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Research Article

Uncommon species of non - fermentative Gram – negative bacilli in the infectious diseases pathology

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Abstract. Introduction: Nonfermentative Gram – negative bacilli, although rarely involved in pathology, represent an important cause of morbidity and mortality in the hospital environment having intrinsic resistance to multiple classes of antibiotics and the ability to gain new resistance factors. Objectives: In this study we proposed to identify the etiology of nonfermentative Gram - negative bacilli in 2014 at the Regional Institute of Gastroenterology and Hepatology "Prof. Dr. O. Fodor" Cluj - Napoca and to analyze their resistance profile. Material and method: We analyzed 357 clinical specimens mono- and plurimicrobials from 215 patients hospitalized between January - December 2014. 405 nonrepetitive strains of nonfermentative Gram - negative bacilli were isolated. Their identification was achieved with automatic system Vitek® 2 Compact (bioMérieux, Marcy - l' Étoile, France) with GN cards. Results: The retrospective study included patients hospitalized in the hospital's wards as follows: 7 (3.3%) in internal medicine, 77 (35.8%) in gastroenterology, 111 (51.6%) in the surgical ones, 8 (3.7%) in intensive care units, 12 (5.6%) ambulatory. We have identified several bacterial species of different pathological samples: *Achromobacter xylosoxidans* (n=1, 0.3%), *Achromobacter denitrificans* (n=1, 0.3%), *Burkholderia cepacia* (n=1, 0.3%), *Burkholderia pseudomallei* (n=1, 0.3%), C*hryseobacterium indologenes* (n=2, 0.5%), *Elizabethkingia meningoseptica* (n=12, 3%), *Myroides* spp. (n=3, 0.8%), *Pseudomonas aeruginosa* (n=314, 77.6%), *P. fluorescens* (n=2, 0.5%), *P. putida* (n=4, 1%), *Rhizobium radiobacter* (n=1, 0.3%), *Shewanella putrefaciens* (n=1, 0.3%), *Shingomonas paucimobilis* (n=2, 0.5%), *Shingomonas paucimobilis* / *Roseomonas gilardii* (n=1, 0.3%) and *S. maltophilia* (n=59, 14.6%), the most common of them being represented by: pus (n=73, 20.5%), tracheal secretions (n=58, 16.2%), stool (n=41, 11.5%), urine (n=36, 10.1%), blood (n=25, 7%), central venous catheter (n=24, 6.7%), sputum (n=23, 6.4%), bile (n=21, 5.9%), peritoneal fluid (n=18, 5%), the ascitic fluid (n=7, 2%), other secretions (n=9, 4.5%). Conclusions: Among nonfermentative gram - negative bacilli, *P. aeruginosa* was the main pathogen isolated, followed by *S. maltophilia* and *E. meningoseptica.* Many other uncommon species involved in infectious diseases at immunosuppressed patients were isolated .

Key Words: non-fermentative Gram – negative bacilli, intrinsic resistance, *Stenotrophomonas maltophilia*, antibiotic resistance

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Introduction

Nonfermentative Gram - negative bacilli (NF - GNB), other than *Pseudomonas aeruginosa*, belong to many bacterial genera and species rarely isolated from clinical specimens. Nowadays there has been an increased incidence of infections caused by these bacteria that are important opportunistic agents in the hospitals. Many species are important throughout the intrinsic high level resistance to multiple classes of antibiotics, but also throughout the ease with which they can acquire resistance factors.

Achromobacter genus includes seven species: *A. xylosoxidans, A. denitrificans, A. insolitus, A. marplatensis, A. piechaudii, A. spanius* and *A. ruhlandii* (Otta et al 2014). *A. xylosoxidans* is more frequently involved in infections production than A. *denitrificans* (Lelli et al 2014). They are Gram – negative bacilli, strictly aerobic, mobile, oxidase and catalase positive, opportunistic pathogens, first described by Yabuuchi in 1971 (Duggan et al 1996; Claassen et al 2011; Otta et al 2014; Kim et al 2008). They are rarely isolated from pathological samples and can easily be confused with *Pseudomonas* spp. (Otta et al 2014). Nosocomial infection source may be represented by disinfectant solutions, mainly quaternary ammonium compounds, saline solutions, dialysis fluids, deionized water (Otta et al 2014; Kim et al 2008). *A. xylosoxidans* was involved in different areas infections, such as: urinary tract, biliary tract, wound, peritonitis, pneumonia, bacteremia (Otta et al 2014; Claassen et al.2011). *A. denitrificans* was occasionally bacteremia involved in patients with cancer, kidney abscesses, osteomyelitis (Lelli et al 2014). *Burkholderia cepacia* are aerobic, mobile bacteria, isolated in infections from hospitalized and immunocompromised patients. Their transmission is achieved throughout medical devices used mainly in ICU (Saiman et al 2014). In 1950, it was discovered by WH Burkholder (Matthaiou et al 2011). It is associated with

lung infections, chronic granulomatous diseases and cystic fibrosis (Matthaiou et al 2011).

