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Short report

Dissemination of NDM-1 carbapenemase-producer *Providencia stuartii* strains in Romanian hospitals: a multicentre study

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SUMMARY

Several Romanian hospitals have noted increasing isolation of *Providencia stuartii* strains in recent years, with an alarming rate of carbapenem resistance. In order to provide molecular epidemiological data regarding their dissemination, 77 *P. stuartii* strains collected from five hospitals located in different regions of Romania were analysed. All strains harboured IncA/C plasmid, and 67 carried the blaNDM-1 gene. Six clonal clusters were differentiated by pulsed-field gel electrophoresis. The predominant subtype was found in all five hospitals. Our study highlights the need for efficient infection-control measures, the optimization of antibiotic use and the targeted surveillance for carbapenemase-producing *P. stuartii*.

Introduction

As with many other members of the former Enterobacteriaceae, *Providencia stuartii* is an opportunistic pathogen that can cause healthcare-associated infections. There are only a few reports detailing the involvement of *P. stuartii* strains in small hospital outbreaks, and the appearance of carbapenem

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resistance in this species is a relatively recent development. However, during the last few years several Romanian hospitals have noted the increasing isolation of *P. stuartii* with a high frequency of carbapenem resistance; as a consequence, *P. stuartii* strains taken from hospitals located in different regions of Romania were analysed in order to establish their degree of relatedness.

Methods

The strains were collected from five tertiary-care hospitals situated in the central, south-eastern, western and north-western regions of Romania (Tîrgu-Mures, Cluj-Napoca, Timisoara, Bucharest, Baia-Mare).

Successive non-duplicate *P. stuartii* strains recovered during routine diagnostic activity between January 2016 and September 2017 were collected. The selection criteria for the strains were: isolate recovered from clinical sample of an inpatient regardless of the site of infection, and reduced susceptibility to ertapenem and/or meropenem. Susceptibility to imipenem was not taken into consideration due to the common low-level resistance in *Providentia* spp. Randomly selected carbapenem-susceptible *P. stuartii* strains isolated from clinical samples (at least one strain from each centre) were included in the study for comparison purposes.

Antibiotic susceptibility tests were performed by Vitek 2 Compact (BioMérieux, France) in all participating hospitals, except the hospital from Bucharest, where the Phoenix Automated Microbiology System (Becton Dickinson, Sparks, MD, USA) was used. Results were interpreted according to EUCAST guidelines version 7.1.

Carbapenemase genes $bla_{OXA\text{-}48\text{-}like}$, $bla_{NDM\text{-}1}$, bla_{KPC}, *respectively* bla_{VIM}, bla_{IMP}, bla_{GIM}, bla_{SPM} were tested for using two multiplex polymerase chain reactions (PCRs), adapted after others [1]. pulsed-field gel electrophoresis (PFGE) typing of all isolates was performed according to a CDC Pulsenet protocol [2]. Macrorestriction fragments obtained after digestion with NotI enzyme (ThermoFisher Scientific, USA) were separated by electrophoresis on a CHEF-DR III System (Bio-Rad Laboratories, USA) using the following parameters: 19 h run time at 10°C, initial switch time 2.2 s, final switch time 63.8 s. Lambda DNA Ladder 48.5 kb - 1 Mb (Lonza, Switzerland) was used as a reference. The similarity of the pulsotypes was evaluated with BioNumerics Seven software (Applied Maths, Belgium: temporary evaluation license) using the UPGMA (unweighted pair group method with averages) cluster analysis method and Dice coefficient with 1.5% optimization and 2% band tolerance. PFGE-type clusters were defined by a similarity coefficient of 90%.

Molecular identification of plasmids was carried out with a PCR based replicon typing method (PBRT 2.0 kit, Diatheva, Italy) according to the manufacturer's instructions.

Isolation of plasmid DNA was performed using an alkalyne lysis method (PureLink® HiPure Plasmid DNA Purification Kit, Thermo Fisher Scientific, USA). Detection of $bla_{NDM\text{-}1}$ was repeated using the isolated plasmids.

