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FULL PAPER

Retrospective analysis of nosocomial infections in an Italian tertiary care hospital

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SUMMARY

Nosocomial infections are one of the leading causes of morbidity and mortality in hospitalized patients. Studies of their prevalence in single institutions can reveal trends over time and help to identify risk factors. The aim of this study was to investigate the nosocomial infections trend and identify the prevalence of predominant bacterial microorganisms and their drug resistance patterns in an Italian tertiary care hospital. Infections were classified according to the Centres for Disease Control and Prevention definitions. A retrospective study was carried out from March 2011 to June 2014, based on the bacterial isolate reports of a hospital located in Central Italy. During the 40-month study period, a total of 1547 isolates were obtained from 1046 hospitalized patients and tested for their antibiotic sensitivity. The most common isolates belonged to the *Enterobacteriaceae* family (61.7%), followed by *Enterococcus* species (12.4%), *Pseudomonas* species (10.7%) and *S. aureus* (10.0%). The incidence density rate of nosocomial infections was 7.4 per 1000 patient days, with a significant difference among the 3 annual infection rates (P<0.001). The highest infection prevalence rate was found in Internal Medicine Unit (41.3%), followed by Intensive Care Units (12.4%), Surgical Units (9.0%,) and Cardiology (7.1%).

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INTRODUCTION

Nosocomial infections are one of the leading causes of death among hospitalized patients and remain a major problem in all health care centres across the world (Wenzel, 2007). Many types of microorganisms cause infections in humans. Over the past decades, most pathogenic species have developed resistance to one or more antimicrobials becoming a public health problem. Members of the *Enterobacteriaceae* family are among the most important bacterial human pathogens. *Escherichia coli, Klebsiella spp. and Proteus spp*. account for the majority of *Enterobacteriaceae* isolated from clinical specimens and their antimicrobial resistance is increasing (Eisenstein *et al.*, 2000). Current antimicrobial issues for *Enterobacteriaceae* include the emergence and proliferation of resistances to many antibiotics classes including broad-spectrum penicillins, third-generation cephalosporins, fluoroquinolones, aminoglycosides and carbapenems (Bradford *et al.*, 2001).

Key words:
Healthcare-associated infections, Nosocomial infections, Antibiotic resistance, Multidrug resistance, Prevalence, Susceptibility, Incidence.

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Surveillance data indicate that the *Enterococcus* species are one of the most isolated nosocomial pathogens (12.0% of all hospital infections) (Sievert *et al.*, 2013) and its rise in prevalence is influenced by the ability to resist and escape the action of the most commonly used antibiotics.
Other important bacterial pathogens most frequently responsible for nosocomial infections are *Acinetobacter* species, *Pseudomonas* species and *Staphylococcus aureus* (Livermore *et al.*, 2001, Karageorgopoulos *et al.*, 2008, Mera *et al.*, 2011). *Acinetobacter* causes various nosocomial infections with high mortality, and the infection caused by multidrug-resistant *A. baumannii* is currently one of the most difficult to treat (Maragakis, 2008). We also need to consider *Pseudomonas spp*. resistance patterns because of the strong evidence that inappropriate empiric therapy leads to increased hospital mortality (Iregui *et al.*, 2002), and patients with a resistant infection are less likely to receive appropriate initial treatment (Micek *et al.*, 2005).
S. aureus strains, in particular methicillin-resistant (MRSA) strains associated with hospitals, are one of the most common causes of hospital-acquired infections and their prevalence has increased over the last 10 years. (Durai *et al.*, 2010). MRSA drug resistance continues to evolve. New multiresistant MRSA phenotypes show rifampicin and trimethoprim-sulfamethoxazole resistance. To date, more than 50% of MRSA strains are resistant to drugs such as macrolides, lincosamides, fluoroquinolones

and aminoglycosides (Archer *et al.*, 1998). Among human pathogens, multidrug-resistant strains (MDR) are a major concern because of their therapeutic complications, acquisition of additional resistance and high potential for a large-scale dissemination. (Sievert *et al.*, 2013).
Many therapies are available and mortality has fallen drastically, but the high rate of antibiotic resistance and intrinsic resistance could increase the risk of inappropriate empirical therapy. Intrinsic resistance, the innate ability of a bacterial species to resist the activity of a particular antimicrobial agent due to certain resistance-conferring genes allows the development of tolerance to a particular drug or antimicrobial class.
A deeper understanding of the hospital bacterial population, relative resistances and incidence rates may help to adopt the best possible antibiotic therapy. The aim of this study was to investigate the nosocomial infections and to identify the prevalence of predominant bacterial microorganisms and their drug sensitivity and resistance in an Italian tertiary care hospital.

METHODS

A retrospective study was carried out based on the bacterial isolates reports of a hospital located in Central Italy with 288 beds and a mean of 31,000 inpatient days per semester. All patients admitted from March 2011 to June 2014 (40 months) were included in the study. Isolates collected from infections that occurred 48 hours after admission were identified as nosocomial infections, as defined by the Centres for Disease Control and Prevention (CDC/NHSN, 2013). A total of 1046 patients and 1286 clinical samples were considered for this study. The clinical specimens were urine samples, pulmonary samples, blood samples, surgical samples, cutaneous swabs, urethral/vaginal swabs, catheter tip and throat swabs. Specimens were processed according to good laboratory practice and standard methods for identification. Blood cultures were collected using Bactec aerobic and anaerobic blood culture bottles (BD Blood Culture System, Becton, Dickinson and Company) and incubated at 37°C in BD BACTEC™ 9120. Blood samples were obtained from 2 separate sites within minutes of each other from patients who were acutely ill or those in whom the likelihood of continuous bacteraemia was high. By contrast, in patients suspected to have intermittent bacteraemia, multiple blood cultures 6-36 hours apart were obtained. Positive bottles were subcultured on blood, chocolate and MacConkey agar. All antimicrobial susceptibility tests were performed with VITEK®2 (BioMérieux, Marcy l'Etoile, France). Resistance to glycopeptides or others drugs were not confirmed by tests different from VITEK2 because confirmation tests are not routinely performed in our laboratory.
The 1547 isolates were grouped and organized in 7 major pathogen classes: *Acinetobacter* spp., *E. coli*, *Enterococcus* spp., *Klebsiella* spp., *Pseudomonas* spp., *S. aureus*, *Streptococcus* spp. and other bacteria belonging to the *Enterobacteriaceae* family. The most representative species in this family were *Proteus spp*., *Enterobacter spp*. and *Citrobacter spp*. An isolate was considered resistant to an antimicrobial agent when tested and interpreted as resistant (R) in accordance with the EUCAST breakpoints criteria adopted by our laboratory. An isolate was considered non-susceptible to an antimicrobial agent when tested and found resistant (R) or with intermediate susceptibility (I) using the same clinical breakpoints as interpretive criteria. Multidrug resistance was defined as being resistant to 3 or more antimicrobial classes among piperacillin (±tazobactam), ceftazidime, fluoroquinolones, aminoglycosides and carbapenems (ECDC, 2012).
Duplicate data were discarded using the Bio-Mérieux VIGIguard™ software if all the following conditions were true: isolate collected from the same patient, same specimen, same ward, same species and similar antibiotic pattern (S/R=1; I/R–S/I=2) within 20 days. After duplicate elimination, data were checked with Microsoft® Excel and verified. Tables for sensitivity patterns, and antimicrobial susceptibility graphics were built with Microsoft® Excel. For selected analyses, a 95% confidence interval was determined for percentages by applying an exact confidence interval for binomial data. The number of inpatient-days and the sum of each daily inpatient census for each semester were obtained from hospital administrative records.

