Antimicrobial consumption and antimicrobial resistance: a snapshot of an Italian neuromuscular rehabilitation center

Giuseppina Barberi[1], Maria Cristina De Cola[1], Pia Dell'Utri[1], Sara Melardi[1], Bernardo Alagna[1], Placido Bramanti[1], Antonio Cascio[2]

[1]IRCCS Centro Neurolesi "Bonino-Pulejo", Messina (ME), Italy;
[2]Department of Health Promotion Sciences and Mother and Child Care "G. D'Alessandro", University of Palermo, Palermo, Italy

SUMMARY

The paper presents a snapshot of the incidence of antimicrobial-resistant microorganisms and antimicrobial consumption in an Italian rehabilitation center over a two-year period (2014-2015). Data on microorganism identification and antimicrobial susceptibility testing were obtained from the diagnostic laboratory of the hospital. A set of indicators was assessed, including the incidence density of resistant isolates *per* 1000 patient-days (IDRI). Data on antimicrobial consumption, semi-annually, obtained from the hospital pharmacy, were expressed as *Defined Daily Dose* (DDD) *per* 1000 patient-days. The most frequently isolated microorganism was *Klebsiella pneumoniae* (19.3%), and a significant increase in piperacillin/tazobactam-resistant *K. pneumoniae* (p=0.04) was observed. Among all antimicrobials used, carbapenems were the most prescribed antibiotic class (31%).

INTRODUCTION

Antimicrobial resistance (AMR) is a serious threat to public health. The number of organisms exhibiting AMR, in particular resistance to multiple antibiotics, continues to increase in Europe (ECDC, 2015a). In the last few years, institutions have started to attach more importance to healthcare-associated infections (HAI), which do not occur only in intensive care units (ICUs), surgery, and long-term care facilities (LTCF), (Stillo *et al.,* 2014; Reilly *et al.,* 2015; *Droz et al.,* 1996; ECDC, 2014; ASSR, 2012).

The rehabilitation phase of a patient's recovery often requires long pathways within specific settings, during which eventual HAI can prolong or compromise the rehabilitation, as well as its outcome.

The aim of the present study is to present a snapshot on the incidence of AMR and systemic antimicrobial consumption at IRCCS Centro Neurolesi "Bonino Pulejo", an Italian rehabilitation center, to obtain an internal benchmark for future surveillance, and useful data to compare with those of other rehabilitation hospitals.

METHODS

The IRCCS Centro Neurolesi "Bonino Pulejo" of Messina is an Italian 80-bed highly qualified rehabilitation hospital which receives patients with ischemic pathologies, traumatic brain injury, brain hemorrhage, brain surgery and other serious neurological diseases referred by ICUs, neurosurgery, neurology, and other rehabilitation centers. The hospital has a 20-bed ward for severe brain-injured patients and two other wards for spinal patients, intensive rehabilitation patients and vegetative and minimally conscious state patients. All microbiological data of the patients consecutively admitted from January 2014 to December 2015 were retrospectively collected and inserted into a database.

Microorganism identification was performed using the automatic system Vitek® 2 Compact (Biomerieux, Marcy - l'Etoile, France) with GP and GN cards. Antimicrobial susceptibility testing was performed with the automatic Vitek® 2 Compact (AST - P632, AST - P586, AST - N204, AST - N202, AST - YS07).

Duplicate strains from the phenotypic point of view and with identical antibiotic resistance patterns, isolated within a month in the same patient and from the same site were excluded from the study. Microorganisms isolated were classified as susceptible (S), intermediate (I) or resistant (R) according to the European Committee on Antimicrobial Susceptibility Testing guidelines (EUCAST, 2016). For each identified species, the isolation density (ID) (number of isolates *per* 1000 patient-days), antibiotic resistance rates (RR) (calculated by dividing the number of resistant/intermediate isolates by the total isolate number of the same species tested against the corresponding antibiotic multiplied by 100), and the incidence density of resistant isolates (IDRI) (defined as the number of resistant or intermediate isolates *per* 1000 patient-days) were assessed (Meyer *et al.,* 2010; Agodi *et al.,* 2015).

Data on antimicrobial consumption were obtained from the hospital pharmacy on a six-monthly basis. Antibiotic consumption (antibiotic usage density, AD) was expressed as *Defined Daily Dose* (DDD) *per* 1000 patient-days to conform to the use of the ATC/DDD system, recognized

Corresponding author:
Giuseppina Barberi
E-mail, giusybarberi@gmail.com; giusy.barberi@irccsme.it

by WHO as an international standard for drug utilization studies since 1996 (WHO, 2016). We focused on consumption of penicillins, third and fourth generation cephalosporins, carbapenems, glycopeptides, aminoglycosides, fluoroquinolones and polymixins. The number of patient-days for each semester was acquired from hospital administrative records. The association between selected antibiotic consumption and antibiotic resistance was examined at six-month intervals (time lag).

A positive time lag for any result indicated that specific antibiotic consumption had preceded a specific antibiotic resistance. Primary and secondary antibiotics were correlated to antibiotic resistance, i.e. microorganism resistance to a specific antibiotic was correlated to the use of the same antibiotic (primary) or to a different antibiotic (secondary) (Bosso *et al.*, 2010). Quantitative variables were expressed as means ± standard deviations, whereas qualitative variables as frequencies and percentages. Trends over time of IDRI and AD were analyzed by regression analysis. Linear correlations between variables were assessed by Pearson's coefficients. A p value <0.05 was considered statistically significant. All analyses were performed using the STATISTICA software (StatSoft).

