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FULL PAPER

Trend of MDR-microorganisms isolated from the biological samples of patients with HAI and from the surfaces around that patient

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SUMMARY

Healthcare-associated infections (HAI) continue to be a major public health concern. A number of epidemiologically relevant HAI microorganisms are multidrug-resistant (MDR) germs that can spread rapidly and/or carry multiple resistance to antibiotics. They are the cause of high mortality and possible nosocomial epidemics. For this reason, we implemented microbiological surveillance acquiring samples from patients with HAI and environmental samples from the surfaces surrounding those patients. A retrospective study was carried out from January 2014 to December 2016 in two departments of the University Hospital in Messina, Italy: the Microbiology and the Hygiene Laboratories. A comparison was made between the microbiological isolates found on the patients and the microorganisms typed further to environmental sampling on the surfaces adjacent to the patient with HAI. There was a 24% match in 2014, 22% in 2015 and 20% in 2016 on total isolates. The most common isolates belonged to the *Enterobacteriacae* family: in particular, an ever-increasing trend has been registered for *Klebsiella* spp; *Acinetobacter baumannii* and multiresistant *Pseudomonas aeruginosa* have seen a growing trend for both patient and environmental samples. During the three years, the highest infection prevalence rate was found in Anaesthesia and Resuscitation, followed by Thoracic and Vascular Surgery.

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INTRODUCTION

Healthcare-associated infections (HAI) continue to be a major public health concern. A study published by ECDC has estimated that the impact of six healthcare-related infections (pneumonia, urinary tract infections, surgical site infections, *Clostridium difficile* infections, neonatal sepsis and blood infections) is greater than that of diseases like flu, HIV/AIDS and tuberculosis. More than 2.5 million cases of nosocomial infections occur in the European Union every year (Cassini *et al.,* 2016).

Italian data are in line with the European average: 6.6% in Italy versus 6.0% in Europe (2.3-10.8%), although the frequency of some infections (such as intravascular catheter-associated infections) is higher than the European average (Ministry of Health of Italy, 2014**)**.

The most commonly isolated HAI causing microorganisms in recent years were, in descending order: *E. coli, Staphylococcus aureus*, *Enterococcus* spp., *Pseudomonas aeruginosa*, *Klebsiella* spp., Coagulase-negative staphylococci, *Candida* spp., *C. difficile*, *Enterobacter* spp., *Proteus* spp. and *Acinetobacter* spp. (ECDC, 2013); HAI are more frequent in high-risk patients, such as those admitted to intensive care units, burn victims, transplant candidates and infants.

According to several European multicentre studies, about 30% of intensive care patients in high-income countries have suffered an episode of HAI. (Vincent *et al.,* 2009). Increased morbidity is observed in addition to the increase in mortality. Contraction of an infection during hospitalization, in general, determines a significant deterioration of the patient's quality of life and an extension of their hospital stay. It has been estimated that HAI alone are responsible for about 16 million extra days in hospital each year in Europe (WHO, 2011).

According to Italy's National Surveillance of Intensive Care Infections (Report 2011-2012**)**, the most commonly isolated germ was *Klebsiella* spp., followed by *P. aeruginosa* and *S. aureus*. A number of epidemiologically important HAI microorganisms are multidrug-resistant (MDR) germs that can spread rapidly and/or carry multiple resistance to antibiotics, causing high mortality and possible nosocomial epidemics. The increased frequency of their isolation is due to the high use of antibiotics and the increase in the use of invasive procedures, particularly in hospital departments with high-risk patients such as Intensive Care Units, Oncohematology, Transplant Units, but also in external clinics.

Key words:
Drug-resistant microorganisms, Nosocomial infections, Surveillance.

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The WHO has drawn up a list of the world's most antibiotic-resistant bacteria, with the aim of helping countries address national surveillance, research, and development activities. Pathogens, prioritised according to the need for new antibiotics, were divided into three groups: Priority 1- Critical *(A. baumannii, P. aeruginosa, Enterobacteriaceae)*, Priority 2- High *(Enterococcus faecium, S. aureus, Helicobacter pylori, Campylobacter, Salmonella spp., Neisseria gonorrhoeae)* Priority 3- Medium *(Streptococcus pneumoniae, H. influenzae, Shigella spp.)* (WHO, 2017).