Results

Seventy-seven *P.stuartii* strains were characterized in the study: 66 carbapenem-resistant and 11 carbapenem-susceptible strains. The carbapenem-resistant *P. stuartii* (CRPS) strains were recovered from the following clinical samples: 21 respiratory tract specimens, 13 urine sample, 12 samples from infected surgical wounds, 11 blood culture, six central venous catheter tips, two peritoneal fluid samples and one pleural fluid sample. The 11 carbapenem-susceptible *P. stuartii* (CSPS) strains were isolated from respiratory tract samples (3), blood cultures (3), surgical wounds (2), central venous catheter tips (2) and urine (1). The majority (49) of the CRPS strains was recovered from intensive care units (ICU); eight strains were recovered from surgical units and nine from medical wards. All carbapenem-susceptible strains were isolated from ICU patients.

Minimum inhibitory concentrations of meropenem and ertapenem for the tested strains are shown in Table I. All phenotypically carbapenem-resistant strains carried $bla_{NDM\text{-}1}$, and one $bla_{NDM\text{-}1}$-positive strain was phenotypically susceptible to carbapenems, this finding being confirmed by repeated testing. No other carbapenemase genes were detected. Strains carrying $bla_{NDM\text{-}1}$ genes were extremely drug-resistant; all 67 were resistant to piperacillin/tazobactam, cefotaxime, ceftazidime, cefepime, ciprofloxacin and co-trimoxazole. The only susceptibilities detected were to amikacin (12 out of 67 strains), fosfomycin (45/67 strains) and aztreonam (seven/10 strains tested). Multidrug resistance was also common amongst the 10 carbapenem-sensitive $bla_{NDM\text{-}1}$-negative strains: four were amikacin-resistant; two were fosfomycin-resistant; three were resistant to piperacillin/tazobactam, cefotaxime, ceftazidime and cefepime, nine were co-trimoxazole-resitant; and all 109 were resistant to ciprofloxacin.

The PFGE dendrogram and cluster analysis is shown in Figure 1. The 77 strains belonged to six clonal clusters (A to F), when a 90% similarity cut-off was used. The dominant cluster (C) consisted of 65 strains and it was closely related to clusters A, B, D and E (similarity of over 80%). Strains belonging to cluster F were all CRPS with pulsotypes significantly different from the other clusters (similarity of 66.1%).

Within cluster C several subtypes were identified. The description of subtypes up to a similarity level of 96% is

Table I
Minimum inhibitory concentrations (MICs) of meropenem and ertapenem, and correlation with the presence of bla_{NDM}-1, for 77 *Providencia stuartii* strains

MIC of meropenem or ertapenem	No. of isolates	No. of isolates carrying bla_{NDM}
Meropenem-susceptible	11	1
MIC ≤0.25 mg/L	7	0
MIC = 0.5 mg/L	2	0
MIC = 1 mg/L	2	1
Meropenem-resistant	66	66
MIC ≥16 mg/L	66	66
Ertapenem-susceptible	11	1
MIC ≤0.25 mg/L	1	0
MIC = 0.5 mg/L	10	1
Ertapenem-resistant	66	66
MIC ≥8 mg/L	38	38
MIC = 4 mg/L	10	10
MIC >1 mg/L*	18	18

* MIC determined with Phoenix automated system.