RESULTS

During the 40-month study period 1286 clinical samples were collected from 1046 patients. The gender distribution was 37.1% male and 62.9% female. The mean age was 74.6±18.1 years. The highest infection prevalence was found in Internal Medicine Unit (41.3%, 95% CI 38.8 to 43.8), followed by Intensive Care Units (12.4%, 95% CI 10.7 to 14.1), Surgical Units (9.0%, 95% CI 7.4 to 10.4) and Cardiology (7.1%, 95% CI 5.8 to 8.5).
Overall, the most common isolates belonged to the *Enterobacteriaceae* family (500 *E.coli* 32.3%, 296 other *Enterobacteriaceae* 19.1%, 158 *Klebsiella spp* 10.2%), followed by *Enterococcus* species (193, 12.4%), *Pseudomonas* species (166, 10.7%) and *S. aureus* (155, 10%). While *Acinetobacter* (52, 3.4%) and *Streptococcus* species (27, 1.8%) were the least isolated microorganisms. The microorganisms isolated from the 8 different clinical specimen were 1008 in urine samples (65.5%), 208 in pulmonary samples (12.9%), 95 in blood samples (6.2%), 93 in surgical samples (6.1%), 74 in cutaneous swabs (4.8%), 40 in urethral/vaginal swabs (2.6%), 26 in catheter tip (1.7%) and 3 in throat swabs (0.2%).
E.coli was the most common isolated bacteria from urine samples (39.4%) and in surgical samples (48.4%). Other common isolations in urine were other *Enterobacteriaceae* (22.3%), *Enterococcus spp*. (15.9%) and *Klebsiella spp*. (10.0%). Among pulmonary specimens *Pseudomonas spp*. (31.7%), *S. aureus* (16.8%) and *Acinetobacter spp*. (12.5%) had the highest isolation rate. In blood samples 36.8% of all cultures resulted positive to *S. aureus* followed by *E.coli* (15.8%) and *Klebsiella spp.* (14.7%). *S. aureus* was also highly prevalent in catheter tip samples and cutaneous swabs with 65.4% and 28.4% respectively of all isolations by sample. Isolates from urethral and vaginal swabs reported 45.0% on *E.coli*, 15.0% on *Streptococcus spp.* and 10.0% on *Streptococcus spp*.
The incidence density rate of nosocomial infections was 7.4 per 1000 patient days, with a significant difference among the 3 annual infection rates (P<0.001). Rates have increased from 6.1 in 2011 to over 10 per 1000 patient-days to date. *E. coli* had the higher incidence with 3.41 infections in 2014. In general, during the 40-month study period all the analyzed microorganisms had a positive rise. The *Enterobacteriaceae* family (*E. coli*, *Klebsiella* spp. and others) rose from 4 to 6.29 infections, *Pseudomo-*

nas spp. from 0.67 to 0.93, *S.aureus* from 0.73 to 0.83. The first semester of 2011 reported a lack of *Acinetobacter spp* infections, while in 2014 this rate reached 0.20 infections. Lastly *Enterococcus spp*. increased from 0.67 to 1.32 and *Streptococcus spp*. from 0.06 to 0.20 (*Table 1*).
The antibiotic sensitivity patterns are listed in *Table 2*. Data were expressed as a sensitivity percentage (S), that is the percentage of sensitive isolates out of all isolates with antimicrobial susceptibility testing information on that specific organism-antimicrobial agent combination (T). If these were fewer than 50.0% of all isolates (n), data for that specific organism-antimicrobial agent combination were not displayed. Several microorganisms showed 100% antibiotic resistance. *Acinetobacter* species were fully resistant to ertapenem and fosfomycin, *Enterococcus* species to clindamycin and *Pseudomonas* species to ampicillin, ertapenem and nitrofurantoin.
The most effective drug against *Acinetobacter spp* was Colistin with 91.4% of sensitivity. Colistin was also very effective to *E. coli* (98.2%) and *Pseudomonas* spp. (94.2%). Tigecycline was the drug with the highest efficacy among all tested drugs, results showed 4 bacteria families (*Enterococcus spp., E. coli, Klebsiella* spp. and *S. aureus*) up to 90.0% of sensitivity. Ertapenem, imipenem and meropenem were the most effective to *E.coli* isolates with percentages ranging from 99.6% to 99.8%.
Data on antimicrobial resistance patterns are shown in *Figure 1* and expressed as the percentage of non-suscep-

Table 1 - *Incidence density adjusted for total inpatioent-days every 6 months.*

Microorganism	*2011*		*2112*		*2013*		*2014*	*Trend 2011-2014*
	s1	*s2*	*s1*	*s2*	*s1*	*s2*	*s1*	
E.coli	2,73	2,57	1,46	2,51	2,13	2,41	3,41	3,40 2,40 1,40
Enterobacteriaceae	1,09	1,07	1,11	1,71	1,28	1,31	2,12	2,40 1,70 1,00
Pseudomonas spp.	0,67	0,64	0,77	0,98	0,67	0,95	0,93	2,40 1,70 1,00
Klebsiella spp.	0,18	0,86	0,43	0,95	0,73	1,13	0,76	1,10 0,60 0,10
Enterococcus spp.	0,67	0,89	0,97	0,92	0,91	0,66	1,32	1,50 1,00 0,50
S. aureus	0,73	0,86	0,49	0,73	0,85	0,66	0,83	0,85 0,65 0,45
Acinetobacter spp.	0,00	0,21	0,06	0,22	0,12	0,47	0,56	0,60 0,30 0,00
Streptococcus spp.	0,06	0,06	0,06	0,13	0,21	0,18	0,20	0,25 0,15 0,05
Total	6,12	7,15	5,34	8,16	6,90	7,78	10,13	9,30 7,30 5,30