In European Union countries, *E. coli* and *K. pneumoniae* have continued to show an increase in third-generation cephalosporin resistance percentages, fluoroquinolones and aminoglycosides, with phenomena often combined to generate multiresistant bacteria. This was aggravated by the spread of resistance to carbapenems, especially in *K. pneumoniae* (by 2015 the average percentage of resistant strains was 8.1%). Gram-positive bacteria methicillin-resistant *Staphylococcus aureus* (MRSA) has been and continues to be a public health priority despite its spread showing a downward trend, from 18.8% in 2012 to 16.8% in 2015 (ECDC, 2015).

In Italy, the spread of multiresistant microorganisms is significantly higher than in other European countries. This is also attributable to high antibiotic use: the prevalence of patients with at least one antibiotic treatment is 44.0% in Italy, against a European average of 35.0% (range 21.4 - 54.7%) (Ministry of Health of Italy, 2014).

Surveillance, in line with the guidelines provided by the WHO, the CDC and the European Council, is the basis for the HAI control activity. Only by implementing this strategy, especially over a prolonged time, can we fully comprehend the magnitude of the problem and then choose the necessary measures to apply in each local situation. For this reason, we implemented microbiological surveillance acquiring samples from patients with HAI, environmental samples from the surfaces surrounding those patients and samples from the hands of the healthcare providers who dealt with the patient. The aim of our study was to identify, in a timely manner, any epidemiologically significant bacterial strains that cause diffusion and multi-resistance (alert microorganisms), to prevent the epidemics caused by such germs by choosing the necessary measures each time to help keep HAI under control and to become aware of our hospital microbial flora.

METHODS

Our study was conducted from January 2014 to December 2016 in two departments of the University Hospital (A.O.U. Policlinico "G. Martino") in Messina, Italy. The biological samples of the hospitalised patients were tested in the Microbiology Laboratory and the environmental samples of the surfaces around those patients were analysed in the Hospital Hygiene Laboratory.

First, pathogens isolated from biological samples taken from patients who had developed an infection 48 h after hospitalisation were considered, in accordance with the criteria of the Centers for Disease Control and Prevention (CDC/NHSH, 2013). MDR microorganisms identified according to their characteristics and local microbiological status are:

- *Staphylococcus aureus* resistant to methicillin (MRSA);
- Carbapenemase producing and/or Colistin-resistant *Enterobacteriaceae* (*Enterobacter* spp., *Klebsiella* spp., *Serratia* spp., *Escherichia coli*, *Proteus* spp. and other Enterobacteria) ESBL;
- Glycopeptide-resistant *Enterococcus faecalis/faecium* (VRE);
- Multidrug-resistant *Acinetobacter baumannii*;
- Carbapenem and Colistin-resistant *Pseudomonas aeruginosa*;
- *Clostridium difficile* toxin manufacturer A and/or B (toxins detection in diarrheal stool specimens).

Samples from the patient were identified and subjected to sensitivity tests to different antimicrobials with VITEK® 2 (Bio-Mérieux, Marcy l'Etoile, France). Multiresistance was defined based on the presence of germs resistant to three or more classes of antimicrobials including piperacillin (± tazobactam), ceftazidime, fluoroquinolones, aminoglycosides and carbapenems (ECDC, 2015). In case of positivity, the germ was identified as MDR to the HIC (Hospital Infection Committee), the Health Care Department and the sample department. The Health Care Department, assessing the need to apply an environmental monitoring protocol in a critical or non-critical area, requested microbiological control of the surfaces around that patient. Environmental sampling was carried out to evaluate the presence of the same MDR germ on the surfaces around the patient with HAI. Pre-moistened sterile swabs were used at separate collection points based on the infectious risk of 10x10 cm^2. The areas the samples were taken from were: the side and/or lower side of the patient's bed, its bedside table, the crib walls (if a newborn), the humidifier (if used by the patient), the monitor (if a patient is admitted, for example, into resuscitation), the drugs trolley and the hands of health workers. The swabs were taken to the laboratory immediately; they were cultured in a brain-heart infusion broth and incubated at 37° for 24-48 h. Positive samples were prepared for further cultures on different growth media: Mannitol salt agar (Oxoid) to isolate Staphylococci, MacConkey agar (bioMérieux) to isolate Gram negative bacteria and Enterococcosel-agar (bioMérieux) to isolate faecal Enterococci. The isolated microorganisms were then identified and the resistance profile was also evaluated by the same method used for patient samples, VITEK® 2 (BioMérieux, France). The outcome of the tests was then communicated to the Health Care Department and the hospital unit involved to implement immediate containment/isolation measures needed to keep HAI under control.