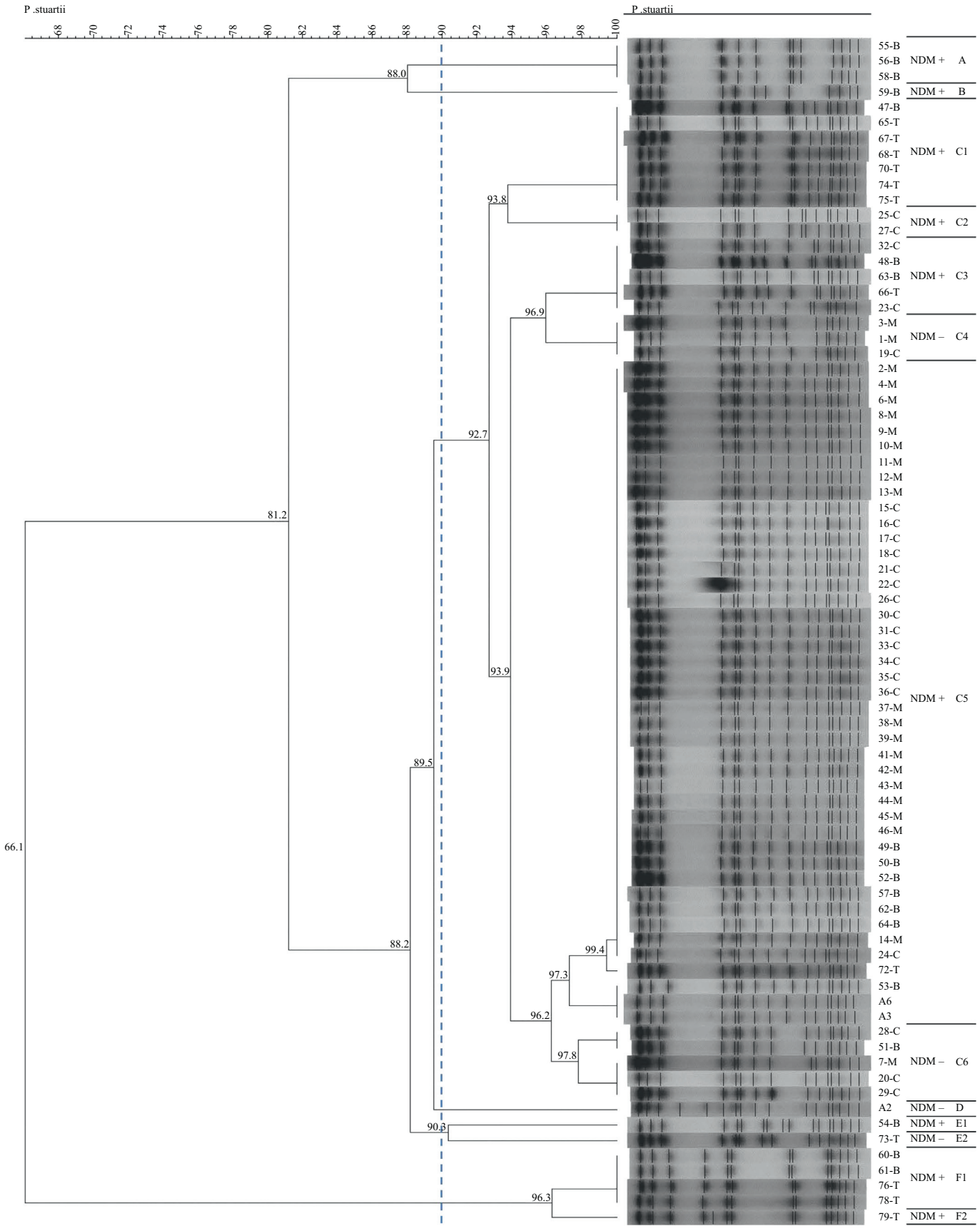

Figure 1. Pulsed-field gel electrophoresis dendrogram and cluster analysis. Origin of the strains: B, Bucharest; C, Cluj-Napoca; M, Tîrgu-Mureș; T, Timișoara; A2, A3, A6, Baia Mare; PFGE Clusters: A, B, C1, C2, C3, C4, C5, C6, D, E1, E2, F1, F2.

noteworthy in our study. Using this cut-off, six subtypes were identified within cluster C, exhibiting one to three band differences. The predominant subtype (C5) was isolated in all five centres participating in the study and comprised 44 bla_{NDM-1}-positive strains with identical pulsotypes. Subtype C6 consisted of 5 bla_{NDM-1}-negative strains and shared a band pattern similarity of 96.2% with C5 due to one missing band. Approximately the same similarity level was observed between the C3 bla_{NDM-1}-positive cluster and C4 bla_{NDM-1}-negative cluster.

