

# 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%).

Received April 4, 2015

Accepted November 11, 2015

## 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).

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

### Key words:

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

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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 (Bio-Mérieux, 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 VIGuard™ 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 ful-

ly 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 inpatient-days every 6 months.

Microorganism	2011		2012		2013		2014		Trend 2011-2014
	s1	s2	s1	s2	s1	s2	s1		
<i>E.coli</i>	2,73	2,57	1,46	2,51	2,13	2,41	3,41		
<i>Enterobacteriaceae</i>	1,09	1,07	1,11	1,71	1,28	1,31	2,12		
<i>Pseudomonas</i> spp.	0,67	0,64	0,77	0,98	0,67	0,95	0,93		
<i>Klebsiella</i> spp.	0,18	0,86	0,43	0,95	0,73	1,13	0,76		
<i>Enterococcus</i> spp.	0,67	0,89	0,97	0,92	0,91	0,66	1,32		
<i>S. aureus</i>	0,73	0,86	0,49	0,73	0,85	0,66	0,83		
<i>Acinetobacter</i> spp.	0,00	0,21	0,06	0,22	0,12	0,47	0,56		
<i>Streptococcus</i> spp.	0,06	0,06	0,06	0,13	0,21	0,18	0,20		
<b>Total</b>	<b>6,12</b>	<b>7,15</b>	<b>5,34</b>	<b>8,16</b>	<b>6,90</b>	<b>7,78</b>	<b>10,13</b>		
	<i>E. coli</i>	<i>Enterobacteriaceae</i>	<i>Pseudomonas</i> spp.	<i>Klebsiella</i> spp.	<i>Enterococcus</i> spp.	<i>S. aureus</i>	<i>Acinetobacter</i> spp.	<i>Streptococcus</i> 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	Microorganism															
	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	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 (93,2-99,1)	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	-	-	-	-	-	-	-	-
Fosfomicin	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)



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 3<sup>rd</sup> 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 fluoroquinolo-

nes, 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 3<sup>rd</sup> 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

