiological data (circulating high-risk clones and associated resistance profiles). Likewise, the occurrence of false-positive results should be considered (>30% of O4 isolates were non-MDR) and false-negative results (20% of ST175 isolates were classified as non-typeable).Thus, it might be useful to combine O-antigen serotyping with other simple procedures, such as MALDI-TOF biomarker peak analysis,[18] for the rapid detection of high-risk clones in the clinical microbiology laboratory.

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Supplementary data

Figure S1 and Table S1 are available as Supplementary data at *JAC* Online.

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