Nationwide multicentric survey on multidrug-resistant *Pseudomonas aeruginosa* isolates in Belgian hospitals in 2015-2016

Youri Glupczynski,[1] Catherine Berhin,[1] Ariane Deplano,[2] Caroline Bauraing,[1] Te-Din Daniel Huang,[2] Bea Jans,[3] Sandrine Roisin,[2] Olivier Denis,[2] Pierre Bogaerts[1]

[1] Reference Laboratory for monitoring of antimicrobial resistance in Gram-negative bacteria, CHU UCL Mont-Godinne, Yvoir
[2] Associated reference Laboratory, Hôpital Universitaire ULB-Erasme, [3] Public Health Institute (ISP-WIV) Brussels, Belgium

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Mailing address:
Youri Glupczynski
Microbiology Laboratory
CHU Dinant-Godinne UCL
1 Avenue Dr. G. Therasse,
5530 Yvoir, Belgium
Gerald.glupczynski@uclouvain.be

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Introduction and purpose

Pseudomonas aeruginosa is one of the leading causes of bacteraemia and pneumonia in hospitalized patients especially in intensive care units (ICUs). In Belgium, a national surveillance programme of antimicrobial resistance in acute hospitals has shown that this organism was the first cause of late onset nosocomial nosocomial pneumonias in ICUs and that it accounted for 5% of all nosocomial bacteraemia (hospital-wide) (NSIH surveillance programme, Scientific Institute of Public Health, http://www.nsih.be/surv_sep/docs/SEP_Rapport_2016.pdf).

In addition to being instrinsically resistant to several antimicrobial agents, *P. aeruginosa* can rapidly develop in vivo resistance to most conventional antipseudomonal antibiotics during treatment [1, 2, 3].

Morevover, the increasing prevalence of nosocomial infections produced by multidrug resistant (MDR) *Pseudomonas aeruginosa* (PA) can severely compromise the selection of appropriate treatment and is associated with significant morbidity and mortality [2].

The aims of this study were to determine the proportion and incidence of MDR PA isolates in Belgian hospitals in 2015 and to assess in vitro susceptibility patterns and the β-lactam resistance mechanisms in a large collection of MDR clinical PA strains isolated during the first semester of 2016.

Methods

All hospitals laboratories participating to the national surveillance programme of nosocomial infection (were invited to collect data on the proportion and incidence (/1000 admissions) of MDR PA isolates (defined by resistance to ≥4 classes of antimicrobials among expanded-spectrum cephalosporins, broad-spectrum penicillins, carbapenems, aminoglycosides, quinolones, polymyxins) in 2015 and to collect prospectively up to 5 nonduplicated MDR PA isolates over a 3 month period in 2016. Identification of all isolates was verified by MALDI-TOF MS on a microflex LT (Bruker Daltonik, Bremen, Germany) by means of the MALDI Biotyper (3.0 software, database version 3.1.2). In vitro Antimicrobial susceptibility was checked by disc diffusion using CLSI guidelines and interrpretative criteria (CLSI M100-S26 document; January 2016) and MICs of 12 antipseudomonal antibiotics were determined by microdilution using customized Sensititre® panels (GNX2F panels, Sensititre, TREK diagnostic Systems, Cleveland, USA). All isolates were analyzed by multiplex PCR and sequencing with specific primers targeting most class A, B and D beta-lactamase coding genes. The epidemiological relatedness of the strains was studied by O serotyping, PFGE and MLST.

Results

- **192 putative MDR *P. aeruginosa* collected (PA) in 53 Belgian hospitals in 2016 (Semester 1)**
  - **143 isolates were confirmed as MDR *P. aeruginosa***
    (R to ≥ 4 class of agents among 3rd/4th gen. cephalosporins, broad-spectrum penicillins, carbapenems, aminoglycosides and fluoroquinolones)
- **72 of 143 MDR PA isolates (50%) harboured carbapenemase encoding genes , VIM type metallo-β-lactamase being the most frequent (VIM-2 [n=60] , VIM-4 [n=8]).**
- **MDR PA isolates were recovered from clinical /screening specimens in 28 hospitals (53 % of participating centra) in all provinces/regions across the country**
- **106 of 125 hospitals (85%) who collected data on the proportion (% MDR PA/total PA) and on the incidence (Nr MDR PA/1000 patients admission) in 2015 reported a total of 1260 non-duplicated MDR PA culture isolates (range : 1- 158; Mean: 11,9)**

- Incidence of MDR PA (2015): **Mean: 1.02/1000 adm.**; Median: 0.44/1000 patients adm. Range; 0 to 7.35/1000 patients adm.

