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Linezolid-resistant staphylococcal bacteraemia: A multicentre case–case–control study in Italy

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ABSTRACT

The aim of this multicentre study was to analyse the characteristics of patients with bloodstream infections due to staphylococcal strains resistant to linezolid. This was a retrospective case–case–control study of patients hospitalised in three large teaching hospitals in Italy. A linezolid-resistant (LIN-R) *Staphylococcus* spp. group and a linezolid-susceptible (LIN-S) *Staphylococcus* spp. group were compared with control patients to determine the clinical features and factors associated with isolation of LIN-R strains. All LIN-R *Staphylococcus* spp. strains underwent molecular typing. Compared with the LIN-S group, central venous catheters were the main source of infection in the LIN-R group. The LIN-R and LIN-S groups showed a similar incidence of severe sepsis or septic shock, and both showed a higher incidence of these compared with the control group. Overall, patients in the LIN-R group had a higher 30-day mortality rate. Multivariate analysis found previous linezolid therapy, linezolid therapy >14 days, antibiotic therapy in the previous 30 days, antibiotic therapy >14 days, previous use of at least two antibiotics and hospitalisation in the previous 90 days as independent risk factors associated with isolation of a LIN-R strain. The G2576T mutation in domain V of 23S rRNA was the principal mechanism of resistance; only one strain of *Staphylococcus epidermidis* carried the *cfr* methylase gene (A2503), together with L4 insertion ($_{71}$GGR$_{72}$) and L3 substitution (H146Q). LIN-R strains are associated with severe impairment of clinical conditions and unfavourable patient outcomes. Reinforcement of infection control measures may have an important role in preventing these infections.

1. Introduction

Gram-positive bacteria have become the predominant causative organisms of sepsis, with coagulase-negative staphylococci, *Staphylococcus aureus* and *Enterococcus* spp. accounting for 50–60% of pathogens responsible for all cases of nosocomial infection [1–3]. Until recently, the glycopeptide antibiotics vancomycin and teicoplanin were effective options for the treatment of infections caused by meticillin-resistant Gram-positive pathogens. However, there have been numerous reports of strains with reduced susceptibility, or outright resistance, to glycopeptides, and evidence of a significant relationship between higher vancomycin minimum inhibitory concentrations (MICs), ranging between 1 mg/L and 2 mg/L, and treatment failure have led to calls for alternative therapies [4–7].

Linezolid is an important antimicrobial agent for the therapy of difficult-to-treat infections, especially pneumonia and skin and soft-tissue infections (SSTIs), caused by meticillin-resistant *S. aureus* (MRSA) and vancomycin-resistant enterococci. Because of the spread of drug-resistant Gram-positive bacteria, the use of linezolid for treating severe infections is increasing, but in the last years linezolid-resistant (LIN-R) strains have emerged [8–10].

Since the introduction of linezolid into clinical practice, several mechanisms of resistance have been described, with mutations in domain V of the 23S rRNA recognised as the major mechanism of resistance, and recently the *cfr*-mediated resistance mechanism has become predominant. Despite the potential importance of these

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infections, no systematic studies of risk factors, clinical outcomes and mechanisms of linezolid resistance have been conducted.

The aim of this multicentre case–case–control study was to analyse the characteristics of patients with bloodstream infections (BSIs) due to strains of staphylococci resistant to linezolid in order to identify the main risk factors associated with isolation of LIN-R strains. The clinical features, microbiological and molecular typing, and outcome of the patients were also analysed.

2. Materials and methods

2.1. Study design and patients

This was a retrospective case–case–control study of patients hospitalised in three large teaching hospitals in Italy, namely Policlinico Umberto I of Rome, University Hospital of Udine and University Hospital of Pisa. Data were collected from November 2012 to November 2013. The Ethics Committee of Policlinico Umberto I, 'Sapienza' University of Rome (Rome, Italy) (principal investigator) approved the study.

Patients aged >18 years who satisfied the criteria for staphylococcal bacteraemia were included in the study. Only patients with isolates obtained >48 h after hospitalisation were included.

Data were extracted from the medical records of patients and from hospital computerised databases, or from clinical charts by means of a questionnaire. Cases and controls were compared with regard to: demographics (age and sex); co-morbid conditions (diabetes mellitus, cardiovascular disease, pulmonary disease, renal disease, hepatic disease, central nervous system disease and malignancy as well as the overall number of co-morbid conditions); treatment and medical procedures (during hospitalisation and/or in the previous 90 days) prior to a positive culture [immunosuppressive therapy, placement of a central venous catheter (CVC) or a urinary catheter, stay in an intensive care unit (ICU), dialysis, instrumentation (including cardiovascular and endovascular catheterisation, endoscopic procedures and tracheostomy), surgery and mechanical ventilation]; admission from home or from an institution; the source of the sample positive by culture; functional status on admission; recent receipt of any antibiotics (received on or after admission, before the positive culture was obtained); the classes of antibiotics received before the positive culture; and the sequential organ failure assessment (SOFA) score at the time of infection.