RESULTS

During the study period, 420 patients generating 57,737 patient-days with an average length of stay of 104.61 days were admitted to our hospital. *Table 1* presents potential risk factors for the admitted patients.

On average, for each semester, 216±17.2 microbial isolates (191 in the first and 225 in the second semester of 2014, 229 in the first and 219 in the second semester of 2015), were isolated; of these, 64.6% were gram-negative, 23.1% gram-positive and 12.3% were fungal species. *Figure 1* shows the isolation rate of all the microbial species.

The most commonly isolated microorganisms, all belonging to the *Enterobacteriaceae* family, were *K. pneumoniae* (19.3%), *Proteus mirabilis* (18.2%) and *E. coli* (9.5%). A non significant increase in *K. pneumoniae* ID, from 2014 (2.7 *per* 1000 patient-days) to 2015 (3.5 *per* 1000 patient-days) and in *Acinetobacter baumannii* ID from 2014 (0.6 *per* 1000 patient-days) to 2015 (0.8 *per* 1000 patient-days) was observed, above all among gram-negative isolates. Among gram-positive isolates an increase in *Enterococcus faecalis* ID, from 2014 (0.4 *per* 1000 patient-days) to 2015 (0.7 *per* 1000 patient-days), in *Staphylococcus haemolyticus* ID, from 2014 (0.3 *per* 1000 patient-days) to 2015 (0.6 *per* 1000 patient-days, p=0.03), and in *Staphylococcus hominis* ID, from 2014 (0.4 *per* 1000 patient-days) to 2015 (0.7 *per* 1000 patient-days) was observed (*Table 2*). No fluctuation in the frequency of *E. coli* and *P. mirabilis* was observed. Among the fungal species, an increase in *Candida glabrata* ID, followed by *Candida parapsilosis* ID and *Candida albicans* ID (from 0.3, 0, 0.6 in 2014 to 1, 0.2. 0.9, in 2015, respectively) was also observed. *Table 3* describes the IDRI assessed on a six-monthly basis. Piperacillin/tazobactam-resistant *K. pneumoniae* had the highest IDRI *per* 1000 patient-days with 3.32 isolate resistance in the second semester of 2015. This species showed an increasing trend from 2014 to 2015 in IDRI for each antibiotic class, except for colistin and trimethoprim/sulfamethoxazole, differently from what was reported for *P. mirabilis*, *E. coli*, *Pseudomonas aeruginosa*. In particular, we observed a significant in-

Table 1 - *Potential risk factors of patients admitted.*

Potential risk factors	%
Invasive devices at admission:	
CVC	28.2
Urinary catheter	66.7
Tracheotomy	25.6
Nasogastric tube	25.6
PEG	15.4
Parenteral nutrition	5.1
Simultaneous presence of invasive devices:	
N. 2 invasive devices	20.5
N. 3 invasive devices	25.6
N. 4 invasive devices	7.7
Admission from:	
UTIs	42
Neurosurgery and neurology	22.3
Other wards	12.1
Home	15.5
Stroke units	6.9
Other rehabilitation units	1.2

creasing trend for piperacillin/tazobactam-resistant *K. pneumoniae* from 1.47 in the first semester of 2014 to 3.32 in the second semester of 2015 (p=0.0419). An increasing trend was also observed for fluoroquinolones, aminoglycosides, and carbapenem-resistant *A. baumannii*, for rifampicin-resistant *Staphylococcus aureus* and for high-level aminoglycoside resistant-*E. faecalis* from 2014 to 2015. On the contrary, a significant decreasing trend for ampicillin/sulbactam-resistant *E. faecalis* from 0.21 in the first semester of 2014 to zero in the second semester of 2015 (p=0.00004) was also observed. Despite not being significant, *P. aeruginosa* also showed a slightly decreasing trend from 2014 to 2015 in IDRI for each tested antimicrobial class, above all for carbapenems, extended-spectrum cephalosporins and aminoglycosides. In the two years studied, 631 and 625 DDD *per* 1000 patient-days were administered, respectively. *Table 4* shows the consumption of all antimicrobial agents, which increased from 282.7 in the first semester of 2014 to 353.5 in the second semester of 2015. Among all antimicrobials used, carbapenems were always the most prescribed antibiotic class (31% of the total AD), followed by penicillins (13%), fluoroquinolones (11.3%), glycopeptides (10.6%), azoles (8.3%) and colistin (8.2%) (data not shown). The consumption of carbapenems was the highest among all systemic antimicrobials. Interestingly, the consumption of ceftriaxone increased from 5.4 to 16.3 DDD (p=0.06) , e.g. piperacillin/tazobactam from 1.7 to 6.4 (p=0.0121), teicoplanin from 7.5 to 11.0 (p=0.0225), and IV ciprofloxacin from 0 to 9.5 (p=0.0629). A higher reduction for tigecycline and rifampicin (p=0.0237) and a slight increase for daptomycin were observed during the study period. Antimicrobial resistance trends over time were not statistically associated with the primary antimicrobial agent. The correlation analysis shown in *Table 5* demonstrates that the use of piperacillin/tazobactam is statistically correlated to aminoglycoside-resistant *K. pneumoniae* IDRI
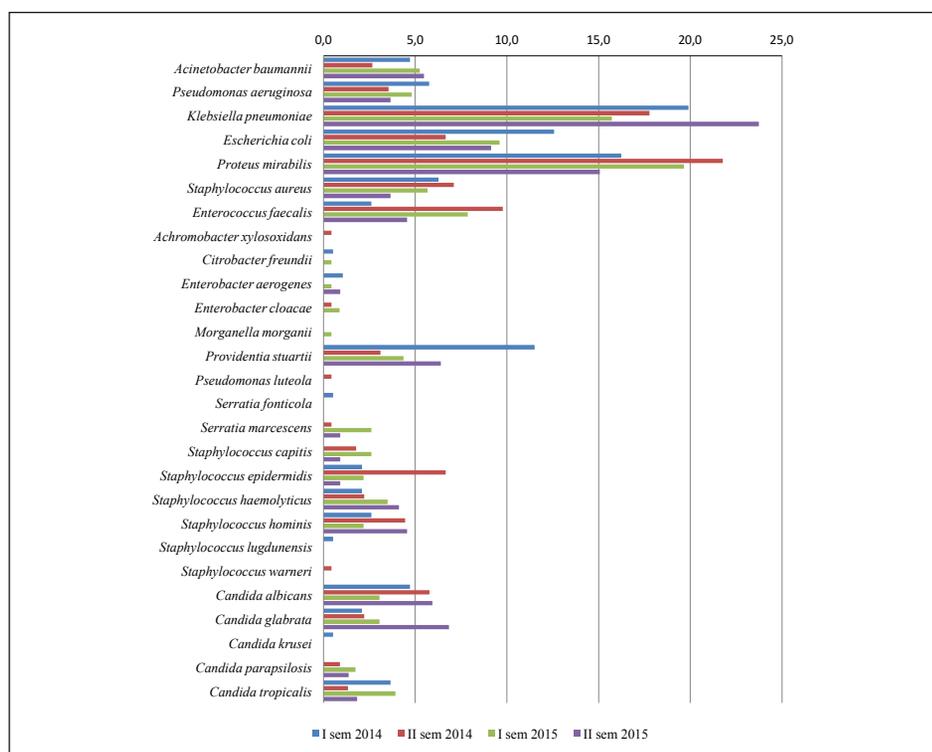
Figure 1 - *Isolation rate of all hospital microbial species. Sem, semester.*

