J Infect Chemother (2013) 19:799–805
DOI 10.1007/s10156-013-0563-3

ORIGINAL ARTICLE

Characteristics of bacteremia caused by extended-spectrum beta-lactamase-producing *Proteus mirabilis*

Yoko Kurihara · Shigemi Hitomi · Tsuyoshi Oishi · Tsukasa Kondo · Tsugio Ebihara · Yasunori Funayama · Yasushi Kawakami

Received: 15 November 2012 / Accepted: 15 January 2013 / Published online: 1 February 2013

Abstract Although *Proteus mirabilis* is a common human pathogen, bacteremia caused by the organism, especially strains producing extended-spectrum beta-lactamase (ESBL), has rarely been investigated. We examined 64 cases of *P. mirabilis* bacteremia identified in the Minami Ibaraki Area, Japan, between 2001 and 2010 and compared the characteristics of cases with ESBL-producing and ESBL-non-producing strains (13 and 51 cases, respectively). All ESBL-producing strains with the gene encoding the CTX-M-2-group were genetically nonidentical. Isolation of ESBL-producing strains was significantly associated with onset in a hospital ($p = 0.030$), receiving hemodialysis ($p = 0.0050$), and previous antibiotic use within 1 month ($p = 0.036$; especially penicillin and/or cephalosporin ($p = 0.010$) and fluoroquinolone ($p = 0.0069$)). Isolation was also associated with inappropriate antibiotic therapy on the 1st and 4th days ($p = 0.011$ and 0.032, respectively) but not with mortality on the 30th day. These findings indicate that, for *P. mirabilis* bacteremia, isolation of ESBL-producing strains causes delay of initiating appropriate antimicrobial therapy but may not be associated with mortality.

Keywords *Proteus mirabilis* · Extended-spectrum beta-lactamase · Bacteremia · Risk factor · Mortality

Y. Kurihara · S. Hitomi (✉)
Department of Infectious Diseases, University of Tsukuba Hospital, 2-1-1 Amakubo, Tsukuba, Ibaraki 305-8576, Japan
e-mail: shitomi@md.tsukuba.ac.jp

T. Oishi
Department of Infectious Diseases, Tokyo Medical University Ibaraki Medical Center, Tokyo, Japan

T. Kondo
Department of Anesthesiology and Critical Care Medicine, Tsuchiura Kyodo General Hospital, Tsuchiura, Japan

T. Ebihara
Department of Gastroenterology and Hepatology, Ryugasaki Saiseikai Hospital, Ryugasaki, Japan

Y. Funayama
Department of Respiratory Medicine, Tsukuba Gakuen Hospital, Tsukuba, Japan

Y. Kawakami
Department of Clinical Laboratories, University of Tsukuba Hospital, Tsukuba, Japan

Introduction

Proteus species are common pathogens causing urinary tract infection, especially among patients with indwelling urinary catheters or anatomic or functional abnormalities of the urinary tract [1]. Of these species, *Proteus mirabilis* is the organism most frequently recovered from blood culture [2–4]. This organism is usually susceptible to commonly used antibiotics, including beta-lactam, aminoglycoside, fluoroquinolone, and trimethoprim-sulfamethoxazole, except for tetracycline [5]. However, strains producing extended-spectrum beta-lactamase (ESBL), enzymes capable of hydrolyzing penicillin, cephalosporin, and monobactam, have increasingly been isolated worldwide [6, 7].

To identify ESBL production among *Enterobacteriaceae*, including *Escherichia coli*, *Klebsiella* spp., and *P. mirabilis*, authorities have published phenotypic criteria [8], which have been used for a variety of epidemiological studies. However, characteristics of infection caused by ESBL-producing *P. mirabilis* have rarely been investigated. A previous study of 25 episodes of *P. mirabilis* bacteremia in Italy, including 11 with ESBL-producing

strains, reported that infection caused by ESBL-producing strains was significantly associated with previous hospitalization in nursing homes, use of bladder catheters, and higher mortality [9]. However, this study included only cases encountered in a single hospital. In this study, therefore, we conducted multi-institutional surveillance of *P. mirabilis* bacteremia for further characterization of infection caused by ESBL-producing *P. mirabilis*.