B. pseudomallei are Gram - negative intracellular bacilli which cause melioidosis or Whitmore's disease that can affect both humans and animals.

Chryseobacterium indologenes are aerobic, immobile, oxidase positive, catalase positive species (Christakis et al 2005). Most of their infections have been described in immunocompromised patients with cancer or diabetes, to whom intravascular catheters have been applied to, have been intubated or administered with prolonged antibiotic therapy (Lin et al 2010).

Elizabethkingia meningoseptica are NF - GNB, immobile, oxidase positive, which are commonly found in hospital environments. They are involved in producing infections, such as pneumonia, endocarditis, bacteremia in patients with malignancies or diabetes (Shinha & Ahuja 2015), being described by american microbiologist Elizabeth O. King in 1959 (Shinha & Ahuja 2015).

Myroides spp., part of the Flavobacteriaceae family, which comprises a heterogeneous group of NF - GNB, immobile, oxidase positive bacilli (Deepa et al 2014), with a yellow pigment (Dharni et al 2008; Mammeri et al 2002; Maraki et al 2012) aren't normal constituents of the human flora, but exist on the ground and in the water (Maraki et al 2012). Their name comes from the words "myron" - perfume and "oides" - like, these bacteria releasing a specific fruit flavor (Deepa et al 2014). Although they have a low virulence, *Myroides* spp. are multi-resistant to antibiotics, making it difficult to treat infections caused by them. They were reported in cases of endocarditis, ventriculitis, soft tissue, urinary tract or central venous catheter infections (Maraki et al 2012).

Pseudomonas fluorescens are NF – GNB that cause nosocomial infections by contamination of the water sources (Wong et al 2011). It is very difficult to differentiate the *P. aeruginosa*, an important culture character being the growth at 4°C (Wong et al 2011).

Pseudomonas putida is a member of the fluorescent group of the pseudomonads that was isolated in the hospital and found in infections such as pneumonia, catheter infections, bacteremia, cholecystitis, tonsillitis, thrombophlebitis, skin and soft tissue infections (Thomas et al 2013).

Rhizobium radiobacter are Gram – negative, aerobic bacilli that exist in soils, worldwide. They are described mainly as plant pathogens, being rarely reported as human pathogens in the systemic human infections, such as peritonitis, urinary infections, cellulitis, myositis (Gruszecki et al 2002; Romano et al 2011). Between 1995 and 2000, there were identified only 8 strains in the Birmingham Medical Center, 4 of them being isolated from blood cultures in immunosuppressed patients (Gruszecki et al 2002).

Shewanella putrefaciens (was *P. putrefaciens*) comprises a group of Gram - negative, oxidative and non-oxidative, catalase positive bacilli that have the property of hydrogen sulfide production on the slope of TSI – Triple Sugar Iron Medium (Khashe & Janda 1998). Their isolation has been documented related to soft tissue infections, bacteremia and otitis media (Khashe & Janda 1998).

Sphingomonas paucimobilis was first named by Yabuuchi (Ryan & Adley 2010). They are NF - GNB, oxidase positive, yellow pigment, spread mainly in the sea, rivers, ice, in wastewater and mineral water (Ryan & Adley 2010). Isolates have been reported in various pathological products such as urine, blood, sputum and cerebrospinal fluid (Ryan & Adley 2010), being rarely involved in the human pathology (Pagani et al 2003).

Stenotrophomonas maltophilia are NF - GNB found in wet environments, in sources of water, soil, plants (Chang et al 2014). They represent an important opportunistic pathogen in the human infections. Risk factors for these agents infections are malignancies, the use of different medical devices, chronic respiratory diseases, immunocompromised status, prolonged antibiotic therapy, long periods of hospitalization, particularly in ICU (Chang et al 2014; Kash et al 2015; Fihman et al 2012). In this study unfold over a year we proposed to determine the incidence of infections with NF – GNB in the immunosuppressed patients and to analyze their resistance profile.

