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Non-fermentative gram-negative bacteria in hospital tap water and water used for haemodialysis and bronchoscope flushing: Prevalence and distribution of antibiotic resistant strains☆

Sara Vincenti, Gianluigi Quaranta, Concetta De Meo *, Stefania Bruno, Maria Giovanna Ficarra, Serena Carovillano, Walter Ricciardi, Patrizia Laurenti

Institute of Public Health, Section of Hygiene, Università Cattolica del Sacro Cuore, Rome 00168, Italy

HIGHLIGHTS

- NFGNB were present in 4.56% of water sources analyzed from hospital wards.
- We assessed their susceptibility for a large number of antibiotics.
- More than half (55.56%) of the isolates showed antibiotic resistance.

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ABSTRACT

This study provides a detailed description of the distribution of non-fermentative gram-negative bacteria (NFGNB) collected in water sources (tap water and water used for haemodialysis and bronchoscope flushing) from different wards of a tertiary care hospital. The aim is to identify risk practices for patients or to alert clinicians to the possible contamination of environment and medical devices. The resistance profile of NFGNB environmental isolates has shown that more than half (55.56%) of the strains isolated were resistant to one or more antibiotics tested in different antimicrobial categories. In particular, 38.89% of these strains were multidrug resistant (MDR) and 16.67% were extensively drug resistant (XDR). The most prevalent bacterial species recovered in water samples were *Pseudomonas aeruginosa*, *Pseudomonas fluorescens*, *Ralstonia pickettii* and *Stenotrophomonas maltophilia*. Analysis of antibiotic resistance rates has shown remarkable differences between Pseudomonadaceae (*P. aeruginosa* and *P. fluorescens*) and emerging pathogens, such as *S. maltophilia* and *R. pickettii*. Multidrug resistance can be relatively common among nosocomial isolates of *P. aeruginosa*, which represent the large majority of clinical isolates; moreover, our findings highlight that the emergent antibiotic resistant opportunistic pathogens, such as *R. pickettii* and *S. maltophilia*, isolated from hospital environments could be potentially more dangerous than other more known waterborne pathogens, if not subjected to surveillance to direct the decontamination procedures.

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1. Introduction

The environment is a vast reservoir of antibiotic resistant organisms (D'Costa et al., 2006). This antibiotic resistance is a mechanism that involves part of the global microbial population and predates the modern selective pressure of clinical human use of antibiotics (D'Costa et al., 2011). There is growing evidence that antibiotic resistance genes in pathogenic organisms have arisen in the environment (Wright, 2010). The natural environment serves as a reservoir for the transfer of resistance genes between environmental and pathogenic bacteria by horizontal gene transfer (HGT) events (D'Costa et al., 2007).

Moreover, antibiotic resistance is not limited to the surface of the globe. In a previous study, Brown and Balkwill (Brown and Balkwill, 2009) identified antibiotic resistant bacteria isolated from within the

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* Corresponding author. Tel.: +39 0630154396; fax: +39 0635001522.
E-mail address: concetta.demeo87@gmail.com (C. De Meo).

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deep terrestrial subsurface between 173 m and 259 m; the majority of these isolates were multi-drug resistant, suggesting a different mechanism from the mere exposition to human source of antibiotics (Allen et al., 2010; Sengupta et al., 2013). Indeed, environmental bacteria carrying genes encoding resistance important in Medicine predate the modern antibiotic era by billions of years (Allen et al., 2010; Brown and Balkwill, 2009).

Antibiotic resistance is not restricted to pathogenic bacteria. In fact, environmental bacteria, such as the opportunistic *Pseudomonas aeruginosa*, often exhibit multiple antibiotic resistance in comparison to their pathogenic counterparts (Alonso et al., 2001; Enoch et al., 2007; Levy, 2002).

These resistant environmental bacteria can transfer the resistance genes to human pathogens (D'Costa et al., 2007; Muniesa et al., 2013; O'Brien, 2002; Okeke and Edelman, 2001; Vaz-Moreira et al., 2014).

Additionally, antibiotic resistance in the hospital environment is an increasingly daunting issue, especially among gram-negative bacilli (Lautenbach and Polk, 2007). Non-fermentative gram-negative bacteria (NFGNB), including the best-studied *P. aeruginosa* and emerging pathogens such as *Stenotrophomonas maltophilia* and *Ralstonia pickettii*, cause serious infections in hospital environments (Ferrara, 2006; Guervil and Chau, 2013). NFGNB are a growing concern in clinical environments, being one of the most common causes of nosocomial infections. The major outbreaks are related to the antibiotic resistance to a wide range of different classes of antibiotics and the capability they may acquire as additional resistances (Enoch et al., 2007). Resistant infections are becoming more difficult or even impossible to treat with current antibiotics, leading to infections causing higher morbidity and mortality. Intrinsic resistance in the bacteria of the hospital environment is problematic because it limits therapeutic options (Alanis, 2005).