Table 2 - *Isolation density per 1000 patient days (ID) of resistant isolates during the period 2014-2015.*

Isolation density (per 1000 patient-days)	I sem 2014	II sem 2014	I sem 2015	II sem 2015	p-value
A. baumannii	0.6	0.4	0.8	0.8	0.3612
P. aeruginosa	0.8	0.6	0.8	0.5	0.5251
K. pneumoniae	2.7	2.8	2.5	3.5	0.3314
E. coli	1.7	1.0	1.5	1.4	0.7759
P. mirabilis	2.2	3.4	3.1	2.2	0.9881
S. aureus	0.8	1.1	0.9	0.5	0.3955
E. faecalis	0.4	1.5	1.3	0.7	0.8267
A. xylosoxidans	0.0	0.1	0.0	0.0	0.7418
C. freundii	0.1	0.0	0.1	0.0	0.5514
E. aerogenes	0.1	0.0	0.1	0.1	0.8907
E. cloacae	0.0	0.1	0.1	0.0	0.8637
M. morganii	0.0	0.0	0.1	0.0	0.7418
P. stuartii	1.5	0.5	0.7	0.9	0.5575
P. luteola	0.0	0.1	0.0	0.0	0.7418
S. fonticola	0.1	0.0	0.0	0.0	0.2254
S. marcescens	0.0	0.1	0.4	0.1	0.4692
S. capitis	0.0	0.3	0.4	0.1	0.6076
S. epidermidis	0.3	1.0	0.3	0.1	0.6388
S. haemolyticus	0.3	0.3	0.6	0.6	0.0320
S. hominis	0.4	0.7	0.3	0.7	0.5736
S. lugdunensis	0.1	0.0	0.0	0.0	0.2254
S. warneri	0.0	0.1	0.0	0.0	0.7418
C. albicans	0.6	0.9	0.5	0.9	0.7802
C. glabrata	0.3	0.3	0.5	1.0	0.0908
C. krusei	0.1	0.0	0.0	0.0	0.2254
C. parapsilosis	0.0	0.1	0.3	0.2	0.1820
C. tropicalis	0.5	0.2	0.6	0.3	0.8428

Sem=semester.

Table 3 - *Incidence density of resistant isolates per 1000 patient-days (IDRI) assessed on a six-monthly basis.*