RESULTS

Biological samples from patients with HAI

MDR microorganisms isolated in the years 2014, 2015 and 2016 were 155, 275 and 49 respectively. The wards from which MDR germs were most frequently reported in 2014 were the wards of Anaesthesia and Resuscitation (22.6%, or 35/155 reports), followed by the ward of General Surgery (21.3%, 33/155 reports) and Thoracic and Vascular Surgery (14.8%, 23/155). In 2015 most of the reports concerned the ward of Thoracic and Vascular Surgery (19.6%, 54/275 reports), Anaesthesia and Resuscitation (17.8%, 49/275) and General Surgery (15.3%, 42/275). In 2016 the cases reported in the Anaesthesia and Resuscitation ward were 30.6% (15/49 reports), followed by Thoracic and Vascular Surgery (24.5%) and Internal Medicine (14.3%).

MDR microorganisms most often isolated from clinical samples were: in 2014: *Acinetobacter baumannii* 42.6%

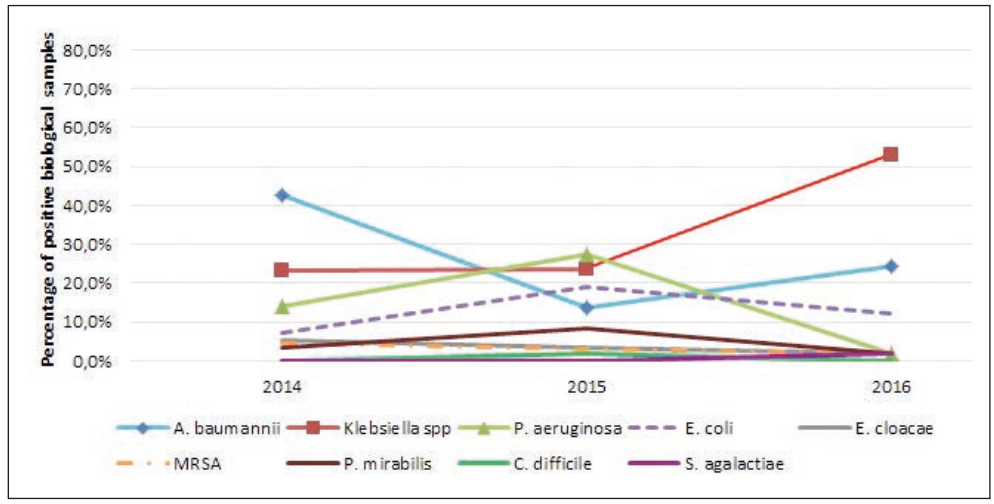

Figure 1 - *Trend of MDR microorganisms isolated from patients' biological samples. Trend for each microorganism isolated from the biological samples of patients with HAI over the course of the three years.*

