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ORIGINAL ARTICLE

O-antigen serotyping and MALDI-TOF, potentially useful tools for optimizing semi-empiric antipseudomonal treatments through the early detection of high-risk clones

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Abstract

The increasing prevalence of extensively drug-resistant (XDR) *Pseudomonas aeruginosa* infections is due to the global spread of defined high-risk clones (HRC). Among them, ST175 is particularly frequent in Spain and France. Here, we evaluated O-antigen serotyping and MALDI-TOF as typing methods for the early identification of ST175. O-antigen (O4) serotyping and MALDI-TOF biomarker peak-based recognition models were tested in several strain collections, including 206 non-duplicated *P. aeruginosa* clinical isolates collected in 2016. Resistance profiles were determined by broth microdilution and clonal epidemiology by pulsed-field gel electrophoresis (PFGE) and multilocus sequence typing (MLST). Up to 24.3% of the isolates were XDR and 28.2% non-susceptible to meropenem, while resistance to ceftolozane/tazobactam (2.9%) and colistin (0.5%) was infrequent. Half of all XDR isolates belonged to ST175 and most of them were only susceptible to ceftolozane/tazobactam and colistin. A model based on the detection of one MALDI-TOF biomarker peak yielded negative and positive predicted values (NPV/PPV) for the detection of ST175 of 100%/51.9%, whereas NPV/PPV for a model based on two biomarker peaks were 99.4%/87.1% and for O4 serotyping, 99.4%/84.1%. Both, O4 serotyping and MALDI-TOF biomarker peak analysis, proved to be sensitive and specific methods that could be easily incorporated in the routine workflow for the early detection of ST175 HCR. Since ST175 is associated with defined XDR profiles, with most isolates only being susceptible to colistin and ceftolozane/tazobactam, these simple techniques could be useful for optimizing semi-empiric antipseudomonal treatments in areas where this HRC is prevalent.

Keywords *Pseudomonas aeruginosa* · High-risk clones · Antibiotic resistance · Ceftolozane-tazobactam · ST175

Introduction

The increasing prevalence of chronic and hospital-acquired infections produced by multidrug-resistant (MDR) or extensively drug-resistant (XDR) *Pseudomonas aeruginosa* is known to be associated with high morbidity and mortality [1]. These infections represent a major threat due to the limited available therapeutic options. *P. aeruginosa* has a non-clonal epidemic population structure composed of a limited number of widespread clones which are selected from a background of a large number of rare and unrelated genotypes that are recombining at a high frequency [2]. Moreover, recent works have provided evidence of the existence of MDR/XDR global clones, denominated high-risk clones, disseminated in hospitals worldwide, among which ST175 is one of the most widespread, particularly in Spain and France [2–4]. Additionally, available data shows a strong association between this high-risk clone and defined resistance genotypes and phenotypes [5, 6]. Thus, the objective of this work was to evaluate two approaches, O-antigen serotyping and MALDI-TOF analysis, for the early detection of ST175, in order to guide semi-empiric treatment of MDR/XDR infections in areas of high prevalence of this clone.

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Materials and methods

Bacterial strains and MALDI-TOF analysis

Training set Fifteen characterized ST175 and fifteen non-ST175 isolates from the Hospital Son Espases (Palma de Mallorca, Spain) were studied in order to develop a robust recognition model for the ST175 high-risk clone. The non-ST175 isolates included nine susceptible isolates belonging to unique clones and three isolates from each of the epidemic high-risk clones ST111 and ST235.

Validation set Two hundred six consecutive non-duplicated isolates (one per patient) recovered at the Hospital Son Espases from March to December of 2016.

External strain set Twenty-eight characterized ST175 from different Spanish and French hospitals were also analyzed [6, 7].

The MALDI-TOF spectra, using Bruker Daltonics™ equipment of the training set, were studied in order to develop a robust recognition model for the ST175 high-risk clone. Strains were cultured for 18 h at 37 °C in Columbia agar supplemented with 5% sheep blood (BioMérieux™), and protein extracts were obtained with ethanol-formic extraction following Bruker™ recommendations. A consensus spectrum was then obtained from 24 independent spectra per strain, and data was analyzed using Bruker™ provided software (FlexAnalysis™). Then, the spectra of the ST175 strains were carefully compared visually against the non-ST175 group looking for biomarker peaks shared by the ST175 strains but completely absent in the other. After the initial development of this model based on the presence of some biomarker peaks, the validation set was analyzed in the following way: two MALDI-TOF spectra for each strain were acquired after a one-step in situ extraction with formic acid followed by the addition of the matrix solution. Finally, the spectra of the external strain set were also analyzed.