Material and methods

In the retrospective study were included 357 mono- and pluribacterials clinical specimens from 215 patients admitted to the Regional Institute of Gastroenterology and Hepatology (RIGH) "O. Fodor" Cluj - Napoca between January - December 2014. The inclusion criterion in the study was isolation NF - GNB from the clinical specimens. NF - GNB strains were isolated on the: Levine m edium (Eosin Methylene Blue Agar), Mac Conkey Agar and chromogenic medium Brillance UTI Agar (Oxoid, United Kingdom). Their identification was done with automatic system Vitek® 2 Compact (bioMérieux, Marcy - l' Étoile, France) with GN cards. Conventional tests were not enough to include them into genus and species. The most accurate identification of the strains allowed the interpretation of the antibiograme and the resistance phenotypes. Antibiotic susceptibility testing was performed both with the automatic VITEK®2 Compact (AST - XN05, AST - GN75, AST - N204, N222 AST cards) and with the disc diffusion method, Kirby – Bauer. For the disc diffusion method were used antibiotic disks manufactured by Oxoid. Antibiotic susceptibility testing for the *Stenotrophomonas maltophilia* isolates was achieved by the discs diffusion method with Levofloxacin (LVX) 5 µg., Minocycline (MIN) 30 µg. and Sulphamethoxazol - Trimethoprim (SXT) 1.25/23.75 µg. Antibiotic susceptibility testing by the disc diffusion method can be made only for a few nonfermentative species, such as *P. aeruginosa, Burkholderia cepacia, S. maltophilia*, and this kind of testing is limited to a few antibiotics. For the other nonfermentatives it is accepted only the testing through the dilution method (Clinical and Laboratory Standard Institute 2014). Interpretation sensitivity testing was achieved according to the current Clinical and Laboratory Standard Institute 2014 (CLSI). Internal quality control was performed with control strains: *P. aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922, *Proteus vulgaris* group ATCC 6380. This study had been achieved in accordance with the Head of the RIGH Cluj – Napoca. We mentione that the included patients in this study gave their knowingly consent.

Results

The retrospective study included 215 hospitalized patients in the hospital's wards: 7 in internal medicine (3.3%), 77 in gastroenterology (35.8 %), 111 in the surgical ones (51.6%), 8 in the intensive care - ICU (3.7%), and 12 from outpatients (5.5%). 100

Table 1. Includes NF - GNB strains resistance to: Penicillin (Ticarcillin – TIC, Piperacillin – PIP) and combined Penicillin with β – lactamases inhibitors (Amoxicyllin + Clavulanic acid – AMC, Ticarcillin + Clavulanic acid – TIM, Piperacillin + Tazobactam – TZP), 3th and 4th generation (Ceftazidime – CAZ, Cefepime – FEP) Cephalosporins, Monobactams (Aztreonam – ATM) and Carbapenems (Imipenem – IMP, Meropenem – MRP). MIC - minimum inhibitory concentration. R_I - intrinsic resistance; R – resistant

Bacterial species	*n* tested strains	AMC	TIC	TIM	PIP	TZP	CAZ	FEP	ATM	IMP	MRP
		R	R	R	R	R	R	R	R	R	R
Achromobacter xylosoxidans	1	-	-	-	-	-	-	100%	100%	-	100%
MIC (mg/l)							-	≥ 64	≥ 64	-	4
Achromobacter denitrificans	1	-	-	-	-	-	-	100%	100%	-	100%
MIC (mg/l)							-	≥ 64	≥ 64	-	4
Burkholderia cepacia	1	R_I	R_I	R_I	100%	-	-	100%	100%	R_I	100%
MIC (mg/l)					≥ 128		-	≥ 64	≥ 64	-	≥ 16
Burkholderia pseudomallei	1	-	-	-	-	-	-	-	-	-	-
MIC (mg/l)							-	-	-	-	-
Chryseobacterium indologenes	2	-	100%	-	100%	100%	100%	100%	100%	100%	100%
MIC (mg/l)			≥ 128		≥ 128	≥ 128	≥ 64	≥ 64	≥ 64	≥ 16	≥ 16
Elizabethkingia meningoseptica	12	16.6%	100% R_I	100% R_I	55.5%	100%	100% R_I	92.9%	-	100% R_I	100% R_I
MIC (mg/l)		≥ 32	≥ 128	≥ 128	≥ 128	≥ 128	≥ 64	≥ 64	-	≥ 16	≥ 16
***Myroides* spp.**	3	-	100%	-	100%	100%	-	100%	-	-	100%
MIC (mg/l)			≥ 128		≥ 128	≥ 128		≥ 64			≥ 16
Pseudomonas fluorescens	2	-	50%	-	-	-	-	-	-	-	-
MIC (mg/l)			≥ 128				-	-	-	-	-
Pseudomonas putida	4	-	100%	-	50%	50%	50%	50%	-	25%	75%
MIC (mg/l)			≥ 128		≥ 128	≥ 128	≥ 64	≥ 64	-	-	≥ 16
Shewanella putrefaciens	1	-	-	-	-	-	-	-	-	-	-
MIC (mg/l)							-	-	-	-	-
Sphingomonas paucimobilis	3	-	25%	-	25%	-	25%	25%	100%	-	-
MIC (mg/l)			≥ 128		≥ 128		-	-	-	-	-