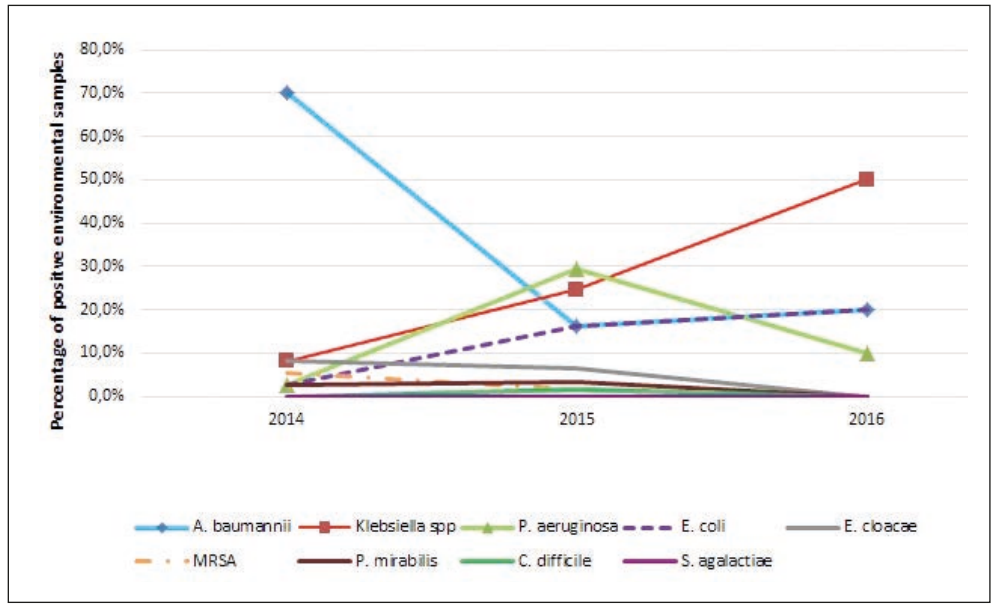

Figure 2 - *Trend of environmental MDR microorganisms. Trend for each microorganism isolated on the surfaces around the patient with HAI over the course of the three years.*

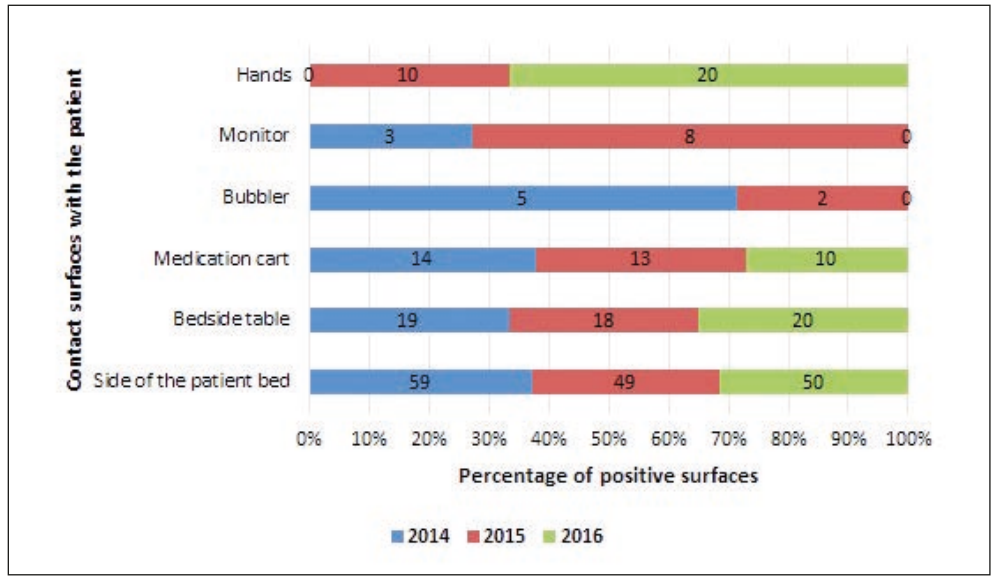

Figure 3 - *Percentage of colonized surfaces from MDR microorganisms. Percentage of positive surfaces around the patient with HAI following environmental sampling.*

(66/155), *Klebsiella* spp. 23.2% (36/155) and *P. aeruginosa* 14.2% (22/155); In 2015: *P. aeruginosa* 27.3% (75/275) and *Klebsiella* spp. 23.6% (65/275); In 2016: *Klebsiella* spp. 53.1% (26/49) and *A. baumannii* 24.5% (12/49) *(Figure 1)*. In particular, an ever-increasing trend has been registered for *Klebsiella* spp., which has risen from an average of 23% in 2014 and 2015 to 53.1% of the isolates in 2016, resulting in the most identified germ in the latter year. Multiresistant *A. baumannii* was the most isolated germ in 2014: 42.6% of clinical samples; then in 2015 it recorded a sharp decrease: 13.8% of the isolates, and a subsequent increase in 2016 (24.5%). Multiresistant *Pseudomonas aeruginosa* was the most isolated microorganism in 2015, present in 27.3% of clinical samples, although in 2016 it dropped drastically to 2%. As far as MRSA is concerned, it went from 4.5% of isolates in 2014 to 2% in 2016.