	E. coli	*Enterobacteriaceae*	*Pseudomonas spp.*	*Klebsiella spp.*	*Enterococcus spp.*	*S. aureus*	*Acinetobacter spp.*	*Streptococcus spp.*
s1 2011	2,73	1,09	0,67	0,18	0,67	0,73	0,00	0,06
s2 2011	2,57	1,07	0,64	0,86	0,89	0,86	0,21	0,06
s1 2012	1,46	1,11	0,77	0,43	0,97	0,49	0,06	0,06
s2 2012	2,51	1,71	0,98	0,95	0,92	0,73	0,22	0,13
s1 2013	2,13	1,28	0,67	0,73	0,91	0,85	0,12	0,21
s2 2013	2,41	1,31	0,95	1,13	0,66	0,66	0,47	0,18
s1 2014	3,41	2,12	0,93	0,76	1,32	0,83	0,56	0,20

Table 2 - *Antimicrobial sensitivity by microorganism. *Isolates tested for that antimicrobial agent. **Isolates sensitive for that antimicrobial agent.*

Antibiotic	*Mcroorganism*															
	Acinetobacter spp. (n=52)		*Other Enterobacteriaceae (n=296)*		*Enterococcus spp. (n=193)*		*E. coli (n=500)*		*Klebsiella spp. (n=158)*		*Pseudomonas spp. (n=166)*		*S. aureus (n=155)*		*Streptococcus spp. (n=27)*	
	T (%)*	*S** % (95% CI)*	*T (%)*	*S % (95% CI)*	*T (%)*	*S % (95% CI)*	*T (%)*	*S % (95% CI)*	*T (%)*	*S % (95% CI)*	*T (%)*	*S % (95% CI)*	*T (%)*	*S % (95% CI)*	*T (%)*	*S % (95% CI)*
AMC	52	1	218	114	_	_	496	369	158	85	158	4	_	_	_	_
Amox+ac.clavul.	100,0%	1,9 (0-5,7)	73,6%	52,3 (45,7-58,9)			99,2%	74,4 (70,6-78,2)	100,0%	53,8 (46,0-61,6)	95,2%	2,5 (0,1-5,0)				
AM	_	_	_	_	190	165	343	120	_	_	100	0	_	_	_	_
Ampicillin					98,4%	86,8 (82,0-91,6)	68,6%	35,0 (29,9-40,0)			60,2%	0,0%				
AN	_	_	296	279	_	_	496	471	158	101	159	122	_	_	_	_
Amikacin			100,0%	94,3 (91,6-96,9)			99,2%	95,0 (93,0-96,9)	100,0%	63,9 (56,4-71,4)	95,8%	76,7 (70,2-83,3)				
CIP	52	5	296	184	_	_	496	329	159	85	159	92	_	_	_	_
Ciprofloxacin	100,0%	9,6 (1,6-17,6)	100,0%	62,2 (56,6-67,7)			99,2%	66,3 (62,2-70,5)	100,6%	53,5 (45,7-61,2)	95,8%	57,9 (50,2-65,5)				
CLI	_	_	_	_	193	0	_	_	_	_	_	_	153	83	25	19
Clindamycin					100,0%	0,0%							98,7%	54,2 (46,4-62,1)	92,6%	76,0 (59,3-92,7)
CS	48	44	240	83	_	_	387	380	138	87	138	130	_	_	_	_
Colistin	92,3%	91,7 (83,8-99,5)	81,1%	34,6 (27,5-39,2)			77,4%	98,2 (96,9-99,5)	87,3%	63,0 (55,0-71,1)	83,1%	94,2 (90,3-98,1)				
CTX	48	1	271	173	_	_	479	381	148	73	143	3	_	_	_	_
Cefotaxime	92,3%	2,1 (0-6,1)	91,6%	63,8 (58,1-69,6)			95,8%	79,5 (75,9-83,2)	93,7%	49,3 (41,3-57,4)	86,1%	2,1 (0-4,4)				
E	_	_	_	_	193	186	_	_	_	_	_	_	154	87	_	_
Erythromycin					100,0%	96,4 (93,7-99,0)							99,4%	56,5 (48,7-64,3)		
ETP	51	0	280	272	_	_	487	485	129	104	143	0	_	_	_	_
Ertapenem	98,1%	0,0%	94,6%	97,1 (95,2-99,1)			97,4%	99,6 (99,0 -100)	81,6%	80,6 (73,8-87,4)	86,1%	0,0%				
FA	_	_	_	_	_	_	_	_	_	_	_	_	149	135	_	_
Fusidic Acid													96,1%	90,6 (85,9-95,3)		
FAM	_	_	_	_	189	172	_	_	_	_	_	_	_	_	_	_
Ampicillin-sulb.					97,9%	91,0 (91,9-98,2)										
FEP	47	1	296	235	_	_	496	418	158	83	159	122	_	_	_	_
Cefepime	90,4%	2,1 (0-6,3)	100,0%	79,4 (74,8-84,0)			99,2%	84,3 (81,1-87,5)	100,0%	52,5 (44,7-60,3)	95,8%	76,7 (70,2-83,3)				
FOS	39	0	198	91	_	_	283	277	108	46	_	_	_	_	_	_
Fosfomycin	75,0%	0,0%	66,9%	46,0 (39,0-52,9)			56,6%	97,9 (96,2-99,6)	68,4%	42,6 (33,3-51,9)						
FTN	_	_	_	_	180	173	280	274	_	_	79	0	_	_	15	15
Nitrofurantoin					93,3%	96,1 (93,3-98,9)	56,0%	97,9 (96,2-99,6)			47,6%	0,0%			55,6%	100,0%
GEN	_	_	_	_	189	90	_	_	_	_	_	_	_	_	_	_
Gentamicin HR					97,9%	47,6 (40,5-54,7)										
GM	48	5	273	172	_	_	479	421	148	88	147	106	148	103	_	_
Gentamicin	92,3%	10,4 (1,8-19,1)	92,2%	63,0 (57,3-68,7)			95,8%	87,9 (85,0-90,8)	93,7%	59,5 (51,5-67,4)	88,6%	72,1 (64,9-79,4)	95,5%	69,6 (62,2-77,0)		
IPM	52	4	_	_	189	172	495	494	158	106	159	120	_	_	_	_
Imipenem	100,0%	7,7 (0,4-14,9)			97,9%	91,0 (86,9-95,1)	99,0%	99,8 (99,4-100)	100,0%	67,1 (59,8-74,4)	95,8%	75,5 (68,8-82,2)				
LEV	_	_	_	_	192	22	_	_	_	_	_	_	153	83	24	21
Levofloxacin					99,5%	11,5 (7,0-16,0)							98,7%	54,2 (46,4-62,1)	88,9%	87,5 (74,3-100,7)