Incidence density of resistant isolates (per 1000 patient-days)	Antibiotics	I sem 2014	II sem 2014	I sem 2015	II sem 2015	p-value
A. baumannii	Carbapenems	0.56	0.42	0.84	0.68	0.4431
	Aminoglycosides	0.56	0.35	0.84	0.68	0.4750
	Fluoroquinolones	0.56	0.42	0.84	0.81	0.2538
	Colistin	0.07	0.00	0.00	0.00	0.2254
P. aeruginosa	Extended-spectrum cephalosporins	0.68	0.48	0.58	0.41	0.2101
	Carbapenems	0.70	0.42	0.56	0.41	0.3143
	Aminoglycosides	0.67	0.42	0.52	0.34	0.2069
	Fluoroquinolones	0.56	0.48	0.70	0.47	0.9439
	Piperacillin-tazobactam	0.56	0.48	0.56	0.41	0.3168
	Colistin	0.07	0.00	0.00	0.00	0.2254
K. pneumoniae	Third-generation cephalosporins	2.39	2.29	2.38	3.12	0.2358
	Fourth-generation cephalosporins	2.39	2.15	2.24	3.05	0.3410
	Carbapenems	1.68	1.52	1.54	2.44	0.3222
	Aminoglycosides	1.58	1.11	1.61	2.37	0.2888
	Fluoroquinolones	2.46	2.35	2.31	3.26	0.3184
	Piperacillin-tazobactam	1.47	2.22	2.31	3.32	0.0419
	Fosfomycin	1.61	1.59	1.75	2.92	0.1752
	Trimethoprim/sulfamethoxazole	1.61	1.52	1.61	0.68	0.2257
	Colistin	0.56	0.62	0.49	0.27	0.1555
E. coli	Third-generation cephalosporins	1.12	0.69	1.12	0.81	0.7039
	Fourth-generation cephalosporins	0.84	0.55	1.05	0.81	0.7389
	Carbapenems	0.14	0.14	0.14	0.00	0.2238
	Aminoglycosides	0.46	0.21	0.56	0.14	0.6081
	-lactam/inhibitor	0.84	0.42	0.63	0.68	0.7963
	Piperacillin-tazobactam	0.14	0.14	0.14	0.27	0.2272
	Fosfomycin	0.21	0.07	0.14	0.00	0.6984
	Trimethoprim/sulfamethoxazole	0.91	0.48	0.77	0.88	0.9042
	Fluoroquinolones	1.26	0.83	1.26	0.95	0.6984
P. mirabilis	Third-generation cephalosporins	2.03	2.98	2.80	1.97	0.9042
	Fourth-generation cephalosporins	1.96	2.49	2.31	1.76	0.6907
	Carbapenems	0.49	0.76	0.63	0.27	0.5122
S. aureus	Methicillin	0.70	0.90	0.91	0.54	0.6559
	Rifampicin	0.28	0.90	0.42	0.41	0.9518
	Fluoroquinolones	0.84	0.97	0.91	0.54	0.3470
	Linezolid	0.00	0.00	0.00	0.00	-
	Vancomycin	0.00	0.00	0.00	0.00	-
E. faecalis	Hlr - aminoglycosides resistance	0.21	1.25	0.63	0.47	0.9484
	Vancomycin	0.28	0.35	0.00	0.07	0.2338
	Aminopenicillins	0.28	0.35	0.28	0.07	0.2495
	β-lactam/inhibitor	0.21	0.14	0.07	0.00	0.00004
	Teicoplanin	0.28	0.28	0.00	0.00	0.1027
	Linezolid	0.00	0.00	0.00	0.00	-

Sem=semester.

Table 4 - *Consumption (DDDs per 1000 patient-days), ATC code and trend for each antibiotic consumed over the study period.*

Antibiotic	Atc Code	DDDs per 1000 patients-days					Trend 2014-2015
		1° sem 2014	2° sem 2014	1° sem 2015	2° sem 2015	P-value	
Oxacillin	J01CF04	0.0	9.0	2.4	0.0	0.7997	
Ampicillin/sulbactam	J01CR01	0.0	0.0	0.7	3.7	0.1373	
Amoxicillin / clavulanate	J01CR02	32.0	32.4	28.5	38.2	0.5257	
Piperacillin / tazobactam	J01CR05	1.7	2.7	5.1	6.4	0.0121	
Ceftriaxone	J01DD04	5.4	8.4	9.1	16.3	0.0666	
Cefepime	J01DE01	6.4	6.7	5.8	3.6	0.1431	
Meropenem	J01DH02	85.6	107.7	82.7	95.6	0.9423	
Imipenem/cilastatin	J01DH51	3.4	3.5	1.4	5.6	0.6621	
Ertapenem	J01DH03	3.8	0.0	0.0	0.0	0.2254	
Vancomycin	J01XA01	20.8	27.9	17.1	28.1	0.7341	
Teicoplanin	J01XA02	7.5	9.6	10.2	11.6	0.0225	
Amikacin	J01GB06	6.7	15.6	5.2	7.1	0.7491	
Gentamicin	J01GB03	3.5	9.9	8.2	13.1	0.1260	
PO Ciprofloxacin	J01MA02	5.1	6.0	5.0	14.4	0.2349	
Ciprofloxacin	J01MA02	0.0	0.0	7.3	9.5	0.0629	
Levofloxacin	J01MA12	0.5	0.3	0.3	0.3	0.3459	
PO Levofloxacin	J01MA12	35.4	38.4	15.4	4.4	0.0795	
Rifampicin	J04AB02	5.8	4.2	1.5	0.9	0.0237	
Trimethoprim / sulfamethoxazole	J01EE01	4.0	1.2	3.2	3.5	0.9625	
PO Trimethoprim / sulfamethoxazole	J01EE01	1.3	0.0	0.9	1.3	0.8429	
Colistin	J01XB	32.3	19.4	17.2	34.5	0.9376	
Daptomycin	J01XX09	0.0	1.9	0.0	3.0	0.3798	
Metronidazole	J01XD01	1.9	6.9	0.0	2.3	0.7436	
PO Claritromycin	J01FA	2.0	0.0	5.9	2.8	0.5554	
Tigecycline	J01AA12	8.1	8.0	4.6	4.7	0.1045	
Linezolid	J01XX08	0.0	0.0	0.0	0.01	0.2254	
Liposomal amphotericin B	J02AA01	0.9	0.9	0.3	0.0	0.0533	
PO Fluconazole	J02AC01	2.8	1.4	0.0	9.8	0.4179	
Fluconazole	J02AC01	3.7	23.3	27.1	27.5	0.1410	
Voriconazole	J02AC03	1.6	0.3	2.7	3.8	0.2250	
Caspofungin	J02AX04	0.4	1.2	0.4	0.0	0.4870	
Anidulafungin	J02AX06	0.0	1.5	0.3	0.0	0.7831	
Micafungin	J02AX05	0.0	0.0	2.4	1.4	0.2660	

Sem=semester; PO=per os.