O4 antigen determination

In addition to the MALDI-TOF analysis, the presence of the O4 antigen was determined in parallel in all the strains belonging to the training and validation set through slide agglutination with O4 antisera (Bio-Rad™).

Susceptibility testing

The MICs of piperacillin/tazobactam, ceftazidime, cefepime, imipenem, meropenem, ciprofloxacin, gentamicin, tobramycin, amikacin, and colistin were determined by broth microdilution using Microscan™ panels (Beckman Coulter™). MICs of ceftolozane/tazobactam were determined using gradient strips (Liofilchem™). Clinical susceptibility categories were interpreted following v7.1 2017 EUCAST guidelines (www.eucast.org). MDR and XDR profiles were defined according to previously described criteria [8]. AmpC hyperproduction, OprD deficiency, and the presence of horizontally acquired β-lactamases were evaluated using previously established phenotypic (cloxacillin and EDTA inhibition tests) and molecular (PCR) methods [5, 9].

Clonal epidemiology

Clonal relatedness was evaluated by pulsed-field gel electrophoresis (PFGE) and multilocus sequence typing (MLST). For PFGE, bacterial DNA embedded in agarose plugs prepared as described previously [10] was digested with SpeI. After DNA separation by electrophoresis in a DRIII apparatus (Bio-Rad, La Jolla, CA), DNA macrorestriction patterns were interpreted according to the criteria established by Tenover et al. [11]. For MLST analysis, previously described schemes and protocols [12] and available databases and tools (http://pubmlst.org/paeruginosa) were used.

Results

Spectra analysis of the training set revealed two biomarker peaks, at *m/z* 6911 and 7359, present in all ST175 strains that most of the susceptible strains lacked. One of the peaks, *m/z* 7359, has already been associated with ST175 in a previous work [13]. Thus, we further analyzed two models, one based on the detection of the peak described by Cabrolier et al. and the other based on the simultaneous presence of the two biomarker peaks (*m/z* 7359 and *m/z* 6911). We additionally evaluated the usefulness of O4 serotyping, since the initial analysis performed in the training set showed that it was nearly universal among ST175 isolates and infrequent among non-ST175 isolates.

Table 1 shows the susceptibility data for the 206 clinical isolates tested. Up to 29.6% were MDR and 24.3% were XDR. Non-susceptibility to meropenem and amikacin was documented in the 28.2% and 25.7% of the isolates, respectively, whereas non-susceptibility to ceftolozane/tazobactam and colistin was only documented in 2.9% and 0.5% of the isolates, respectively. Only two (1%) isolates were carbapenemase-producers and both showed a positive PCR for VIM-2 cluster. Molecular typing revealed that 28 (13.6%) isolates belonged to ST175 (Table 1). All of them were XDR except one that was MDR, and ST175 accounted for 54.0% of all the XDR clinical isolates from that period (Table 1). All ST175 isolates were meropenem non-susceptible and 32.1% were amikacin non-susceptible; resistance to colistin and ceftolozane/tazobactam was documented only in one isolate reach (Table 1).

Table 1 Resistance profiles and characteristics of the collection of clinical isolates tested

Antibiotic/profile	Total isolates ($n = 206$)	ST175 ($n = 28$)	Non-S175 ($n = 178$)
PIP/TAZ (R)	52 (25.2%)	24 (85.7%)	28 (16.0%)
CAZ (R)	30 (14.6%)	22 (78.6%)	8 (4.6%)
FEP (R)	57 (27.7%)	23 (82.1%)	34 (19.4%)
IP (I/R)	55 (26.7%)	27 (96.4%)	28 (16.0%)
MER (I + R)	58 (28.2%)	28 (100%)	30 (17.1%)
GM (R)	79 (38.3%)	28 (100%)	51 (29.1%)
TOB (R)	56 (27.2%)	28 (100%)	28 (16.0%)
AK (I + R)	53 (25.7%)	9 (32.1%)	44 (25.1%)
CIP (I + R)	78 (37.9%)	28 (100%)	50 (28.6%)
COL (R)	1 (0.5%)	1 (3.6%)	0 (0%)
TOL/TAZ (R)	6 (2.9%)	1 (3.6%)	5 (2.9%)
MDR	61 (29.6%)	28 (45.9%)	33 (18.9%)
XDR	50 (24.3%)	27 (96.4%)	23 (13.1%)
MBL+	2 (1.0%)	0 (0%)	2 (1.1%)