Antibiotic	*Mcroorganism*															
	Acinetobacter spp. (n=52)		*Other Enterobacteriaceae (n=296)*		*Enterococcus spp. (n=193)*		*E. coli (n=500)*		*Klebsiella spp. (n=158)*		*Pseudomonas spp. (n=166)*		*S. aureus (n=155)*		*Streptococcus spp. (n=27)*	
	T (%)*	*S** % (95% CI)*	*T (%)*	*S % (95% CI)*	*T (%)*	*S % (95% CI)*	*T (%)*	*S % (95% CI)*	*T (%)*	*S % (95% CI)*	*T (%)*	*S % (95% CI)*	*T (%)*	*S % (95% CI)*	*T (%)*	*S % (95% CI)*
MEM	_	_	295	286	_	_	490	489	158	106	158	121	_	_	_	_
Meropenem			99,7%	96,9 (95,0-98,9)			98,0%	99,8 (99,4-100)	100,0%	67,1 (59,8-74,4)	95,2%	76,6 (70,0-83,2)				
MXF	_	_	_	_	179	14	_	_	_	_	_	_	134	74	19	18
Moxifloxacin					92,7%	7,8 (3,9-11,8)							86,5%	55,2 (46,8-63,6)	70,4%	94,7 (84,7-100)
OXA	_	_	_	_	_	_	_	_	_	_	_	_	149	73	_	_
Oxacillin													96,1%	49,0 (41,0-57,0)		
OXS	_	_	_	_	_	_	_	_	_	_	_	_	149	73	_	_
Cefoxitin													96,1%	49,0 (41,0-57,0)		
PEN	_	_	_	_	_	_	_	_	_	_	_	_	148	21	19	17
Penicillin G													95,5%	14,2 (8,6-19,8)	70,4%	89,5 (75,7-100)
RC	_	_	_	_	193	193	_	_	_	_	_	_	_	_	_	_
Cefuroxime-Sodium					100,0%	100,0%										
RIF	_	_	_	_	_	_	_	_	_	_	_	_	69	28	_	_
Rifampicin													44,5%	40,6 (29,0-52,2)		
SRH	_	_	_	_	188	123	_	_	_	_	_	_	_	_	_	_
Streptomycin HR					97,4%	65,4 (58,6-72,2)										
SXT	52	17	296	196	106	1	496	325	158	92	160	9	150	133	22	20
Trimeth-sulfa.	100,0%	32,7 (19,9-45,4)	100,0%	66,2 (60,8-71,6)	54,9%	0,9 (0-2,8)	99,2%	65,5 (61,3-69,7)	100,0%	58,2 (50,5-65,9)	96,4%	5,6 (2,1-9,2)	96,8%	88,7 (83,6-93,7)	81,5%	90,9 (78,9-100)
TAZ	47	1	296	199	_	_	496	409	158	79	159	117	_	_	_	_
Ceftazidime	90,4%	2,1 (0-6,3)	100,0%	67,2 (61,9-72,6)			99,2%	82,5 (79,1-85,8)	100,0%	50,0 (42,2-57,8)	95,8%	73,6 (66,7-80,4)				
TEC	_	_	_	_	193	192	_	_	_	_	_	_	155	142	16	16
Teicoplanin					100,0%	99,5 (98,5-100)							100,0%	91,6 (87,2-96,0)	59,3%	100,0%
TET	_	_	_	_	_	_	_	_	_	_	_	_	154	120	26	9
Tetracycline													99,4%	77,9 (71,4-84,5)	96,3%	34,6 (16,3-52,9)
TGC	_	_	200	67	190	190	364	364	92	83	_	_	153	153	15	15
Tigecycline			67,6%	33,5 (27,0-40,0)	98,4%	100,0%	72,8%	100,0%	58,2%	90,2 (84,1-96,3)			98,7%	100,0%	55,6%	100,0%
TOB	_	_	_	_	_	_	_	_	_	_	_	_	127	74	_	_
Tobramycin													81,9%	58,3 (49,7-66,8)		
TZP	_	_	294	246	_	_	226	199	91	39	153	86	_	_	_	_
Pip-Tazobactam			99,3%	83,7 (79,4-87,9)			45,2%	88,1 (83,8-92,3)	57,6%	42,9 (32,7-53,0)	92,2%	56,2 (48,3-64,1)				
VA	_	_	_	_	193	188	_	_	_	_	_	_	155	144	24	24
Vancomycin					100,0%	97,4 (95,2-99,7)							100,0%	92,9 (88,9-96,9)	88,9%	100,0%

tible (I+R) isolates to at least one antimicrobial agent of the antimicrobial class (cephalosporins, fluoroquinolones, aminoglycosides and carbapenems) out of all the strains tested for that precise combination bacterial species-antimicrobial class. The graph shows the highest resistance of *Acinetobacter spp*. to all the 4 considered antimicrobial families. Tests reported that 98.1% of isolates were resistant to 3rd generation cephalosporins, 90.4% to fluoroquinolones, 86.6% to aminoglycosides and all the species were resistant to the carbapenems family. *Acinetobacter spp*. overcame the multidrug resistant section with 78.8% of resistance to fluoroquinolones, third-generation cephalosporins and aminoglycosides combined together. *Klebsiella* spp. was the second most resistant bacteria to antimicrobials: 51.6% of all isolates were resistant to 3rd generation cephalosporins, 46.5% to fluoroquinolones, 44.0% to aminoglycosides and 34.0% to carbapenems.