- Proportion of MDR PA (2015) : **Mean: 6.1%;** (Median: 4.1%; Range: 0 to 32.8%)

- **The incidence rates and the proportion (%) of MDR PA/total PA were not found to differ by region nor by size of hospital**

Results

Table 1. Types of units and anatomical sites of isolation for the 143 MDR *P. aeruginosa isolates*

Type of unit	Nr isolates	Percentage
ICU	50	35,0
Medicine	43	30,1
Geriatrics/Rehabilitation	16	11,2
Surgery	12	8,4
Burns	9	6,3
Others	19	13,3
Total	**143**	**100,0**

Isolation site	Nr isolates	Percentage
Lower resp. tract	75	52,4
Urine	32	22,4
Wound/Pus/fluid	22	15,4
Blood culture	6	4,2
Others	18	12,6
Total	**143**	**100,0**

Over 50 % of the isolates originated from respiratory tract specimens and one third of the specimens were collected from patients in ICU. MDR *P. aeruginosa* were associated with deep-seated infections in almost 20% of the cases (5% bacteriemia/septicemia)

Table 2a & 2b: MIC of MDR *P. aeruginosa* isolates (n=131) by microdilution and in vitro susceptibility rates according to EUCAST breakpoints (Breakpoint tables V.6.0, Jan. 2016)

Antimicrobial agents	MIC range (µg/ml)	MIC_{50} (µg/ml)	MIC_{90} (µg/ml)
Imipenem	1 ->256	32	>256
Meropenem	0.25 - - >256	32	>256
Piperacillin-tazob.	2 ->256	128	>256
Ceftazidime	0.5 - >256	32	128
Cefepime	1 ->236	32	128
Ceftolozane/tazob.	0.5 - >256	32	>256
Aztreonam	0.25 - >256	32	128
Amikacin	1 ->256	32	128
Tobramycin	0.5 - >64	64	>64
Gentamicin	0.5 - >64	16	>64
Ciprofloxacin	0.12 - >64	16	>64
Levofloxacin	0.5 - >64	32	>64
Colistin	0.5 - 4	2	2

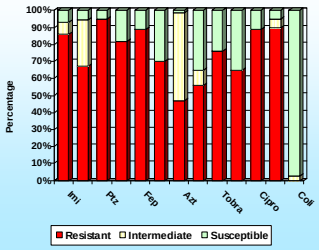
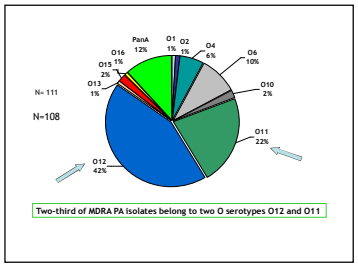


Fig. 3 Serotyping of MDR *P. aeruginosa* isolates



O-serotyping was determined by slide agglutination test using polyvalent antisera and 16 monovalent antisera numbered O1-O16 according to the manufacturer's instructions (Bio-Rad, Marnes-La-Coquette, France).

Table 3. Secondary* β-lactamases coding genes detected in the MDR *P. aeruginosa* isolates

Secondary β-lactamases[a]	Nr of isolates
VIM-2	47
VIM-2, OXA-10	4
VIM-2, OXA-9	2
VIM-2, PER-1	2
VIM-2, GES-1/GES-26	2
VIM-2, BEL-1	3
VIM-4	8
IMP-13	1
GES-5, GES-18	4
PER-1, OXA-2, OXA-20	7
BEL-1	1
OXA-2/OXA-2a	12
OXA-31/-35/-47	8
OXA-13	1
None/Unknown	52

*secondary β-lactamases with extended spectrum activity are shown in bold character.