Table 5 - *Pearson correlation between AD and IDRI (time lag corresponding to one semester). In the table, only cases with an r correlation index >0.6 are included.*

Antibiotic class	Incidence density of resistant isolates (IDRI)		Pearson	
	Microorganism	*Antimicrobial*	*r*	*p*
Penicillins	*S. aureus*	Rifampicin	-0.693	NS
	S. aureus	Methicillin	0.996	0.03
	P. aeruginosa	Third-generation cephalosporins	0.873	NS
		Carbapenems	0.643	NS
		Aminoglycosides	0.890	NS
		Fluoroquinolones	0.626	NS
		Piperacillin/tazobactam	0.902	NS
	K. pneumoniae	Third-generation cephalosporins	-0.977	NS
		Fourth-generation cephalosporins	-0.979	NS
		Carbapenems	-0.991	0.04
		Aminoglycosides	-0.868	NS
β-Lactam/Inhibitor		Fluoroquinolones	-0.997	0.02
		Piperacillin/tazobactam	-0.982	NS
		Fosfomycin	-0.975	NS
		Trimethoprim/sulfamethoxazole	1	NS
		Colistin	0.882	NS
	E. coli	Carbapenems	0.993	0.03
		Aminoglycosides	0.714	NS
		Piperacillin/tazobactam	-0.993	0.03
		Fosfomycin	0.918	NS
		Trimethoprim/sulfamethoxazole	-0.627	NS
	P. mirabilis	Third-generation cephalosporins	0.960	NS
		Fourth-generation cephalosporins	0.938	NS
		Carbapenems	0.931	NS
	A. baumannii	Fluoroquinolones	0.681	NS
	P. aeruginosa	Third-generation cephalosporins	-0.610	NS
		Aminoglycosides	-0.639	NS
		Piperacillin/tazobactam	-0.658	NS
	K. pneumoniae	Third-generation cephalosporins	0.982	NS
		Fourth-generation cephalosporins	0.980	NS
		Carbapenems	0.963	NS
		Aminoglycosides	0.993	0.03
Piperacillin / Tazobactam		Fluoroquinolones	0.947	NS
		Piperacillin/tazobactam	0.977	NS
		Fosfomycin	0.984	NS
		Trimethoprim/sulfamethoxazole	-0.929	NS
		Colistin	-0.996	NS
	E. coli	Carbapenems	-0.958	NS
		Amoxicillin/clavulanate	0.839	NS
		Piperacillin/tazobactam	0.958	NS
		Fosfomycin	-0.687	NS
		Trimethoprim/sulfamethoxazole	0.883	NS
	P. mirabilis	Third-generation cephalosporins	-0.993	0.03
		Fourth-generation cephalosporins	-0.999	0.01
		Carbapenems	-1	0.009
Third-Generation Cephalosporins	*A. baumannii*	Carbapenems	0.844	NS
		Aminoglycosides	0.875	NS
		Fluoroquinolones	0.971	NS

NS=not significant.

Antibiotic class	Incidence density of resistant isolates (IDRI)		Pearson	
	Microorganism	Antimicrobial	r	p
Third-Generation Cephalosporins	K. pneumoniae	Third-generation cephalosporins	0.718	NS
		Fourth-generation cephalosporins	0.713	NS
		Carbapenems	0.661	NS
		Aminoglycosides	0.895	NS
		Fluoroquinolones	0.617	NS
		Piperacillin/tazobactam	0.701	NS
		Fosfomycin	0.726	NS
		Colistin	-0.881	NS
	E.coli	Fourth-generation cephalosporins	0.778	NS
		Carbapenems	-0.646	NS
		Amoxicillin/clavulanate	1	0.001
		Piperacillin/tazobactam	0.646	NS
		Trimethoprim/sulfamethoxazole	0.996	0.03
	P. mirabilis	Third-generation cephalosporins	-0.765	NS
		Fourth-generation cephalosporins	-0.808	NS
		Carbapenems	-0.820	NS
	E. faecalis	Hlr-gentamcin	-1	0.005
Carbapenems	P. aeruginosa	Third-generation cephalosporins	0.995	0.03
		Carbapenems	0.966	NS
		Fluoroquinolones	0.961	NS
		Piperacillin/tazobactam	0.986	NS
		Aminoglycosides	0.990	0.04
	K. pneumoniae	Third-generation cephalosporins	-0.678	NS
		Fourth-generation cephalosporins	-0.683	NS
		Carbapenems	-0.734	NS
		Ciprofloxacin	-0.771	NS
		Piperacillin/tazobactam	-0.696	NS
		Fosfomycin	-0.669	NS
		Trimethoprim/sulfamethoxazole	0.802	NS
	E. coli	Third-generation cephalosporins	0.829	NS
		Fourth-generation cephalosporins	0.647	NS
		Carbapenems	0.747	NS
		Aminoglycosides	0.987	NS
		Piperacillin/tazobactam	-0.747	NS
		Fosfomycin	0.979	NS
		Fluoroquinolones	0.829	NS
	P. mirabilis	Third-generation cephalosporins	0.625	NS
Fluoroquinolones	P. aeruginosa	Third-generation cephalosporins	0.916	NS
		Carbapenems	0.714	NS
		Aminoglycosides	0.930	NS
		Fluoroquinolones	0.699	NS
		Piperacillin/tazobactam	0.940	NS
Fluoroquinolones	K. pneumoniae	Third-generation cephalosporins	-0.952	NS
		Fourth-generation cephalosporins	-0.954	NS
		Carbapenems	-0.973	NS
		Aminoglycosides	-0.815	NS
		Fluoroquinolones	-0.985	NS
		Piperacillin/tazobactam	-0.959	NS
		Fosfomycin	-0.948	NS
		Colistin	0.832	NS
		Trimethoprim/sulfamethoxazole	0.992	0.03