Concomitant bacterial, fungal or viral infections (e.g. pneumonia, SSTIs, intra-abdominal infections, BSIs) at the time of staphylococcal bacteraemia were recorded. Data on antibiotic therapy in the previous 30 days as well as other risk factors for multidrug-resistant (MDR) organisms were obtained from the following sources: (a) history taken from patients and/or relatives; (b) discharge letters and summaries if patients were previously hospitalised in other facilities; and (c) electronic records if patients were previously hospitalised or seen in the clinics involved in the study.

2.2. Study definitions

BSIs, including sepsis, severe sepsis and septic shock, were defined using standard international criteria [11]. Bacteraemia was defined as the isolation of micro-organisms in two or more separate blood cultures with clinical evidence of infection, and infective endocarditis was diagnosed according to the modified Duke criteria [12,13]. The CVC was considered the likely source of infection if a blood culture obtained from the lumen of the catheter was positive within 2 h of a culture collected at the same time from a peripheral vein also becoming positive, and/or culture of the catheter also being positive. Severe sepsis was defined as sepsis with sepsis-induced organ dysfunction or tissue hypoperfusion (manifesting as hypotension, elevated lactate or decreased urine output); septic shock was defined as severe sepsis plus persistently low blood pressure following administration of intravenous fluids [14].

During the study period, all patients with LIN-R strains isolated from the blood, in which the source of the staphylococcal infection was primary bacteraemia or a bacteraemia secondary to another site of infection, were enrolled.

Length of hospital and ICU stay were calculated as the number of days from the date of admission to the date of discharge or death.

2.3. Microbiological analysis

All strains were tested for their antibiotic susceptibility profiles by the broth microdilution (MIC) method against linezolid (Pfizer Pharma, Milan, Italy) and comparator agents, i.e. oxacillin, erythromycin and lincomycin (Sigma-Aldrich, Milan, Italy), in accordance with Clinical and Laboratory Standards Institute (CLSI) guidelines [15]. European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines were used for categorisation of susceptibility [16].

2.4. Molecular sequence analysis and typing

Chromosomal extraction of whole genomic DNA was performed as previously described [17]. All strains were screened for the presence of *mecA* and *cfr* genes as well as 23S rRNA (domain V), L3 (*rplC*), L4 (*rplD*) and L22 (*rplV*) mutations. Oligonucleotide sequences, PCR conditions and sequence analysis used have been described elsewhere [18,19].

Chromosomal extraction of whole genomic DNA, *Sma*I macrorestriction (New England Biolabs, Hitchin, UK), pulsed-field gel electrophoresis (PFGE) analysis (CHEF-DR® II apparatus; Bio-Rad, Hercules, CA) and multilocus sequence typing (MLST) were performed following a previously reported protocol [17–20].

2.5. Study groups and endpoints

Groups of LIN-R *Staphylococcus* spp. and linezolid-sensitive (LIN-S) *Staphylococcus* spp. were compared with controls to determine the factors associated with isolation of a LIN-R strain.

Three study groups were defined [21,22]:

- LIN-R group: hospitalised patients from whom a LIN-R strain was isolated from blood during hospitalisation;
- LIN-S group: hospitalised patients from whom a LIN-S strain was isolated from blood during hospitalisation; and
- control group: patients without infection and no blood cultures positive for *Staphylococcus* spp. during their hospitalisation.

The obtainable records of all patients meeting the criteria for the LIN-R group were examined, and an equal number of patients were selected to comprise the LIN-S group. Patients in the LIN-S group and the controls were chosen at random from lists of patients hospitalised during the same period in the same wards as the patients of the LIN-R group (case:control ratio = 2).

Using this design, two separate case–control analyses were performed within a single study: the first compared the LIN-R group with control patients without infection caused by the target organism; and the second compared the LIN-S with control patients without infection caused by the target organism. Finally, the LIN-R group was compared with the LIN-S group in order to a provide a better identification of the risk factors specifically associated with isolation of the LIN-R strains.

The clinical endpoints were 30-day mortality, clinical risk factors for selection of resistant strains and length of hospital stay. The