Less than half of the *Klebsiella spp*. isolates were multiresistant compared to *Acinetobacter spp. Pseudomonas* spp., and the other Enterobacteriaceae were the third and the fourth most resistant bacteria with percentages of resistance slightly lower than *Klebsiella*. The other *Enterobacteriaceae* along with *E.coli* alone had the lower resistance

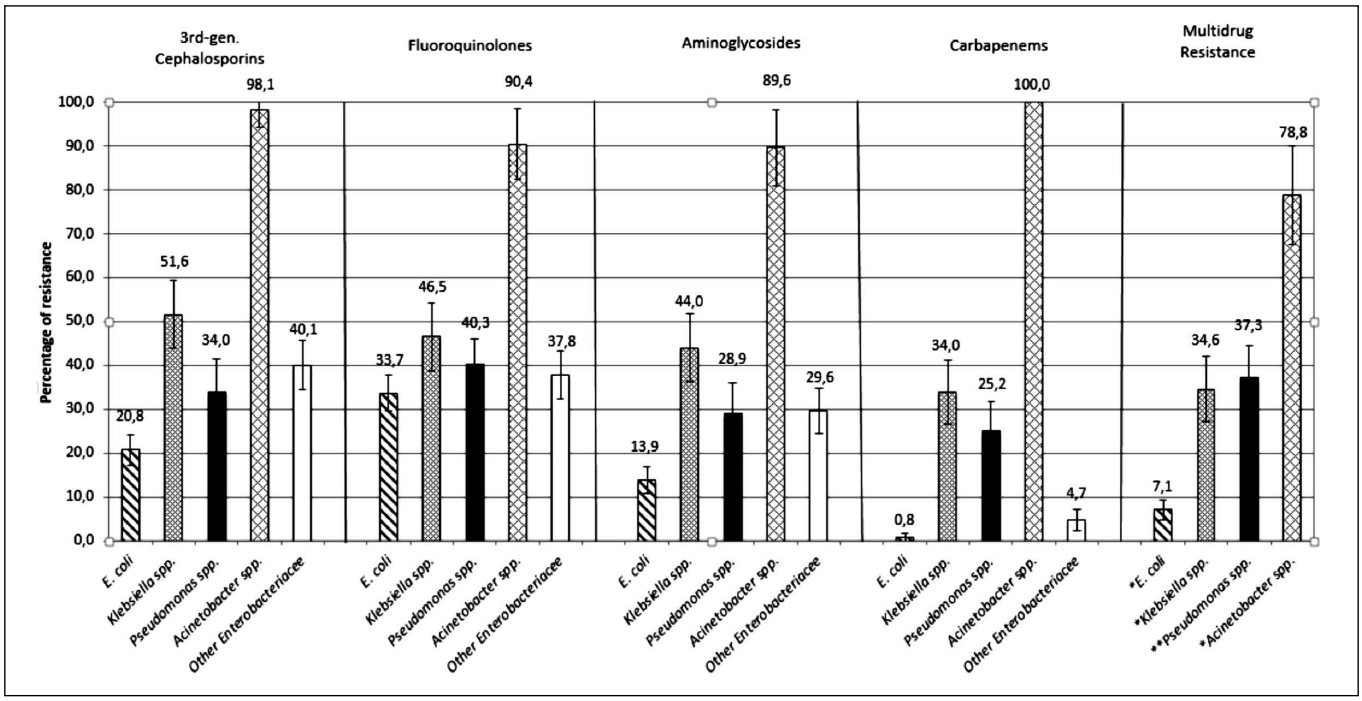

Figure 1 - *Antimicrobial resistance by microorganism.*

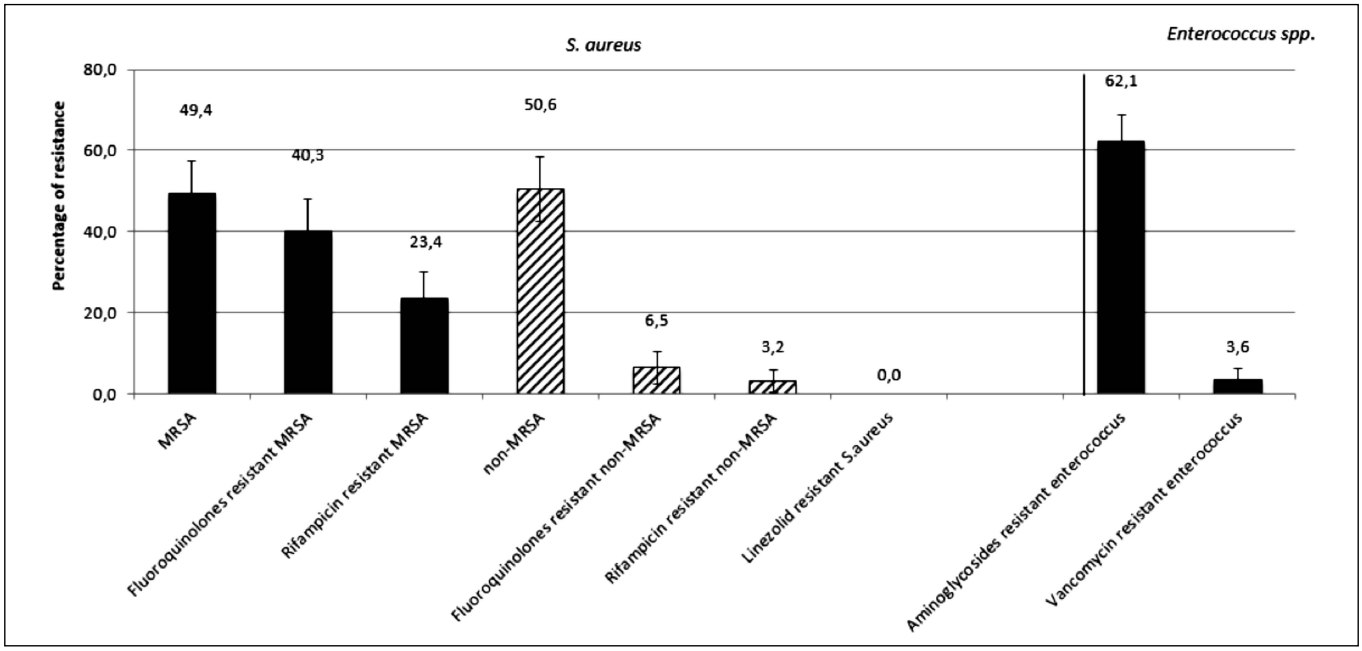

Figure 2 - *S. aureus and Enterococcus spp. resistances.*

rates to carbapenems with 4.7% and 0.8%. *E. coli* resistance rates were the lowest among all the isolates.
In depth analysis of antimicrobial susceptibility patterns of S. aureus and *Enterococcus spp*. are shown in *Figure 2*: 49.4% of all *S. aureus* isolations were methicillin resistant. Among these, 40.3% were resistant to fluoroquinolones and 23.4% to rifampicin. The non-MRSA isolates had a lower resistance to both fluoroquinolones (6.5%) and rifampicin (3.2%). No isolates were resistant to linezolid. Regarding *Enterococcus* spp., 62.1% were resistant to the aminoglycosides and only 3.6% to vancomycin.