Several studies concerning the antibiotic resistance of environmental strains show increasing evidence that clinical strains of these bacteria and/or their modes of resistance often originate from the natural environment, including bacteria within soil and water (Esiobu et al., 2002; Finley et al., 2013; Kummerer, 2004; Vaz-Moreira et al., 2014). In addition, NFGNB appear to develop and spread resistance in water environments (Baquero et al., 2008). An important way of antibiotic resistance dissemination is the water; multidrug-resistant bacteria have been detected from various water sources, including drinking water or tap water (Xi et al., 2009).

Therefore, we performed a cross-sectional study evaluating the recovery of NFGNB in water sources from the hospital environment in different wards of a tertiary care centre and, at the same time, assessing the antibiotic resistance profile of NFGNB in accordance with recent standard definitions (Magiorakos et al., 2012).

Our study provides detailed descriptions of the NFGNB distribution, collected in water sources (tap water and water used for haemodialysis and bronchoscope flushing) from different wards of a tertiary care hospital to identify risk practices for patients, such as haemodialysis, or to alert clinicians to the possible contamination of medical devices, such as bronchoscopes. Moreover, we assessed the resistance profile of NFGNB environmental isolates to determine the antibiotic resistance between different NFGNB in different wards. In particular, we compared the antibiotic resistance of the best known *P. aeruginosa* and *Pseudomonas fluorescens*, with respect to the emerging pathogens *S. maltophilia* (Looney et al., 2009) and *R. pickettii* (Ryan and Adley, 2014).

2. Materials and methods

2.1. Sample collection

The cross-sectional study was conducted on samples collected between September 2004 and February 2013 in a teaching hospital (1183-bed tertiary care centre) in Rome, Italy. A total of 3268 water samples were collected and analysed. The samples were distributed as follows: 860 tap water samples (608 from Maternity-paediatric wards and 252 from Generic clinical wards), 1750 haemodialysis samples (154 bi-osmosis treated water samples and 1596 dialysates) and 658 samples of fluids collected from bronchoscopes in the bronchoscopy unit.

Samples were taken from water sources in both generic and high-risk (hosting susceptible or immunocompromised patients) departments at scheduled time intervals: monthly for the haemodialysis unit, where samples came from bi-osmosis treated water and dialysate, and from the clinical wards, where samples came from the Maternity–paediatric wards and Generic clinical wards such as haematology and orthopaedics wards, and quarterly from bronchoscope flushing in the bronchoscopy unit.

Samples were collected in aseptic conditions using sterile containers from three categories of water reservoirs; in particular, from haemodialysis systems, the water was collected with syringes, while samples flushed through bronchoscopes were collected in sterile tubes (Falcon; Greiner Bio_One, Germany).

Samples were collected according to the following procedures.

One thousand mL of tap water, after running, from the wards under study was collected monthly from taps served by the same municipal water system (Italian Legislative Decree 31–2001).

One hundred mL of dialysate was collected monthly from the effluent connector of the artificial kidneys in the Haemodialysis unit; 100 mL of bi-osmosis treated water was collected monthly from a dedicated tap served by the municipal water system after the double osmosis treatment (Alloatti et al., 2005; Integration on the Resolution of the Regional Council of Lazio n. 1650/95 published in the Official Bulletin of the Lazio Region n. 18 of 30/06/1995, produced in May 1999 by the Department of Health Care and Protection).

Twenty mL of 0.9% sterile saline solution was collected after being flushed through both the operational channel and the aspiration valve of each bronchoscope in the bronchoscopy unit according to the ISO standards on disinfection assays of bronchoscopes (Beilenhoff et al., 2007).

Samples were transported in a cool box protected from direct light and processed within 2 h after collection.

2.2. Microbiological analysis

Each category of samples was treated according to standards reported in laws, GMPs or ISO.

1. For tap water, two amounts of 250 mL of samples were successively concentrated by filtration on a 0.45 μm pore diameter cellulose acetate membrane filter. These filters were aseptically removed, placed on selective media plates (one on Cetrimide Agar for *P. aeruginosa* detection and the other on MacConkey Agar for other NFGNB) and then incubated at 37 °C for 48 h, according to the methods described by the Italian Regulations on Water Safety (Italian Legislative Decree 31–2001).
2. For dialysate and bi-osmosis, two amounts of 100 mL of samples were successively concentrated by filtration on a 0.45 μm pore diameter cellulose acetate membrane filter. These filters were placed on selective media plates (one on Cetrimide Agar for *P. aeruginosa* detection and the other on MacConkey Agar for other NFGNB) and then incubated at 37 °C for 48 h (Alloatti et al., 2005).
3. For liquid from bronchoscopes, samples were collected after flushing through channels; 20 mL of 0.9% sterile saline solution was analysed and then filtered as reported for samples 1 and 2. The filter was placed on TSA (Tryptone Soya Agar) medium and incubated at 37 °C for 48 h according to the European ISO standards (Beilenhoff et al., 2007). The grown colonies showing morphological differences were subcultured on selective media plates (Cetrimide Agar for *P. aeruginosa* detection and MacConkey Agar for other NFGNB).

For the three categories of samples, all colonies macroscopically suggestive on Agar Cetrimide (showing a characteristic blue-green

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