were female (46.5%) and 115 men (53.5%), aged between 19 and 90 years [average: 63 years]. Specimens were represented as follows: pus (n=73, 20.5%), tracheal secretions (n=58, 16.2%), stool (n=41, 11.5%), urine (n=36, 10.1%), blood (n=25, 7%), central venous catheter (n=24, 6.7%), sputum (n=23, 6.4%), bile (n=21, 5.9%), peritoneal fluid (n=18, 5%), the ascitic fluid (n=7, 2%), other secretions (n=9, 2.5%). The rest of the other biological specimens were represented by eschar biopsy secretion (n=2, 0.6%), pharyngeal secretion (n=1, 0.3%), nasal secretion (n=1, 0.3%), vaginal secretion (n=2, 0.6%), necrotic tissue (n=1, 0.3%), bronchial aspirate (n=1, 0.3%), bronchial lavage (n=2, 0.6%), visceral abscess fluid (n=2, 0.6%), pericardial fluid (n=1, 0.3%) pleural fluid (n=3, 0.8%). Clinical outcome was favorable in the case of 153 patients (71.2%) and 62 patients, died (28.8%). Hospitalization was between 2 and 212 days [median: 26 days], and the time between the pathogen isolation and the time of the inpatients was between 2 and 164 days, with a median of 15.5 days.These strains were isolated from immunosuppressed patients by various forms of cancer (hepatocellular carcinoma, cholangiocarcinoma, gastric cancer and esophageal, recto - sigmoid and pancreatic cancer) or from other immunosuppressed medical (cirrhosis, cholecystitis gangrenosum, obstructive jaundice, acute pancreatitis) or surgical (gastric polyps, umbilical hernia, intestinal obstruction), conditions.

Characterization of the isolates: 405 nonrepetitive strains of NF - GNB were isolated and identified from mono- and pluribacterials pathological samples consisting of 2 or 3 bacterial strains. These identified species were: *Achromobacter xylosoxidans* (n=1, 0.3%), *Achromobacter denitrificans* (n=1, 0.3%), *Burkholderia cepacia* (n=1, 0.3%), *Burkholderia pseudomallei* (n=1, 0.3%), *Chryseobacterium indologenes* (2, 0.5%), *Elizabethkingia meningoseptica* (12, 3%), *Myroides* spp. (n=3, 0.8%), *P. aeruginosa* (n=314, 77.6%), *Pseudomonas fluorescens* (n=2, 0.5%), *Pseudomonas putida* (n=4, 1%), *Rhizobium radiobacter* (n=1, 0.3%), *Shewanella putrefaciens* (n=1, 0.3%), *Shingomonas paucimobilis* (n=2, 0.5%), *Shingomonas paucimobilis* / *Roseomonas gilardii* (n=1, 0.3%) and *S. maltophilia* (n=59, 14.6%).

Only *Achromobacter*, *P. fluorescens* and *S. paucimobilis* species showed sensitivity to Penicillin and Penicillin-associated with β - lactamases inhibitors, other species having natural resistance of species (see Table 1 and 4) or acquired resistance.

Achromobacter species, *B. pseudomallei*, *P. fluorescens* were 100% susceptible to CAZ, and *P. putida* and *S. paucimobilis*

Table 2. NF - GNB strains' resistance to Aminoglycosides (Gentamicin – GEN, Tobramycin – TOB, Amikacin – AK) and Fluoroquinolones (Ciprofloxacin – CIP, Levofloxacin – LVX, Pefloxacin – PEF, Norfloxacin – NOR, Moxifloxacin – MXF). MIC - minimum inhibitory concentration; R_I - intrinsic resistance. R – resistant.