PBRT analysis performed on representative strains of each clonal group revealed the presence of an IncA/C plasmid both on bla_{NDM-1} positive and negative strains. The presence of bla_{NDM-1} gene was confirmed on the extracted plasmids of resistant strains.

Discussion

A recent literature review by Abdallah and Balshi identified 39 reported cases of carbapenem-resistant *P. stuartii* infections worldwide. The 11 reports from seven countries (which did not include Romania) described only four strains of NDM-1 carbapenemase-producing *P. stuartii*; the main resistance mechanism being VIM-1 production [3]. There is also another recent report of isolation of an NDM-1-producing *P. stuartii* strain in a burn patient previously treated with colistin [4]. In Romania the first documented NDM-1 producer Enterobacteriaceae strain was isolated in 2010 in Tîrgu-Mureș [5]. In the following years bla_{NDM-1}-positive strains were found sporadically among *Enterobacter cloacae, Klebsiella pneumoniae, Escherichia coli* and *Serratia marcescens* strains [5—7].

In a previous study conducted between 2014 and 2015 in two out of the five hospitals included in our present study, the dissemination of a pKOX_NDM1-like plasmid (IncFIIγ) among *E. cloacae, K. pneumoniae,* and *S. marcescens* strains was found [8]. At that time no carbapenem-resistant *P. stuartii* strains had been detected in those two hospitals, the first CRPS strains being identified in 2016. In contrast to the previously characterized CPEs, the carbapenem-resistant *P. stuartii* strains harboured the IncA/C type plasmid, carrying the bla_{NDM-1} gene. Clonal relations of the tested isolates show the inter-hospital spread of one successful *P. stuartii* strain. In the same cluster of CRPS strains the pulsotypes of CSPS strains are also to be found, suggesting a common genetic background.

Hayakawa et al. found a correlation between increased colistin consumption and increasing prevalence of *P. stuartii* infections [9]. The increasing number of infections due to CPE in Romania has led to increased usage of colistin [10] which might have exerted significant pressure on the hospital flora leading to the selection of *P. stuartii*.

Our study revealed a challenging situation regarding the most appropriate treatment options: only amikacin, fosfomycin and aztreonam remained effective against some of our CRPS strains.

Unfortunately, in Romania only oral fosfomycin-trometamol is available, which is intended only for the treatment of acute lower urinary tract infections. However, the majority of our CRPS strains were isolated from non-urinary sites. Susceptibility to aztreonam was not tested consistently in our study, because this antibiotic is not available in Romania. Although several strains were found to be susceptible in vitro to amikacin, this antibiotic has a questionable in vivo efficacy. According to EUCAST expert rules, these strains should be reported as resistant to aminoglycosides as mutational events lead to phenotypic resistance to them.

In all participating hospitals *P. stuartii* was not the only species of CPE causing healthcare-associated infections, therefore the applied infection-control measures were directed for the prevention of CPE-caused infections in general. Screening for CPE colonization of patients admitted to ICUs performed at the participating hospitals did not detect any CRPS strains during the study period. Isolation of a CRPS from any clinical specimen triggered an alert for the infection-control unit to take measures (isolation of patients, contact precautions, screening of contacts, enhancing hand hygiene compliance). Antibiotic usage at hospital level was analysed and colistin prescription was restricted.

Our study had some limitations. It was a retrospective laboratory-based study analysing strains collected from five hospitals. Clinical data were not available, nor was the outcome of the infections known. Nevertheless, to our knowledge, this is the first study showing the nationwide inter-hospital dissemination of a successful carbapenemase producer *P. stuartii* strain. As colistin use increases, the emergence of carbapenemase production in *Enterobacteriaceae* that are intrinsically resistant to colistin presents a serious threat.

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Conflict of interest statement
None declared.

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