DISCUSSION

Several surveillance studies on antimicrobial resistance have been performed in recent years. Many of them have exclusively examined nosocomial isolates, others have focused on community isolates while others have considered combined or single clinical sources of isolation. The resistance profiles that emerged in the present study were, in most of cases, consistent with the Italian data described in the 2012 European Centre for Disease Prevention and Control antimicrobial resistance surveillance report (ECDC, 2012). A comparison with EARS-Net data may not be appropriate, since the report is exclusively based on invasive isolates collected from blood or cerebrospinal fluid, therefore matching results could not be representative for isolates of the same bacterial species from different specimens. Comparing findings of the present study with those described in the EARS-Net report, some discrepancies emerged: a lower resistance level to cephalosporins, fluoroquilonones and aminoglycosides and a higher multidrug resistance in *E. coli* isolates, than those reported by EARS-Net data. Moreover, *S. aureus* isolates were more resistant to rifampicin and methicillin, while *Pseudomonas spp*. isolates had a higher resistance to fluoroquinolones and a higher rate of multiresistance. Cavallo *et al*. 's analysis carried out in 2001 reported similar rates of fluoroquinolone-resistant *Pseudomonas spp* (Cavallo *et al.*, 2001). A study performed in an Indian Intensive Care Unit (Rath *et al.*, 2014) showed higher resistance rates among *E. coli* isolates: 75.5% were resistant to 3rd generation cephalosporins, 71.0% to fluoroquinolones and aminoglycosides, and 72.2% to carbapenems. Lower resistance rates in *Klebsiella* spp. for cephalosporins (17.2%), fluoroquinolones (12.8%), aminoglycosides (9.2%) and carbapenems (4.3%) had been shown in a study performed in United States (Sanchez *et al.*, 2013). In contrast with our data, a study carried out in Spain showed a peak in 2006 of rifampicin-resistant MRSA (45.0%), more than twice our rates, with fewer MRSA isolates (30.5%) (Mick *et al.*, 2010).
A much higher resistance to macrolides, lincosamides, fluoroquinolones, and aminoglycosides, more than 50%, had been reported in a study in 2005 by Owens *et al*. (Owens *et al.*, 2005). A multicenter epidemiological study showed that 28.0% of enterococci isolated from 25 North American Intensive Care Units were resistant to vancomycin (Zirakzadeh *et al.*, 2006). An Italian multicentre study reported 9.0% of *E. faecium* isolates resistant to vancomycin (Fontana *et al.*, 1998); our vancomicyn-resistant enterococcus rate falls well below this result. Similar rates of resistance in *Acinetobacter* spp. were reported from a hospital-based study in China except for 3rd generation cephalosporins (91.0%) and carbapenems (85.0%) (Takagi *et al.*, 2009).
Some bacteria reported complete resistance to certain antibiotic families. The ability to resist a particular antimicrobial agent is widely discussed in previous publications and our data are in agreement with them. Ertapenem has limited in vitro activity against *Pseudomonas* spp. and *Acinetobacter* spp. (Livermore *et al.*, 2002). Other studies show a very high resistance among *Pseudomonas* spp. to ampicillin, nitrofurantoin, amoxicillin and trimethoprim/sulphamethoxazole (Khan *et al.*, 2008, Loureiro *et al.*, 2002). Trimethoprim/sulphamethoxazole resistance is also common in *Enterococcus spp*. (Gordon *et al.*, 2003, Hoban *et al.*, 2001), which shows high rates of resistance to clindamycin (Duh *et al.*, 2001). Finally, fosfomycin alone showed no significant antibacterial effects on *Acinetobacter baumannii* (Zhang *et al.*, 2013).
The most commonly observed nosocomial infections were similar to observations of a United States study, with a rate of 31.0% for UTIs, followed by 27.0% for pneumonia (Richards *et al.*, 2000), although our UTI rate was almost double. The urinary tract was the most common site of nosocomial infection also in a hospital-based survey carried out in the UK, in the Republic of Ireland (Emmerson *et al.*, 1996) and in a multicentre prevalence survey of nosocomial infections in Greek hospitals (Gikas *et al.*, 2002). A decreasing prevalence of UTIs over the years has already been noted, but our data include a high proportion of patients well over 60 years. Studies on elderly patients reported a higher prevalence of UTIs (Reilly *et al.*, 2008, Pellizzer *et al.*, 2008) than others with a relatively young group of patients.
A multicentre study of the ECDC classifies surgical infections as the second to fourth most common nosocomial infection in agreement with our results (Zarb *et al.*, 2012). In other surveys, surgical infections were the most frequent (Ilić *et al.*, 2009, Fitzpatrick *et al.*, 2008), but our centre is not a surgery-focused hospital.
The isolated microorganisms patterns are similar to those of other industrialized nations studies, where *E. coli*, *Pseudomonas* spp, *Enterococcus* spp, and *Staphylococcus* spp, were predominant (Gikas *et al.*, 2002, Pellizer *et al.*, 2008). Another survey reported that the most common isolated bacteria were *Enterobacter spp*., followed by *E. coli, Pseudomonas spp.* and *Staphylococcus spp* (Assar *et al.*, 2012). By contrast, other studies reported *Acinetobacter spp*. and *S. aureus* as the most frequent pathogens, but these results are related to the high rates of respiratory tract and surgical infections, respectively (Pradhan *et al.*, 2014, Scherbaum *et al.*, 2014). In our study, UTIs represented the most frequent infection and this may be the cause of the high *Enterobacteriacee* isolation rate (Linhares *et al*., 2013).
The incidence density rate of nosocomial infections is increasing. The estimated incidence rate in the United States was 9.3 infections per 1000 patient-days and a recent European multicentre study showed an average of 17 episodes per 1000 patient-days (WHO, 2014). Compared to our average rate, these results are higher but infection rates have increased to date. A study performed in an acute Rehabilitation Unit showed a rate of infection of 6 episodes per 1000 patient-days (Mylotte *et al.*, 2000) and in a Pediatric Intensive Care Unit, Simon *et al*. (2000) reported a rate of 14.5 infections per 1000 inpatient-days (Simon *et al.*, 2000). These differences are due to variations in definitions of infection, the characteristics of the patients studied, or different wards considered.
There are several limitations to this study. The 40 month study period is relatively short. Matching results with the

current literature was quite difficult due to the differences in infection control measures among hospitals, different wards and different specimens evaluated. Our results may not be generalizable to hospitals in other geographic areas. In addition, there are very few multi-ward retrospective record-based infection studies in Italy.