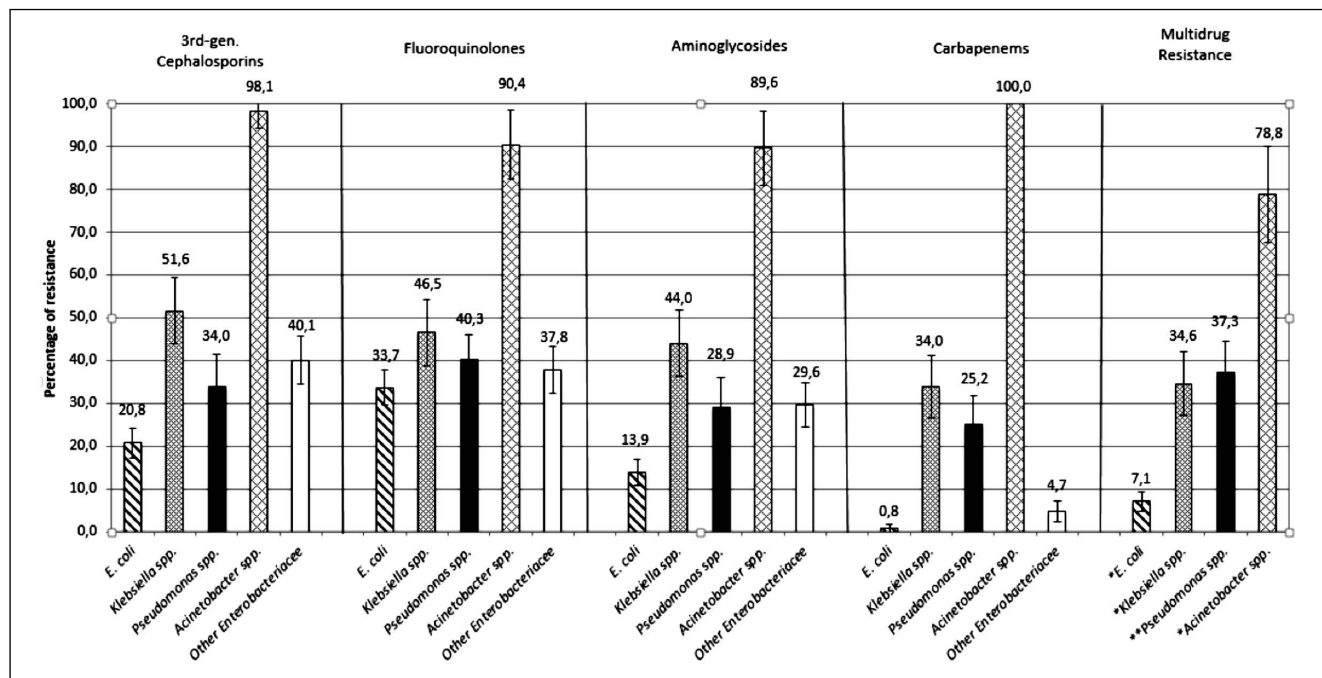


Figure 1 - Antimicrobial resistance by microorganism.

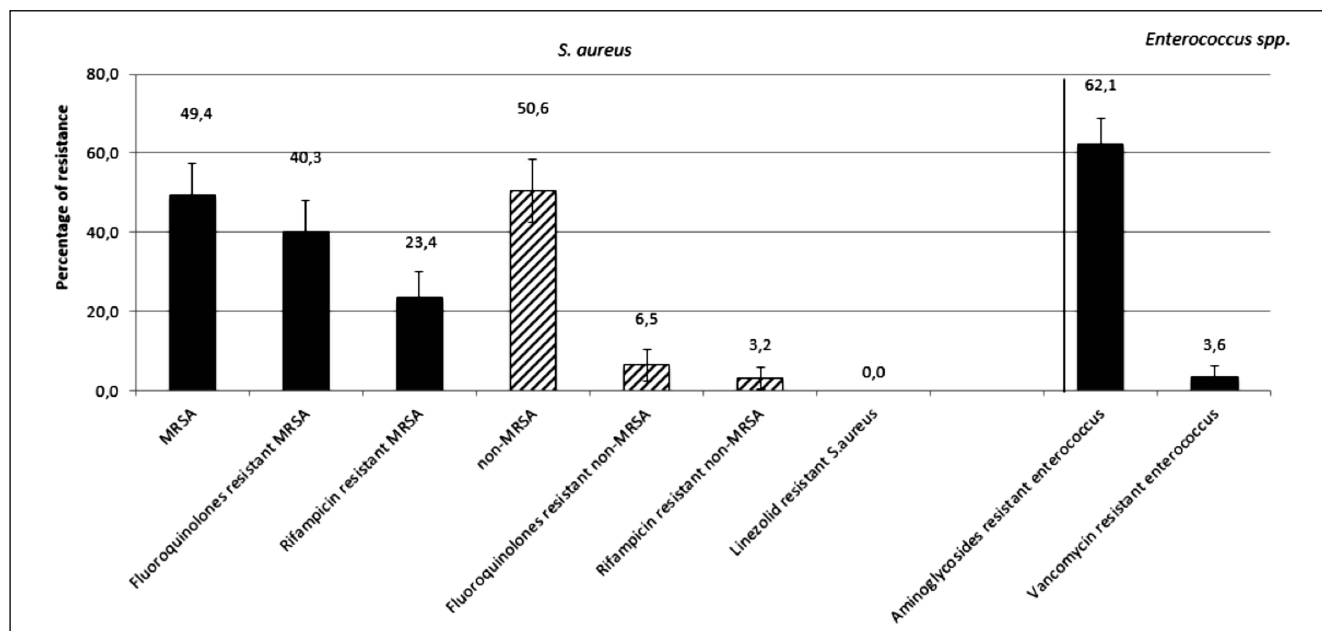


Figure 2 - *S. aureus* and *Enterococcus spp.* resistances.

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, fluoroquinolones 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 3<sup>rd</sup> 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 vancomycin-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 3<sup>rd</sup> generation cephalosporins (91.0%) and carbapenems (85.0%) (Takagi *et al.*, 2009).

Some bacteria reported complete resistance to certain an-

tibiotic 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, Pellizzer *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 *Enterobacteriaceae* 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.

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