| Antibiotic class | Incidence density of resistant isolates (IDRI) | | Pearson | |
	Microorganism	Antimicrobial	r	p
Fluoroquinolones	E. coli	Carbapenems	0.978	NS
		Aminoglycosides	0.779	NS
		Piperacillin/tazobactam	-0.978	NS
		Fosfomycin	0.952	NS
	P. mirabilis	Third-generation cephalosporins	0.929	NS
		Fourth-generation cephalosporins	0.900	NS
		Carbapenems	0.891	NS
	S. aureus	Methicillin	0.982	NS
		Fluoroquinolones	0.942	NS
	E. faecalis	Aminoglycosides	0.898	NS
		Ampicillin/sulbactam	0.741	NS
Aminoglycosides	E. coli	Third-generation cephalosporins	0.997	0.02
		Fourth-generation cephalosporins	0.940	NS
		Aminoglycosides	0.937	NS
		Fosfomycin	0.750	NS
		Fluoroquinolones	0.997	0.02
	A. baumannii	Carbapenems	0.896	NS
		Aminoglycosides	0.867	NS
		Fluoroquinolones	0.708	NS
	P. aeruginosa	Third-generation cephalosporins	0.813	NS
		Carbapenems	0.967	NS
		Aminoglycosides	0.791	NS
		Fluoroquinolones	0.972	NS
		Piperacillin/tazobactam	0.775	NS
Colistin	A. baumannii	Carbapenems	-0.867	NS
		Aminoglycosides	-0.896	NS
		Fluoroquinolones	-0.980	NS
	K. pneumoniae	Third-generation cephalosporins	-0.687	NS
		Fourth-generation cephalosporins	-0.681	NS
		Carbapenems	-0.627	NS
		Aminoglycosides	-0.874	NS
		Piperacillin/tazobactam	-0.669	NS
		Fosfomycin	-0.696	NS
		Colistin	0.860	NS
	E. coli	Third-generation cephalosporins	-0.615	NS
		Fourth-generation cephalosporins	-0.805	NS
		Carbapenems	0.612	NS
		Amoxicillin/clavulanate	-0.999	0.001
		Fluoroquinolones	-0.615	NS
		Piperacillin/tazobactam	-0.612	NS
		Trimethoprim/sulfamethoxazole	-0.991	0.04
	P. mirabilis	Third-generation cephalosporins	0.736	NS
		Fourth-generation cephalosporins	0.782	NS
		Carbapenems	0.794	NS

per 1000 patient-days (p=0.03), and that fluoroquinolone consumption was correlated to trimethoprim/sulfamethoxazole-resistant K. pneumoniae IDRI (p=0.03). β-Lactam/β-lactamase inhibitor combination consumption was correlated to carbapenem-resistant E. coli IDRI (p=0.03). Cephalosporin consumption was correlated to amoxicillin/clavulanate (p=0.001) and trimethoprim/sulfamethoxazole (p=0.03)-resistant E. coli IDRI, as was the use of aminoglycosides correlated to third generation cephalosporins (p=0.02) and ciprofloxacin (p=0.02)-resistant E. coli IDRI, respectively.

Finally, use of carbapenems was correlated to both cephalosporin (p=0.03) and aminoglycoside (p=0.04)-resistant P. aeruginosa IDRI.

DISCUSSION

In recent years, antibiotic resistance and multidrug-resistant (MDR) bacteria have become an emerging worldwide problem compromising the future efficacy of therapeutic tools in the control of bacterial infections, leading to increased additional healthcare costs, prolonged hospitalizations, treatment failures, lost productivity, human suffering and sometimes death (ECDC, 2015b; Sheikh *et al.*, 2015). Although several prevalence studies on HAI and antibiotic use have been conducted (ECDC, 2013; ECDC, 2014), only very few studies have highlighted the relationship between antibiotic use and HAI in rehabilitation units (Rossini *et al.*, 2016; Weiner *et al.*, 2016; Stenzelius *et al.*, 2016). Rossini *et al.* (2016), in their study performed in a rehabilitation hospital, identified some conditions for a greater risk of carbapenemase-producing *Enterobacteriaceae* (CPE) colonization at admission to hospital. The same conditions (risk factors) were present in our patients (see *Table 1*).

In Italy, in the last five years, a significant increase in strains of *K. pneumoniae* and *E. coli* with combined resistance to multiple antibiotic classes has been observed (ECDC, 2015c). Serious infections caused by extended-spectrum beta-lactamases (ESBLs) and fluoroquinolone resistant *Enterobacteriaceae* have led to an increased dependence on carbapenems as therapeutic options. As a consequence, the spread of CPE has been observed. The high prevalence of *K. pneumoniae* and the increased carbapenem-resistant *K. pneumoniae* IDRI we observed reflects the dramatic situation in the Mediterranean area where *Enterobacteriaceae* strains have spread in endemic form (Albiger, *et al.*, 2015). The 2015 EARS-Net surveillance data report found that most European countries showed resistance against carbapenems in *K. pneumoniae* below 1%, with, however, Italy and Romania reporting carbapenem-resistance *K. pneumoniae* rates above 25% and Greece above 50% (ECDC, 2015b). *K. pneumoniae* is an important cause of infection everywhere in patients with an impaired immune system and with indwelling devices (Cascio *et al.*, 2014). Urinary tract infections, respiratory tract infections, and bloodstream infections are frequently encountered, and outbreaks due to CPE *K. pneumoniae* are frequently reported (Campos *et al.*, 2016).