We included patients from the Internal Medicine ward and Intensive Care Units with severe medical disabilities, conditions that might interfere with the ability to collect urine properly. Therefore, the prevalence of UTIs has probably been overestimated. The relatively old age of the sample (mean age =74.6 years) may also play a part.

We excluded *Streptococcus* species, due to their not representative isolation rate. Although we considered only infections that occurred 48h after hospital admission, it is possible that patients were misclassified as hospital-acquired, given the lack of available documentation in the administrative and clinical records. Data like risk factors, diseases at admission, use of anti-peptic ulcer drugs, antibiotic empirical treatments and data on clinical outcomes were not available. Patients who showed infections soon after discharge and carriers of nosocomial infections were not included in this study.

To avoid an even more marked rise in resistance, an active surveillance program combined with proper hand hygiene, environmental cleaning, contact precautions, and antimicrobial stewardship should be improved.

References

Archer G.L. (1998). Staphylococcus aureus: a well-armed pathogen. *Clinical Infectious Diseases : An Official Publication of the Infectious Diseases Society of America*, **26**, 1179-1181.

Assar S., Akhoundzadeh R., Aleali A.M., Latifi S.M., Salemzadeh M. (2012). Survey of nosocomial infections and causative bacteria: A hospital-based study. *Pak J Med Sci.* **28**, 455-458.

Bradford P.A. (2001). Extended-spectrum b-lactamases in the 21st century: Characterization, epidemiology, and detection of this important resistance threat. *Clinical Microbiology Reviews*.

Cavallo J.D., Leblanc F., Fabre R., Fourticq-Esqueöute A. (2001). Survey of the antibiotic sensitivity of Pseudomonas aeruginosa in France and the distribution of beta-lactam resistance mechanisms: the GERPB 1999 study. *Pathologie-Biologie*. **49**, 534-539.

CDC/NHSN Surveillance. (2013). Definition of Healthcare-Associated Infection and Criteria for Specific Types of Infections in the Acute Care Setting;. *Available from: http://www.cdc.gov/nhsn/pdfs/pscmanual/17pscnosinfdef_current.pdf*.

Duh R.W., Singh K.V, Malathum K., Murray B.E. (2001). In vitro activity of 19 antimicrobial agents against enterococci from healthy subjects and hospitalized patients and use of an ace gene probe from Enterococcus faecalis for species identification. *Microbial Drug Resistance (Larchmont, N.Y.)*. **7**, 39-46.

Durai R., Ng P.C.H., Hoque H. (2010). Methicillin-resistant Staphylococcus aureus: an update. *AORN Journal*. **91**, 599-606; quiz 607-609.

ECDC. European Centre for Disease Prevention and Control. (2012). Antimicrobial resistance surveillance in Europe 2012. *Available from: http://ecdc.europa.eu/en/publications/Publications/antimicrobial-resistance-surveillance-europe-2012.pdf*

Eisenstein B.I., Zaleznik D.F. (2000). Enterobacteriaceae. G. L. Mandell, J. E. Bennett, and R. Dolin (ed.). Principles and practice of infectious diseases, 5th ed. Churchill Livingstone, Philadelphia, Pa. 2294-2310.

Emmerson A.M., Enstone J.E., Griffin M., Kelsey M.C., Smyth, E.T. (1996). The Second National Prevalence Survey of infection in hospitals-overview of the results. *J Hosp Infect*. **32**, 175-190.

Fontana R., Ligozzi M., Mazzariol A., Veneri G., Cornaglia G. (1998). Resistance of enterococci to ampicillin and glycopeptide antibiotics in Italy. *The Italian Surveillance Group for Antimicrobial Resistance. Clin Infect Dis*. (Suppl, 1) S84-6. PubMed PMID: 9710675.

Fitzpatrick F., McIlvenny G., Oza A., Newcombe R.G., Humphreys H., Cunney R., Smyth E.T.M. (2008). Hospital Infection Society Prevalence Survey of Healthcare Associated Infection 2006: comparison of results between Northern Ireland and the Republic of Ireland. *Journal of Hospital Infection*. **69**, 265-273.

Gikas A., Pediaditis J., Papadakis J.A., Starakis J., Levidiotou S., Nikolaides P., Apidianaki, N. (2002). Prevalence study of hospital-acquired infections in 14 Greek hospitals: Planning from the local to the national surveillance level. *Journal of Hospital Infection*. **50**, 269-275.

Gordon K.A., Jones R.N. (2003). Susceptibility patterns of orally administered antimicrobials among urinary tract infection pathogens from hospitalized patients in North America: comparison report to Europe and Latin America. Results from the SENTRY Antimicrobial Surveillance Program (2000). *Diagn Microbiol Infect Dis*. **45**, 295-301.

Hoban D., Bouchillon S., Johnson J., Zhanel G., Butler D., Miller L., Poupard J. (2001). Comparative in vitro potency of gemifloxacin and fluoroquinolones against recent European clinical isolates from a global surveillance study. *European Journal of Clinical Microbiology and Infectious Diseases*. **20**, 814-819.

Ilić M., Marković-Denić L. (2009). Nosocomial infections prevalence study in a Serbian university hospital. *Vojnosanitetski Pregled*. **66**, 868-875.

Iregui M., Ward S., Sherman G., Fraser V.J., Kollef M.H. (2002). Clinical importance of delays in the initiation of appropriate antibiotic treatment for ventilator-associated pneumonia. *Chest*. **122**, 262-268.

Karageorgopoulos D.E., Falagas M.E. (2008). Current control and treatment of multidrug-resistant Acinetobacter baumannii infections. *The Lancet Infectious Diseases*.

Khan J.A., Iqbal Z., Ur Rahman S., Farzana K., Khan A. (2008). Prevalence and resistance pattern of Pseudomonas aeruginosa against various antibiotics. *Pakistan Journal of Pharmaceutical Sciences*. **21**, 311-315.

Linhares I., Raposo T., Rodrigues A., Almeida A. (2013). Frequency and antimicrobial resistance patterns of bacteria implicated in community urinary tract infections: a ten-year surveillance study (2000-2009). *BMC Infectious Diseases*. **13**, 19.