Bacterial species	*n* tested strains	GEN	TOB	AK	CIP	LVX	PEF	NOR	MXF
		R	R	R	R	R	R	R	R
Achromobacter xylosoxidans	1	100%	100%	100%	100%	-	-	-	-
MIC (mg/l)		≥ 16	8	≥ 64	≥ 4	-	-	-	-
Achromobacter denitrificans	1	100%	100%	100%	100%	-	-	-	-
MIC (mg/l)		≥ 16	8	≥ 64	≥ 4	-	-	-	-
Burkholderia cepacia	1	R_I	R_I	R_I	R_I	-	-	-	-
MIC (mg/l)					-	-	-	-	-
Burkholderia pseudomallei	1	100%	100%	100%	100%	-	-	-	-
MIC (mg/l)		≥ 16	≥ 16	4	≤0.25	-	-	-	-
Chryseobacterium indologenes	2	100%	100%	100%	50%	-	50%	-	-
MIC (mg/l)		≥ 16	≥ 16	≥ 64	≥ 4	-	≥ 16	-	-
Elizabethkingia meningoseptica	12	100%	100%	85.7%	28.6%	-	-	100%	-
MIC (mg/l)		≥ 16	≥ 16	≥ 64	≥ 4	-	-	≥ 16	-
Myroides spp.	3	100%	100%	100%	100%	100%	-	-	100%
MIC (mg/l)		≥ 16	≥ 16	≥ 64	≥ 16	≥ 8	-	-	≥8
Pseudomonas fluorescens	2	-	-	-	-	-	-	-	-
MIC (mg/l)					-	-	-	-	-
Pseudomonas putida	4	25%	25%	25%	50%	-	100%	-	-
MIC (mg/l)		≥16	≥16	≥ 64	≥4	-	≥ 16	-	-
Shewanella putrefaciens	1	-	-	-	-	-	-	-	-
MIC (mg/l)					-	-	-	-	-
Sphingomonas paucimobilis	3	25%	25%	25%	25%	-	-	-	-
MIC (mg/l)		≥16	≥16	≥ 64	1; ≤0.25	-	-	-	-
Stenotrophomonas maltophilia	59	-	-	-	-	5.1%	-	-	-
MIC (mg/l)						≥ 8			

showed 50%, respectively, 75% sensitivity. To FEP and ATM *Achromobacter* species, *B. cepacia, C. indologenes* were resistant (100%). The resistance to Carbapenems was variable depending on the species (see Table 1).

Most bacterial species were 100% resistant to aminoglycosides. *P. fluorescens* strains were 100% sensitive, whereas *P. putida* strains and *S. paucimobilis* strains were 100% sensitive. 14.3% of the *E. meningoseptica* strains were susceptible to AK. Sensitivity for the most species to Fluoroquinolone was between 50% and 100%, except for the *Myroides* spp. strains which were 100% resistant to all the tested fluoroquinolones.

MIN was effective 100% for most species. *P. putida* was 50% resistant to MIN and 9.6% of the *S. maltophilia* strains were resistant to MIN. Although sensitive to MIN in 100%, *Myroides* spp. strains were resistant to TE and TIG. Strains were 100% resistant to CT, except the species: *Pseudomonas*, *Shewanella* and *Sphingomonas*. The strains tested for F were 100% resistant and of those tested to C, only *S. putrefaciens* was sensitive. *Myroides* spp. and *Achromobacter* species were 100% resistant to SXT, while for other species, resistance varied between 30.2% and 85.7%. While 14.3% of the *E. meningoseptica* strains were susceptible to SXT, they were 100% resistant to trimethoprim (TMP).

Discussion

Intrinsic resistance NF - GNB is known at most bacterial species and it corresponds both to the European Committee on Antimicrobial Susceptibility The Testing (EUCAST) standard and to CLSI. In table 4 is presented the intrinsic resistance in accordance to these standards. NF - GNB are resistant to Benzylpenicillin, Cefoxitin (FOX), Cefamandole (MA), Cefuroxime (CXM), Glycopeptides, Fusidic acid, Macrolides, Lincosamides, Streptogramins, Rifampicin (RIF), Daptomycin, and Linezolid (LNZ) (Leclercq et al 2013; Clinical and Laboratory Standard Institute 2014) .

