A binational cohort study of intestinal colonization with extended-spectrum β-lactamase-producing *Proteus mirabilis* in patients admitted to rehabilitation centres

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Abstract

The aims of our study were to analyse the risk factors for colonization by Extended-spectrum β-lactamases (ESBL)-producing *Proteus mirabilis* (ESBL-PM) in rehabilitation patients and to characterize the molecular features of these strains. The study was conducted in two rehabilitation centres located in Rome, Italy (Fondazione Santa Lucia IRCCS (FSL)), and Tel-Aviv, Israel (Tel-Aviv Sourasky Medical Center (TASMC)). Carriage of ESBL-PM was surveyed by rectal swabs. Strain typing was performed by pulsed-field gel electrophoresis (PFGE). Identification of ESBL genes was done by PCR and sequencing. Patients admitted to the same institutions without ESBL carriage were included as controls. The study group included 70 and 41 patients from FSL and TASMC, respectively. In FSL, the multivariate analysis identified severe acute brain injury (OR = 15, 95% CI = 3.2–69.5, p 0.001), decubitus ulcer (OR = 3.5, 95% CI = 1.2–9.8, p 0.018) and recent treatment with quinolones (OR = 5.7, 95% CI = 1.07–30.1, p 0.042) as independent risk factors. ESBL-PM carriers stayed longer in the hospital on average and were less likely to be discharged home. No significant risk factor was identified in TASMC. There were no similarities in PFGE types or ESBL genes between the ESBL-PM isolates from the two institutions. In both hospitals, a variety of PFGE types existed but a single ESBL type predominated, namely TEM-92 in FSL (*n* = 64/70; 91%) and CTX-M-2 in TASMC (*n* = 37/41; 90%). A new TEM ESBL variant, TEM-177 was identified in FSL. The clonal diversity and the predominance of a single ESBL type suggested that horizontal gene transfer played an important role in dissemination of resistance. The development of a population analysis tool that would allow tracing deeper genetic relationships is required.

Keywords: Clones, colonization, extended-spectrum β-lactamase, *Proteus mirabilis*, rehabilitation centres
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Introduction

Proteus mirabilis is one of the most common agents of urinary tract infection, in particular among patients with urological abnormalities [1]. In addition, it is a frequent cause of other types of hospital-acquired infections, including bacteraemia [2], meningitis, empyema and osteomyelitis [3]. Along with sporadic infection cases, nosocomial outbreaks due to *P. mirabilis* have been reported since the 1970s [4–6]. Gastrointestinal colonization is believed to serve as a reservoir for intra-hospital spread [4]. As with other *Enterobacteriaceae*, resistance to cephalosporins, mediated mostly by extended-spectrum β-lactamases (ESBLs) [5,7,8] or acquired AmpC-type cephalosporinases [9,10], has been spreading in *P. mirabilis* populations in many parts of the world. The molecular epidemiology of ESBL-producing *P. mirabilis* (ESBL-PM) varies widely between different countries. In Italy and France TEM-type enzymes, especially TEM-92, have been the most common [2,7,8],

whereas in Poland, Israel and Japan the CTX-M types have been usually observed [5,11–13]. In some countries, such as Greece, Italy and Poland, AmpC-producing *P. mirabilis* strains have disseminated along with those expressing ESBLs [9].

Only a single study designed to look for the specific risk factors for ESBL-PM infections has been published so far [2]. In this study, the clinical and demographic characteristics of patients with ESBL-PM bacteraemia were compared with those of the patients with antibiotic-susceptible *P. mirabilis* infections in acute-care settings. Previous hospitalization in a nursing home and use of a bladder catheter were identified as risk factors. The study reported here was conducted in two rehabilitation centres in Italy and Israel, with distinct types of patient populations (see below). Rehabilitation wards (RWs) differ significantly from acute-care units, as patients are commonly hospitalized for longer periods, are more ambulant and may share joint facilities, such as physiotherapy suites or pools. Thus, the potential for direct patient-to-patient transmission of resistant bacteria may be more significant. Because patients in our study were monitored throughout their hospitalization by surveillance cultures, we were able to track the acquisition of ESBL-PM prior to evolution of clinical infection. Our aims were: (i) to comparatively assess the risk factors for ESBL-PM carriage in each of the two institutions; (ii) to reveal the clonal structure and ESBL genes of ESBL-PM populations; and (iii) to analyse the acquisition-to-admission ratio for the identified ESBL-PM clones.

Methods

Hospital settings

This study was a part of the project MOSAR (Mastering Hospital Antimicrobial Resistance and its Spread into the Community), a trans-disciplinary network funded by the European Commission and devoted to combating and controlling resistance in bacteria. The project focused on endemic and epidemic nosocomial pathogens in high-risk medical units, including ICUs, RWs and surgery wards in different European countries and Israel. Here we present a part of the results from the Work Package 5 (WP5), based on clinical trials in RWs, and Work Package 2 (WP2), comprising the laboratory work on bacterial isolates collected during the trials.

The study was conducted in two hospitals. The first centre, Fondazione Santa Lucia IRCCS in Rome, Italy (FSL), consists of two wards (106 beds together), and admits patients following spinal, cranial or orthopaedic trauma and with non-traumatic neurological and orthopaedic disorders. The second centre comprises two geriatric RWs (50 beds combined) at the Tel-Aviv Sourasky Medical Center (TASMC) in Tel Aviv, Israel. These wards admit elderly patients (>65 year of age) for rehabilitation following acute care admissions due to orthopaedic or neurological disorders or because of general deterioration in condition. The study included patients admitted to the wards between September 2008 and November 2010 and was approved by the ethics committees of FSL and TASMC.

Design and data collection

This was a prospective case–control study, aimed at examining risk factors for ESBL-PM carriage among RW patients. Surveillance rectal cultures were collected from all patients at admission, 2 weeks later, then once monthly, and at discharge. According to the local infection control policy, contact isolation was not carried out for ESBL-PM carriers. The following data were recorded: patient's age and sex, admission diagnosis, medical history including underlying conditions and co-morbidities, prior hospital or long-term care facility (LTCF) stay and its duration, antibiotic treatment during the last month prior to admission, the presence of medical devices, history of surgery or other invasive procedures, and the discharge destination. ESBL-PM carriers and control patients were randomly selected from the MOSAR database. Molecular typing and identification of ESBL genes were performed on first patient-unique isolates as described below.

Detection of ESBL-PM isolates and their phenotypic characterization

Rectal swabs were streaked onto the Brilliance ESBL Agar (Oxoid, Basingstoke, UK). Putative ESBL-producing *Enterobacteriaceae* colonies (one from each morphotype detected) were identified according to the manufacturer's instructions. Pure cultures were frozen at −80°C and shipped to the MOSAR ESBL laboratory (National Medicines Institute in Warsaw, Poland) for definite identification and further analysis. Species identification was carried out using the Vitek 2 system (bioMérieux, Marcy l'Etoile, France). ESBL production was verified using the double-disk synergy test with disks containing cefotaxime, ceftazidime, cefepime and amoxicillin with clavulanate on Mueller-Hinton agar plates (Oxoid) that were unsupplemented and supplemented with 250 mg/L cloxacillin (Polfa Tarchomin, Warsaw, Poland) as previously described [14].

Molecular typing of ESBL-PM isolates

For pulsed-field gel electrophoresis (PFGE), total DNAs of the isolates were purified as described by Struelens *et al.* [15] and digested sequentially with *Not*I and *Sfi*I restriction enzymes (New England BioLabs, Beverly, MA, USA) [16]. PFGE types and subtypes were discerned by the visual analysis using the