PIP/TAZ, piperacillin/tazobactam; *CAZ*, ceftazidime; *FEP*, cefepime; *IP*, imipenem; *MER*, meropenem; *GM*, gentamicin; *TOB*, tobramycin; *AK*, amikacin; *CIP*, ciprofloxacin; *COL*, colistin; *TOL/TAZ*, ceftolozane/tazobactam; *MDR*, multidrug-resistant; *XDR*, extensively drug resistant; *MBL+*, metallo-β-lactamase producers

As shown in Table 2, all 28 confirmed ST175 isolates showed the *m/z* 7359 peak (100% sensitivity), but it was also present in 26 non-ST175 isolates, yielding a specificity of 85.4% and a PPV of 51.9%. Using our novel approach based on two biomarker peaks, false positive results were reduced from 26 to four isolates increasing specificity to 97.8% and the PPV to 87.1%. On the other hand, only one of the 28 confirmed ST175 lacked the second peak, only decreasing sensitivity to 96.4% and NPV 99.4%.

As shown in Table 2, 27 out of 28 confirmed ST175 were positive for O4 agglutination (sensitivity 96.4%) and all except five non-ST175 clinical isolates were negative (specificity 97.2%). Thus, O4 agglutination as an identifier method of the ST175 high-risk clone yielded a PPV of 84.4% whereas the NPV was 99.4%.

Finally, concerning the collection of 28 characterized ST175 isolates from Spanish and French hospitals, all of them exhibited both biomarker peaks (sensitivity 100%, NPV 100%), and all but two had a positive O4 agglutination (sensitivity 92.9%, NPV 86.7%).

Discussion

In our study, ST175 accounted for over 50% of XDR isolates from our hospital and the vast majority was susceptible to colistin and ceftolozane/tazobactam, and non-susceptible to all other antipseudomonal penicillins, cephalosporins, carbapenems, and fluoroquinolones. The same resistance profile was associated to ST175 in two multicenter studies from Spain, revealing an even higher proportion of this clone among XDR isolates (68% and 75%, respectively) [3, 14]. Thus, the early detection of this clone might be a useful tool for the optimization of semi-empiric antipseudomonal treatments prior to obtaining susceptibility data.

MALDI-TOF analysis is an emerging useful first-line epidemiological tool for bacterial typing that is still under methodological harmonization [15]. The model recently described by Cabrolier et al. considers eight biomarker peaks and is mainly based on the presence of the peak at *m/z* 7359 and the absence of the others. In agreement with our findings, their model yielded a high sensitivity and NPV (100% and 100%, respectively), but specificity and PPV were lower (92.6% and

Table 2 Performances of O4 agglutination and the presence of biomarker peaks (BP), alone and in combination, as recognizing methods of the ST175 high-risk clone

	O4 agglutination	BP 7358 (*m/z*)	BP 7359 and 6911 (*m/z*)	Both BP and O4 agglutination
Sensitivity (%)	96.4	100.0	96.4	92.9
Specificity (%)	97.2	85.4	97.8	100.0
Positive predictive value (%)	84.4	51.9	87.1	100.0
Negative predictive value (%)	99.4	100.0	99.4	98.9

41.2%, respectively) in accordance with our results in the model (85.4% and 51.9%, respectively). The addition of the new biomarker peak at *m/z* 6911 in our model significantly increased specificity and the PPV, maintaining high sensitivity and NPV. Additionally, our work showed that O4 agglutination, a non-expensive method that does not require infrastructures such as mass spectrometry, yielded comparable results for the presumptive identification of ST175. Finally, a model based on the combination of MALDI-TOF and O4 agglutination yielded the best specificity (100%) without a dramatic reduction in sensibility and NPV (92.9% and 98.9%). Moreover, results from the evaluation of the collection of isolates from multiple Spanish and French hospitals suggested that both methods could be implemented broadly for the detection of ST175 in different settings. Thus, results from this single-center pilot study support the development of a future multicenter evaluation of the usefulness of these simple techniques for the presumptive identification high-risk clones to guide semi-empiric treatment of MDR/XDR *P. aeruginosa* infections.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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