Some *B. cepacia* complex strains may appear susceptible in vitro to certain β - lactams, but are clinically resistant. *B. cepacia* and *S. maltophilia* are showing resistance to aminoglycosides. Intrinsic resistance is a result of low permeability or probably of the efflux system. Most of the *S. maltophilia* isolates produce the AAC(6') enzyme that inactivates aminoglicosydes. Even if *S. maltophilia* may have low MIC for CAZ, however it is considered resistant. *S. maltophilia* is SXT sensitive, but resistant to TMP (Leclercq et al 2013).

In our study, we isolated and identified in the tracheal secretions of a patient with liver cirrhosis, diabetes and right pleural effusion a strain of *A. xylosoxidans* and one *A. denitrificans* strain

Table 3. NF - GNB strains' resistance to Tetracyclines (Tetracycline - TE, Minocycline – MIN, Tygecycline – TIG), Colistin (CT), Chloramphenicol (C), Nitrofurantoin (F), Sulphamethoxazol – Trimethoprim (SXT) and Trimethoprim (TMP). MIC - minimum inhibitory concentration; R_I - intrinsic resistance; R – resistant, I – intermediate, S – susceptible; * - disc diffusion testing.

Bacterial species	*n* tested strains	MIN			TE	TIG	CT	F	C	SXT	TMP
		S	I	R	R	R	R	R	R	R	R
Achromobacter xylosoxidans	1	100%	-	-	-	-	100%	-	-	100%	-
MIC (mg/l)		≤1					4	-	-	80	-
Achromobacter denitrificans	1	100%	-	-	-	-	100%	-	-	100%	-
MIC (mg/l)		≤1					4	-	-	80	-
Burkholderia cepacia	1	100%	-	-	100%	-	100% R_I	-	100% R_I	-	R_I
MIC (mg/l)		≤1			≥ 16		≥ 16	-	≥ 64	-	-
Burkholderia pseudomallei	1	-	-	-	-	-	-	100%	-	100%	-
MIC (mg/l)							-	≥ 512	-	80	-
Chryseobacterium indologenes	2	100%	-	-	-	-	100%	-	-	-	-
MIC (mg/l)		≤1					≥ 16	-	-	-	-
Elizabethkingia meningoseptica	12	100%	-	-	100%	-	100% R_I	100%	-	85.7%	100%
MIC (mg/l)		≤1; 2			≥ 16		≥ 16	256 - ≥ 512	-	160	8
Myroides spp.	3	100%	-	-	100%	100%	100%	-	100%	100%	100%
MIC (mg/l)		≤1			≥ 16	≥ 8	≥ 16		≥ 64	≥ 320	≥ 16
Pseudomonas fluorescens	2	50%	-	-	-	-	50%	-	-	50%	-
MIC (mg/l)		≤1; 2					≥ 16	-	-	80	-
Pseudomonas putida	4	50%	-	50%	-	-	0%	-	-	85.7%	-
MIC (mg/l)		4; ≤1		≥ 16			-	-	-	≥ 320	-
Shewanella putrefaciens	1	100%	-	-	-	-	0%	-	-	-	-
MIC (mg/l)		≤1					-	-	-	-	-
Sphingomonas paucimobilis	3	100%	-	-	-	-	0%	-	-	50%	-
MIC (mg/l)		≤ 1; 2					-	-	-	80; ≥ 320	-
Stenotrophomonas maltophilia	59	88.5%	1.9%	9.6%	-	-	-	-	-	30.2%	R_I
MIC (mg/l)		*	*	*			-	-	-	160, *	-

was identified in the blood culture from a patient with operated gastric tumor. *A. xylosoxidans* is sensitive to anti-*Pseudomonas* penicillins carbapenems and aminoglycosides, resistant to fluoroquinolones and variable resistance to SXT (Otta et al 2014; Claassen et al 2011). The isolated *A. xylosoxidans* strain was sensitive to anti-*Pseudomonas* penicillins (TIC, PIP, TZP), CAZ, Carbapenems (IMP) and MIN and was resistant to FEP, MEM, ATM, Fluoroquinolones (CIP), aminoglycosides (GEN, TOB, AK), CT and SXT. The *A. denitrificans* strain, isolated, was sensitive to CAZ and IMP, similar to that reported by D. Lelli et al in a case of osteomyelitis.