Our results are in line with those of the European survey of carbapenemase-producing Enterobacteriaceae (EuSCAPE) in which the isolates resistant to carbapen-

ems were more common in *K. pneumoniae* than in *E. coli* (Grundmann *et al.*, 2016). In our study, the low carbapenem-resistant *E. coli* IDRI is comforting: in these microorganisms, the possible horizontal carbapenemase gene transfer demonstrated by Di Luca *et al.* (2016) seems not yet to have occurred.

Despite *A. baumannii* being ranked 8th among the microorganisms isolated, it showed an increased IDRI to carbapenems, aminoglycosides and fluoroquinolones in the same way as *K. pneumoniae*. According to Magiorakos *et al.* (2013), high RRs found for *A. baumannii* and *K. pneumoniae* to carbapenems are worrisome, since these are last-line antibiotics. *K. pneumoniae* and *P. aeruginosa* RRs exceed those of the EARS-Net study for all the antibiotic classes considered. On the contrary, RRs of *A. baumannii* were similar (ECDC, 2015b).

In consideration of the lack of data coming from rehabilitative hospitals, we compared our data with those coming from other ICUs in which similar risk factors and comorbidities are present. Even though only 23.5% of our hospital beds correspond to ICU-beds, our results are similar to those of a recent study performed in Sicilian ICUs by Agodi *et al.* (2015); RR% - carbapenem-resistant *K. pneumoniae* 65.2% *vs* 59.2%; carbapenem-resistant *A. baumannii* 91.65 *vs* 96.6%; third generation cephalosporin-resistant *K. pneumoniae* 91.45 *vs* 81.6%.

Figure 2 shows a constant low use of the last line molecules, daptomycin and tigecycline, probably justified by the poor circulation of vancomycin-resistant gram-positive microorganisms in our hospital. However, our data, if compared with those of Fondazione Santa Lucia show similar Methicillin-resistant *Staphylococcus aureus* (MRSA) IDRI values (0.54 *vs* 0.42) but different *E. faecalis* ID values (0.7 *vs* 0.07) (Salvia *et al.*, 2007).

Our results, compared with those of the study by Agodi *et al.* (2015) show lower values of DDD for all classes of antibiotics (penicillins, cephalosporins, carbapenems, fluoroquinolones, aminoglycosides) except for glycopeptides (67 *vs* 29). Comparing our data with those of German ICUs, higher DDD values for carbapenems (185.3 *vs* 151), aminoglycosides (33.6 *vs* 24) and glycopeptides (67 *vs* 36), and lower DDD values for penicillins (85.1 *vs* 286), cephalosporins (34.8 *vs* 117) and quinolones (56.6 *vs* 163) were observed (Meyer, *et al.*, 2010).

Comparing our data with those of a recent study on trends of antibiotic use in nursing homes for the elderly by Tedes-
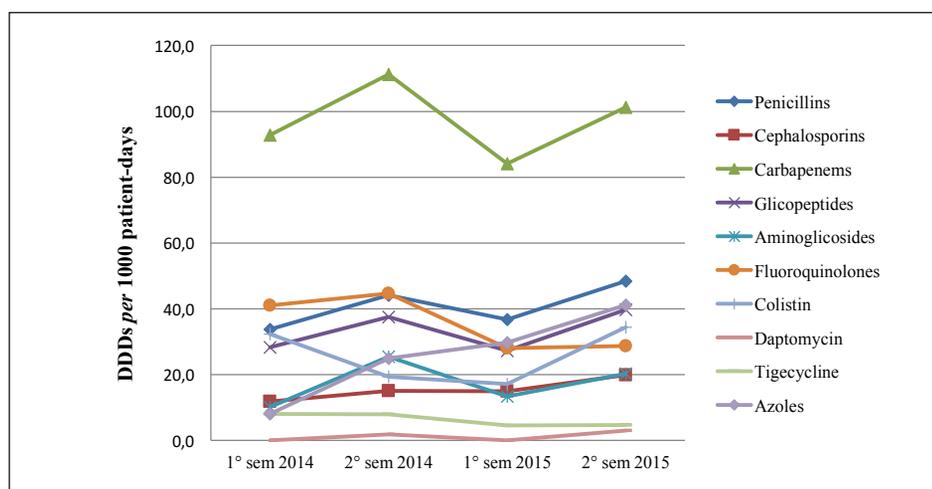

Figure 2 - *Consumption of antibiotic classes (DDDs per 1000 patient-days) over the study period. Sem, semester.*
β= *beta*

co *et al.* (2015), we found higher DDD mean total values (628 *vs* 82.4 DDD), as well as for all antibiotic classes [carbapenems 194.7 *vs* 0.61; penicillins 81.5 *vs* 27.91; cephalosporins 30.9 *vs* 17.8; aminoglycosides 34.7 *vs* 1.38; fluoroquinolones 71.2 *vs* 16.17; glycopeptides 66.4 *vs* 0.47]. The recent study by Grundmann *et al.* (2016), cited above, highlights that physicians depend even more on carbapenems for the treatment of infections caused by MDR microorganisms. Also in our hospital, clinicians have often chosen carbapenems as a treatment option despite the high *K. pneumoniae* resistance rate to this antibiotic class.