Environmental samples

MDR microorganisms isolated in the environmental samples carried out around the patient with HAI in the years 2014, 2015 and 2016 were 37, 61 and 10 respectively. The most isolated germ in 2014 was *A. baumannii*, 70.3%; in 2015: *P. aeruginosa* 29.5% of cases and in 2016 *Klebsiella* spp. in 50%. *(Figure 2)*.

The surfaces that tested positive to environmental sampling are shown in *Figure 3*. The locations that tested positive more often were: bedside bars and tables of patients infected with MDR germs. The percentage of positives found on the hands of healthcare workers was 0% in 2014, 10% in 2015 and 20% in 2016. A comparison was made between the microbiological isolates found on the patients and the microorganisms typed further to environmental sampling on the surfaces adjacent to the patient with HAI. There was a 24% match in 2014 (of which 16.8% was *A. baumannii*), 22% in 2015 (of which 6.5% was *P. aeruginosa*) and 20% in 2016 (of which 10.2% was *Klebsiella* spp) on total isolates.

DISCUSSION

During the three-year period observed, most of the isolated germs on the patient and on the surfaces, in agreement with other studies (Mancini et al., 2016), belonged to the Enterobacteriaceae family. We recorded a growing trend for Klebsiella pneumonia, which ranged from 23.2% to 53.1% in 2014 to 2016 for patient samples and from 1.9% to 10.2% in 2014 to 2016 of correspondence with environmental isolates, in line with recent national and European data. (Circular of the Ministry of Health of Italy, 2013; Gagliotti *et al.,* 2014; ECDC, 2015). Resistance to carbapenems and multiresistance are also common for *Acinetobacter baumannii* and *Pseudomonas aeruginosa* (De Francesco *et al.,* 2013, Boncagni *et al.,* 2015). In our study, *A. baumannii* and multiresistant *P. aeruginosa* showed a growing trend for both patient and environmental samples. The correspondence between patient samples and environmental samples for *P. aeruginosa* was highest in 2015, when a 6.5% match was recorded. Instead, this correspondence dropped to 2% in 2016. There was a positive result also for *A. baumanni*, which dropped from 16.8% in 2014 to 4.1% in 2016, as a consequence of the correct and appropriate application of environmental sanitation procedures.

The decreasing trend for MRSA is in line with what emerged from the above-mentioned ECDC report (ECDC, 2015). There are, by contrast, international studies that have reported *Acinetobacter* spp. and *S. aureus* as the most common pathogens. (Pradhan *et al.,* 2014; Scherbaum *et al.,* 2014). Sexton *et al.* (2006) showed that the MRSA present in dust were also found in the breathable air and thus the very environment, if not suitably sanitised as we indicated, can be a significant source of contagion.

As for the sampled surfaces, bedside bars and tables tested positive more frequently to the presence of MDR germs, throughout the observed period of time. In previous studies we showed the presence of germs, often multiresistant, in recovery rooms (La Fauci *et al.,* 2017; Squeri *et al.,* 2012). An alarming fact that emerged from our research is the microbiological contamination of the hands of healthcare professionals by multiresistant germs, which increased over the three years observed. This indicates a risk for transmission of infections, demonstrating that the proper HAI prevention hand-washing procedures, according to WHO guidelines, have not been yet properly implemented (WHO, 2009; Squeri *et al.,* 2016), despite the continued recommendations to each healthcare worker. A reassuring fact that emerged in these three years is the decreasing trend of the correspondence between the microbiological isolates found on patients and the microorganisms typed further to environmental sampling on the surfaces adjacent to the patient with HAI, which dropped from 24% to 20%. This shows that there has been greater attention to our environmental sanitisation procedures (through the proper use of disinfectants), despite the lack of appropriate handwashing. Given the correspondence of multiresistant gram negative bacteria both in the patient and the environment in all three years, antibiotic prescription should be based on knowledge of our hospital microbial ecosystem and on antibiotic sensitivity profiles of bacterial isolates. Hence, a targeted antibiotic therapy should be advocated in infection cases. To further reduce HAIs, all healthcare workers need to implement the most appropriate actions for the various environmental situations in a timely manner and favour multidisciplinary collaboration.

Conflict of interests

None to declare.

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