Materials and methods

Data collection

We conducted laboratory-based surveillance of *P. mirabilis* bacteremia in the Minami Ibaraki Area, a suburban area approximately 50 km northeast of Tokyo, between 2001 and 2010. The surveillance began with two university-affiliated hospitals and one community hospital, followed by participation of two more community hospitals since 2006. In 2010, these five hospitals had 2,789 beds and a total of approximately 750,000 admissions. When *P. mirabilis* was repeatedly isolated from the same patient, only the first episode of bacteremia was examined. Medical records of patients were retrospectively reviewed for evaluation of demography, underlying diseases, previous medication, primary sources of bacteremia, predisposing factors, treatments, and outcomes.

Definitions of clinical characteristics

Primary causes of bacteremia were determined on the basis of clinical manifestation and/or culture of specimens obtained simultaneously. Infections developing in other hospitals or after hospitalization for 48 h were defined as "hospital-onset" infections. Severity of septicemia was classified according to the criteria of the American College of Chest Physicians/Society of Critical Care Medicine [10]. When organisms other than *P. mirabilis* were isolated from the same blood specimen or from those obtained within 24 h, the case was regarded as "polymicrobial bacteremia". Antimicrobial therapy was regarded as "appropriate" when isolated *P. mirabilis* strains were susceptible to at least one antibiotic administered to patients. The appropriateness of antibiotic therapy was evaluated on the day when positive blood culture was drawn and 3 days later (1st and 4th days of therapy, respectively). "Recurrence" was defined as repeated recovery of *P. mirabilis* from the blood of the same patient after more than 28 days. Mortality was determined 30 days after positive blood culture was drawn or at discharge from a hospital where diagnosis of bacteremia had been made.

Isolation and drug susceptibility of organisms and detection of ESBL production

Blood specimens were cultured with the BacT/Alert® 3D System (BioMérieux Japan, Tokyo, Japan) or BACTEC™ Instrumental Blood Culture Systems (Nihon Becton Dickinson, Tokyo, Japan). Isolated organisms were identified during routine laboratory work with Gram staining and the MicroScan WalkAway System (Dade Behring, Tokyo, Japan), the VITEK 2 Card for Gram-Negative Bacteria (BioMerieux, Marcy-l'Etoile, France), or Enterotube® II (Nihon Becton Dickinson, Fukushima, Japan). All strains identified as *P. mirabilis* were preserved in 10 % skimmed milk at −85 °C until further examination. Minimum inhibitory concentrations (MICs) of antibiotics were assayed by use of the broth microdilution method, using cation-adjusted Mueller–Hinton broth and Dry Plate (Eiken Chemical, Tokyo, Japan), and interpreted in accordance with the recommendation of the Clinical and Laboratory Standards Institute [11]. Production of ESBL was screened by measurement of MICs of cefotaxime, ceftazidime, cefpodoxime, and aztreonam [11] and confirmed phenotypically with the Epsilometer test (Etest® ESBL CT/CTL and ESBL TZ/TZL; Asuka Junyaku, Tokyo, Japan) in accordance with the manufacturer's instructions.

Detection and typing of the ESBL-producing genes

A loopful of organisms was suspended in 200 µl TE buffer (10 mM Tris–Cl, 1 mM ethylenediaminetetraacetic acid (pH 8.0)) and boiled for 10 min. After centrifugation, 1 µl supernatant was used for polymerase chain reaction for detection of the bla_{TEM}, [12] bla_{SHV} [12], $bla_{\text{CTX-M}}$ [13] $bla_{\text{CTX-M-1-group}}$ [14], $bla_{\text{CTX-M-2-group}}$ [15], $bla_{\text{CTX-M-8}}$ [16], and $bla_{\text{CTX-M-9-group}}$ [15] genes.

Genotyping with pulsed-field gel electrophoresis (PFGE)

Organisms cultured on Mueller–Hinton broth agar overnight were suspended in 100 µl PIV buffer (10 mM Tris–Cl (pH 8.0), 1 M NaCl), mixed with an equal volume of 2 % InCert Agarose (Cambrex Bio Science, Rockland, ME, USA), and solidified in plug molds at 4 °C. The agarose plugs were immersed in lysis buffer (6 mM Tris–Cl (pH 8.0), 100 mM ethylenediaminetetraacetic acid, 1 M NaCl, 0.2 % sodium deoxycholate, 0.5 % sodium *N*-lauroyl sarcosinate) supplemented with 0.5 mg proteinase K (Wako Pure Chemicals, Osaka, Japan) per ml and incubated at 50 °C for 18 h. Portions of the plugs were rinsed in TE buffer four times and digested with a