Scientific evidence documents that selective pressure, also resulting from non-prudent antibiotic consumption, is the major cause of the increasing emergence of MDR microorganisms. Many studies have evaluated this relationship and different results have been reported (Joseph *et al.,* 2013; Bergman *et al.,* 2009; Chen *et al.,* 2013; McLaughlin *et al.,* 2013).

We have observed that carbapenem-resistant K. *pneumoniae* and *E. coli* IDRIs showed no significant correlation with the consumption of the primary antibiotic during the study period, similar to *P. aeruginosa* and *A. baumannii* isolates. By contrast, the total consumption trend of ampicillin/sulbactam and piperacillin/tazobactam (p=0.01) increased and a statistically significant change was observed only for piperacillin/tazobactam-resistant *K. pneumoniae* IDRI (p=0.0419). As described in the EARS-Net report, *K. pneumoniae,* similar to *E. coli,* can be resistant to multiple antimicrobial classes, and resistance profiles are usually acquired through plasmids (ECDC, 2015b). In addition, carbapenemase-encoding genes are associated with various mobile genetic elements also conferring aminoglycoside and fluoroquinolone resistance. In view of this, horizontal plasmids and transposon transmission promotes the co-selection and spread of MDR microorganisms, by carrying additional resistant determinants to other non-β-lactam antibiotics, rendering these organisms extensively drug-resistant (XDR) or pan drug-resistant (PDR), leaving few available therapeutic options (ECDC, 2011; Magiorakos *et al.,* 2012). Indeed, a shift towards carbapenem consumption was associated with permeability mutations, above all, in bacterial strains producing ESBLs and other β-lactamases (Nordmann *et al.,* 2009).

As observed in France, Germany, Austria, Malta, Slovakia and Serbia (Mladenovic-Antic *et al.,* 2016), our data also show a slight reduction in aminoglycoside-resistant *P. aeruginosa* IDRI.

In accordance with Lepper *et al.* (2002), in our study the use of carbapenems was statistically correlated to cephalosporin-resistant *P. aeruginosa* IDRI (p=0.03). In contrast to the study by Metlay *et al.* (2003), in which the exposure rate to cephalosporins was not significantly different between patients with trimethoprim/sulfamethoxazole-resistant and -susceptible infections, we found that the use of third-generation cephalosporins was statistically correlated to trimethoprim/sulfamethoxazole-resistant *E. coli* (p=0.03). As suggested by Bosso *et al.* (2010), this significant correlation may be explained by the possibility that resistance mechanisms depend on secondary antibiotic consumption.

A previous study by Friedrich *et al.* (1999) found that extensive use of one antibiotic class could influence resistance rates to other antibiotic classes. Even if this is the case, it is likely that the relationship between antimicrobial use and antimicrobial resistance is multifactorial and may differ from one microorganism - antimicrobial agent pair to another (Bosso *et al.,* 2010). Also, our findings could be explained like those of Friedrich *et al.* (1999), but our study was not able to demonstrate a causal relationship.

It is quite understandable that resistance to a given antibiotic is associated with resistance to other molecules of the same class but the concept of cross-class associations is less obvious. In this regard, it is known that efflux pump systems and the permeability of bacterial envelopes can cause resistance to multiple antibiotic classes (Aeschlimann 2003) and that a plasmid can carry out gene coding for resistance to different antimicrobial classes. As discussed by Kaase *et al.* (2016), carbapenemases may confer resistance to most β-lactam antibiotics and, at the same time, can cause resistance against several non-β-lactam antibiotics. Our findings on the use of a primary antimicrobial agent and occurrence of resistance rates to a secondary antimicrobial agent could be understood as one of the above mechanisms.

As is well known, the evolution of β-lactamase is the result of the misuse and overuse of several generations of β-lactam antibiotics over the last 40-50 years. In particular, exposure to third and fourth generation cephalosporins and carbapenems constitutes a risk factor for infection and colonization with CPE (ECDC, 2011).

Our study presents the following limitations: it was conducted in a single 80-bed institution that cannot be considered representative of all Sicilian rehabilitation hospitals. In addition, it was performed and based on laboratory data collection regardless of the patient's clinical data and without evidence of HAI or colonization for each isolate. It was also not possible to establish epidemiological correlations since bacterial molecular characterization was not performed. Moreover, the limited study period does not allow the observation of significant trends related to both antibiotic consumption and resistant microorganism isolation. The short length of the study and probably the time-period selected for evaluating the "time lag effect" do not allow us to assert, but only to presume, an antibiotic selective action. This study, at best, can suggest potential relationships without establishing cause and effect. Further research on associations between IDRI and AD are needed to design action plans that can slow the spread of MDR microorganisms.

The priority of the patient admitted to a rehabilitation unit is to recover functional autonomy and the occurrence of a HAI might affect the rehabilitation pathway. It is often difficult to stop the spread of MDR bacteria, also considering that patients coming from ICUs may often be colonized. A screening program to identify CPE rectal carriers at patient admissions will be performed, as recommended by the Ministerial Circular Letter "Surveillance and control of infections due to carbapenemase-producing bacteria (CPE)." In consideration of strong antibiotic selective pressure and the lack of, or pending, specific microbiological data, an antibiotic prescription should be based on knowledge of the hospital microbial ecosystem and on antibiotic sensitivity profiles of bacterial isolates, and a targeted antibiotic therapy should be advocated in infection cases.

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