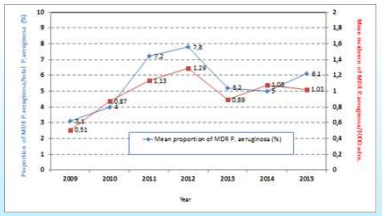
The majority of MDR PA of serotype O12 were VIM-2 producers. On the other hand, MDR PA with serotype O11 displayed much larger variety of transferable/acquired resistance mechanisms to beta-lactams (VIM-2, VIM-4, GES, PER, BEL, OXA)

Overall, MDR *P. aeruginosa* isolates clustered in 23 different PFGE types (13 isolates were singleton by PFGE) and in 9 different MLST types (ST 111 and ST235 being the two most frequent types)

The VIM-2 producing *P. aeruginosa* isolates were distributed in three major PFGE clones (X, Q, W) belonging to MLST type ST111 (clone Q and X) and to ST244 (clone W) . These three PFGE types were widely spread in 18 different hospitals in 11 cities

MDR PA isolates belonging to ST235 type (most of which of O11 serotype) had a wider clonal delineation encompassing 9 different PFGE types and were also largely in 10 different hospitals.

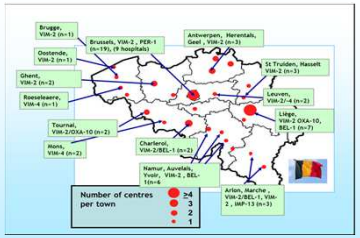
Fig. 1. Evolution of the proportion (%) and incidence (/1000 adm.) of MDR *P. aeruginosa* isolates in Belgium (2009-2015)



The proportion and incidence of MDR *P. aeruginosa almost* doubled in participating hospitals (n=69) between 2009 and 2015 (n=106) (p<0.05) with highest levels being observed in 2012

In contrast to the first surveillances (2009-2011) during which higher proportions and incidences of PA MDR were reported in larger university hodpitals, no difference were observed anymore in 2015 by hospital size nor by region (Wallonia, Flanders, Brussels)

Fig. 2. Distribution of MDR *P. aeruginosa* isolates in Belgium in 2016



Increase of MDR P. *aeruginosa* over years was essentially linked to the spread of few successful international lineages (ST111, ST235 which represent together represent 70% of all MDR PA isolates in Belgium. Since 2013, there has been a diversification of the MDR PA clones with the occurrence of other international lineages (e.g: ST175, ST233 and few others (see Fig.4).

Fig.4. Distribution of MLST types of MDR PA isolates in Belgian hospitals

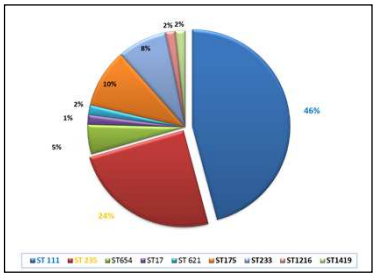
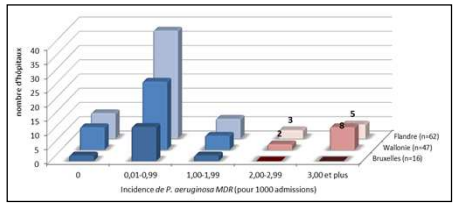


Fig. 5. Distribution of incidence of MDR PA by region in Belgium in 2015



A higher incidence of MDR PA (≥ 2/1000 adm.) was found In 18 out of 125 participating centers (14.4%) , distributed both in Flanders, and in the Walloon region, reflecting the occurrence of nosocomial outbreaks or endemic settings in these institutions.

Conclusions

- **The proportion and incidence of MDR has almost doubled in Belgium since 2009 and reaches 6.1% and 1/1000 patient admission in 2015**
- **Carbapenemase-producing isolates (essentially VIM-2) account for nearly 50% of the MDR PA isolates. Among the antimicrobial agents tested, colistin displayed the best in vitro activity (97% activity at MIC of 2 µg/ml) while other compounds including the recently marketed ceftolozane/tazobactam combination were active against ≤40% of the isolates.**
- **The increase of MDR PA was mostly linked to the probable importation and subsequent spread of a limited number of successful international clones (mostly ST111 and ST235, and more recently ST175, ST233 and few other lineages).**
- **The large diffusion of MDR PA isolates in Belgian hospitals raises concern both for patient management and infection control and underlines the for continuous epidemiological monitoring**

References

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