CT is inactive against *B. cepacia* due to their unusual lipopolysaccharide structure (Enoch et al 2007). It is resistant to disinfectants such as povidone iodine and chlorhexidine (Matthaiou et al 2011). Temocillin therapy was used to treat *B. cepacia* in cystic fibrosis, but this antibiotic is not active for others non-fermentative bacilli (Enoch et al 2007).

In our study one strain was isolated in tracheal secretions from a patient with heart failure hospitalized in the internal medicine ward of RIGH Cluj – Napoca and was sensitive to LVX and MIN. The best treatment for *B. pseudomallei* is initially with IMP and CAZ intravenously for 2 weeks, followed by oral therapy with doxycycline, SXT and C for 3 more months. This treatment plan can prevent relapses, reducing mortality (Northfield 2002). Tigecycline may be an alternative therapy for melioidosis (Enoch et al 2007). One strain was isolated from a wound secretion from a patient with gastric cancer, and was sensitive to CAZ, FEP, LVX and SXT, intermediate to PIP and MEM.

C. indologenes shows resistance to aminoglycoside, penicillin, ATM, Ist, IInd and IIIrd generation of cephalosporins (except CAZ) and variable resistance to IMP. Most active agents used against it were Fluoroquinolones, TZP, FEP and SXT (Lin et al 2010; Christakis et al 2005). The strains identified were, one from tracheal secretion from a patient with gastric cancer and the other from another with respiratory and heart failure. Both strains were sensitive only to MIN and SXT, one of the strains was Fluoroquinolone (PEF and CIP) sensitive and the other one was intermediate. Our strains were resistant to FEP and TZP.

E. meningoseptica produces a chromosomal β - lactamase that can hydrolyze most β - lactams. Thus, it shows intrinsic resistance

Table 4. Intrinsic resistance to NF - GNB (Leclercq et al 2013)

Organism	Ampicillin	Amoxicilin + Clavulanic acid	Ticarcillin	Ticarcillin + Clavulanic acid	Piperacillin	Piperacillin + Tazobactam	Cefazolin	Cefotaxime	Ceftriaxone	Ceftazidime	Ertapenem	Imipenem	Meropenem	Ciprofloxacin	Chloramphenicol	Aminoglycosides	Trimethoprim	Polimyxin B/Colistin	Nitrofurantoin
Achromobacter xylosoxidans	R	-	-	-	-	-	R	R	R	-	R	-	-	-	-	-	-	-	-
Achromobacter denitrificans	R	R	-	-	-	-	R	R	-	-	-	-	-	-	-	-	-	R	-
Burkholderia cepacia complex*	R	R	R	R	-	-	R	-	-	-	R	R	-	R	R	R	R	R	R
Burkholderia pseudomallei	R	R	R	R	-	-	R	-	-	-	R	R	-	R	R	R	R	R	-
Elizabethkingia meningoseptica	R	-	R	R	-	-	R	R	R	R	R	R	R	-	-	-	-	R	-
Stenotrophomonas maltophilia	R	R	R	-	R	R	R	R	R	R	R	R	R	-	-	R	R	-	-

to most β - lactams, including carbapenems, but is sensitive to Vancomycin, Fluoroquinolones, SXT, TIG and RIF, antibiotics that are active against Gram – positive bacteria (Pereira et al 2013; Ray et al 2014). 12 strains of *E. meningoseptica* were isolated from tracheal secretions, central venous catheters, blood cultures, bronchial lavages, pus discharge (drainage tube) in patients with gastric cancer, peritonitis, cirrhosis, intraperitoneal adherents syndrome, intestinal obstruction. 8 strains were provided from the surgical wards (66.5%), 2 from the gastroenterology departments (16.7%) and 2 from the internal medicine wards (16.7%). One strain (14.3%) of the 7 tested was sensitive to AK. 6 strains (85.7%) from the 7 strains tested to LVX were susceptible and one was intermediate. Five strains (71.4%) from 7 tested were sensitive to CIP and two were resistant. All 12 strains (100%) were susceptible to MIN. 7 strains (100%) tested for TMP were susceptible and only one strain (14.3%) was susceptible to SXT.