Livermore D.M., Carter M.W., Bagel S., Wiedemann, B., Baquero, F., Loza, E., … Shungu, D. L. (2001). In vitro activities of ertapenem (MK-0826) against recent clinical bacteria collected in Europe and Australia. *Antimicrobial Agents and Chemotherapy*. **45**, 1860-1867.

Livermore D.M. (2002). Multiple mechanisms of antimicrobial resistance in Pseudomonas aeruginosa: our worst nightmare? *CID: Clinical Infectious Dissease*. **34**, 634-640.

Loureiro M.M., De Moraes B.A., Mendonça V.L.F., Quadra M.R.R., Pinheiro G.S., Asensi M.D. (2002). Pseudomonas aeruginosa: Study of antibiotic resistance and molecular typing in hospital infection cases in a neonatal intensive care unit from Rio de Janeiro City, Brazil. *Memorias Do Instituto Oswaldo Cruz*. **97**, 387-394.

Maragakis L.L., Perl T.M. (2008). Acinetobacter baumannii: epidemiology, antimicrobial resistance, and treatment options. *Clinical Infectious Diseases : An Official Publication of the Infectious Diseases Society of America*. **46**, 1254-1263.

Mera R.M., Suaya J.A., Amrine-Madsen H., Hogea C.S., Miller L.A., Lu E.P., Acosta C.J. (2011). Increasing role of Staphylococcus aureus and community-acquired methicillin-resistant Staphylococcus aureus infections in the United States: a 10-year trend of replacement and expansion. *Microbial Drug Resistance (Larchmont, N.Y.)*. **17**, 321-328.

Micek S.T., Lloyd A.E., Ritchie D.J., Reichley R.M., Fraser V.J., Kollef M.H. (2005). Pseudomonas aeruginosa bloodstream infection: Importance of appropriate initial antimicrobial treatment. *Antimicrobial Agents and Chemotherapy*. **49**, 1306-1311.

Mick V., Domínguez M.A., Tubau F., Liñares J., Pujol M., Martín, R. (2010). Molecular characterization of resistance to Rifampicin in an emerging hospital-associated Methicillin-resistant Staphylococcus aureus clone ST228, Spain. *BMC Microbiology*. **10**, 68.

Mylotte J.M., Graham R., Kahler L., Young,L., Goodnough S. (2000). Epidemiology of nosocomial infection and resistant organisms in patients admitted for the first time to an acute rehabilitation unit. *Clinical Infectious Diseases : An Official Publication of the Infectious Diseases Society of America*. **30**, 425-432.

Owens R.C., Ambrose, P. G. (2005). Antimicrobial safety: focus on fluoroquinolones. *Clinical Infectious Diseases : An Official Publication of the Infectious Diseases Society of America*. **41** (Suppl. 2), S144-S157.

Pellizzer G., Mantoan P., Timillero L., Allegranzi B., Fedeli U., Schievano E., Spolaore P. (2008). Prevalence and risk factors for nosocomial infections in hospitals of the Veneto Region, north-eastern Italy. *Infection*. **36**, 112-119.

Pradhan N.P., Bhat S.M., Ghadage D.P. (2014). Nosocomial Infections in the Medical ICU: A Retrospective Study Highlighting their Prevalence, Microbiological Profile and Impact on ICU Stay and Mortality. *JAPI*. vol. 62.

Rath S., Dubey D., Sahu M.C., Debata N.K., Padhy R.N. (2014). Surveillance of ESBL producing multidrug resistant Escherichia coli in a teaching hospital in India. *Asian Pacific Journal of Tropical Disease*. **4**, 140-149.

Reilly J., Stewart S., Allardice G.A., Noone A., Robertson C., Walker A., Coubrough S. (2008). Results from the Scottish National HAI Prevalence Survey. *Journal of Hospital Infection*. **69**, 62-68.

Richards M.J., Edwards J.R., Culver D.H., Gaynes, R.P. (2000). Nosocomial infections in combined medical-surgical intensive care units in the United States. *Infection Control and Hospital Epidemiology : The Official Journal of the Society of Hospital Epidemiologists of America*. **21**, 510-515.

Sanchez G.V., Master R.N., Clark R.B., Fyyaz M., Duvvuri P., Ekta G., Bordon J. (2013). Klebsiella pneumoniae antimicrobial drug resistance,

United States, 1998-2010. *Emerging Infectious Diseases*. **19**, 133-136.
Scherbaum M., Kösters K., Mürbeth R.E., Ngoa U.A., Kremsner P.G., Lell B., Alabi A. (2014). Incidence, pathogens and resistance patterns of nosocomial infections at a rural hospital in Gabon. *BMC Infectious Diseases*. **14**, 124.
Sievert D.M., Ricks P., Edwards J.R., Schneider A., Patel J., Srinivasan A., Fridkin, S. (2013). Antimicrobial-resistant pathogens associated with healthcare-associated infections: summary of data reported to the National Healthcare Safety Network at the Centers for Disease Control and Prevention, 2009-2010. *Infection Control and Hospital Epidemiology : The Official Journal of the Society of Hospital Epidemiologists of America*. **34**, 1-14.
Simon A., Bindl L., Kramer M.H. (2000). Surveillance of nosocomial infections: prospective study in a pediatric intensive care unit. Background, patients and methods. *Klin Padiatr*. **212**, 2-9.
Takagi E.H., Lincopan N., Cassettari V.C., Passadore L.F., Mamizuka E.M., Martinez M.B. (2009). Carbapenem-resistant Acinetobacter baumannii outbreak at university hospital. *Brazilian Journal of Microbiology*.
WHO. World Health Organization. (2014). Health care-associated infections FACT SHEET. *Available from: http://www.who.int/gpsc/country_work/gpsc_ccisc_fact_sheet_en.pdf*.
Zarb P., Coignard B., Griskeviciene J., Muller A., Vankerckhoven V., Weist K., Suetens C. (2012). The european centre for disease prevention and control (ECDC) pilot point prevalence survey of healthcare-associated infections and antimicrobial use. *Eurosurveillance*. **17**, 1-16.
Zhang Y., Chen F., Sun E., Ma R., Qu C., Ma L. (2013). In vitro antibacterial activity of combinations of fosfomycin, minocycline and polymyxin B on pan-drug-resistant Acinetobacter baumannii. *Experimental and Therapeutic Medicine*. ***5***, 1737-1739.
Zirakzadeh A., Patel R. (2006). Vancomycin-resistant enterococci: colonization, infection, detection, and treat