Myroides spp. is susceptible to SXT and CIP (Deepa et al 2014) and is resistant to β - lactams, monobactams, carbapenems and aminoglycosides (Maraki et al 2012). Their resistance to β - lactams is determined by the production of metallo - β - lactamases (Maraki et al 2012). We isolated three strains, two from the bile and one from the urine of the gastroenterology departments hospitalized patients. These strains showed a significant resistance to multiple antibiotics classes, only 2 of them being susceptible to MIN and resistant to fluoroquinolones (CIP, LEV, MXF) and SXT.

We isolated 2 strains of *P. fluorescens* from the urine of two hospitalized patients in the gastroenterology departments. These strains were susceptible to TIC, PIP, TIM, TZP, CAZ, FEP, GEN, TOB, AK, PEF. One strain was sensitive to IMP, and the other one was intermediate. One strain was sensitive and the other one was resistant to MIN, CT and SXT.

In our study there are four strains of *P. putida* isolated from the tracheal secretion (1), sputum (1) and urine (2) collected from outpatients (2), from hospitalized patients in gastroenterology (1) and from surgery wards (1). Pathologies were, as follows: urinary tract infections, acute necrotic – hemorrhagic pancreatitis and esophagus tumor. All these strains were resistant to TIC, PEF, SXT, 2 strains were susceptible to PIP, TZP, CAZ, FEP, CIP, MIN, the rest being resistant to them. 3 strains were susceptible to IMP, GEN, TOB, AK. All the strains were susceptible to CT.

The therapy of *Rhizobium radiobacter* infections with CIP, TZP, SXT, CRO, GEN, AK was a fair choice for the cases mentioned in the study developed in the Birmingham Medical Center (Gruszecki et al 2002). We have isolated and identified a strain of *Rhizobium radiobacter* from the biliary secretion in a patient with a pancreatic head tumor hospitalized in the surgery department. This strain was susceptible to GEN, PIP, CIP, LVX, IMP, MEM, TOB, SXT, TE and resistant to CAZ and TZP. *Shewanella putrefaciens* was isolated from the bile of a hospitalized patient in the gastroenterology department and that was sensitive to all antibiotics tested.

We isolated three strains of *S. paucimobilis* from blood cultures (2) and from the tracheal secretion (1) in patients with gastric (1) and esophageal (2) tumors, hospitalized in gastroenterology (1), surgery (1) and internal medicine wards (1). To one strain, the identification couldn't be discriminated between *S. paucimobilis* and *Roseomonas gilardii*. *R. gilardii* are Gram - negative coccobacillus, pink, resistant to cephalosporins and broad-spectrum penicillins (PIP, Mezlocillin), but are susceptible to IMP, aminoglycosides (AK, GEN, TOB) and TE (Shokar et al 2002).

The 59 strains of *S.maltophilia* were isolated from immunosuppressed patients with various forms of cancer (hepatocellular carcinoma, cholangiocarcinoma, gastric, esophageal and recto - sigmoid cancer, pancreatic cancer) or other medical conditions (cirrhosis, cholecystitis gangrenosum, obstructive jaundice, acute pancreatitis) and surgery (gastric polyps, umbilical hernia, intestinal obstruction). 20 strains were isolated from wound secretion, 10 from sputum, 7 strains from bile, 5 from tracheal secretions, 5 from central venous catheter, 4 from stool, 4 from blood and 2 strains from urine. 54 strains (91.5%) were susceptible to LVX, 3 (5.1%) were resistant and 2 strains (3.4%) were intermediate. 46 strains (88.5%) were susceptible to MIN, 5 (9.6%) were resistant and one strain (1.9%) was intermediate. 36 strains (67.9%) were susceptible to SXT, 16 strains (30.2%) were resistant and, one strain (1.9%) was intermediate. Most strains remain sensitive to SXT, but the toxicity or the intolerance of this chemotherapeutic agent remains a major problem in these patients (Enoch et al 2007). Fluoroquinolones may be associated with TIM or SXT therapy (Enoch et al 2007). Four from the patients in which was isolated *S. maltophilia*, died.

Conclusions

Among NF - GNB, *P. aeruginosa* was the main pathogen isolated, followed by *S. maltophilia* and *E. meningoseptica*. Many other unusual species were involved in the infectious diseases. These infections had numerous predisposing factors such as: immunosuppression in the case of cancers, diabetes, chronic infections, different surgical conditions or lengthy hospitalizations and antibiotic therapy long overuse and multiple. Although they are less isolated than other bacteria, they raise important therapy issues throughout intrinsic resistance to multiple antibiotics classes, and increased acquired rate of resistance factors that determine their proliferation in the hospital.

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