Title: Carbapenem non-susceptible *Klebsiella pneumoniae*

Authors: Rocchetti A.;[1]

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Abstract

Purpose: This work aims at studying Carbapenem-resistant Klebsiella pneumoniae, which has recently been reported as a new, multidrug-resistant nosocomial pathogen in several hospitals from various Italian regions.

Methods: Through Micronet, a new Italian sentinel laboratory-based surveillance network, researchers studied the trend of non-susceptibility of K. pneumoniae to selected carbapenems (imipenem and/or meropenem) in 14 of the 15 hospitals participating in the network.

Results: Analysis of data from 1 January 2009 to 30 April 2012 revealed a statistically significant increasing trend ($p<0.01$) in the proportion of carbapenem non-susceptible K. pneumoniae isolates from clinical specimens (from 2.2 % in 2009 to 19.4% in 2012). The increase in the proportion of non-susceptibility was very large for isolates from the respiratory tract (from 5.3% in 2009 to 38.5% in 2012) and blood (from 5.4% in 2009 to 29.2% in 2012).

Conclusions: The results demonstrate the urgent need in Italy for infection control, guidelines, antibiotic stewardship programmes and utilisation of surveillance

[1] Hospital "SS. Antonio e Biagio e C. Arrigo" of Alessandria;
Structure of Microbiology
E-mail: arocchetti@ospedale.al.it;

systems, such as Micronet, which are capable of receiving data from hospitals in real time for many pathogens and types of clinical specimens.

1. Background

Micronet is a project of the Italian Institute of Health (i.e. Istuto Superiore di Sanità), which is aimed at controlling infections and microbial agents, involving 15 Italian laboratories of microbiology. The structure of microbiology of the hospital "SS. Antonio e Biagio e Cesare Arrigo" of Alessandria, under the responsibility of Dr. Andrea Rocchetti, has been actively involved in the Micronet project and the study of Carbapenem non-susceptible *Klebsiella pneumoniae*, which has lead to an international scientific publication (Sisto et al., 2012).[2] This Working Paper proposes an extract of that article aimed at presenting the results of the Micronet project and the scientific activity has been performed by this structure of microbiology.

In recent years, carbapenem-resistant Enterobacteriaceae have emerged rapidly in hospitals worldwide (Gupta et al., 2011). Two main mechanisms can lead to reduced susceptibility or resistance to carbapenems in Enterobacteriaceae, namely reduced outer-membrane permeability associated with the production of extended-spectrum beta-lactamases (ESBLs) or AmpCtype beta-lactamases (Ardanuy et al., 1998; Martinez-Martinez et al., 1999) and production of acquired beta-lactamases that degrade carbapenems, i.e. carbapenemases (Nordmann and Poirel, 2002). The most frequent carbapenemases spreading among Enterobacteriaceae currently are the following: the KPC-type serine carbapenemases (belonging to Ambler's molecular class A); the VIMand NDM-type metallo-beta-lactamases (belonging to Ambler's molecular class B); and the OXA-48-like serine carbapenemases (belonging to Ambler's molecular class D). The species most commonly affected is *Klebsiella pneumoniae*. Carbapenemaseproducing Enterobacteriaceae (CPE) usually carry additional resistance determinants to other antimicrobial agents, making these strains resistant to many antibiotics (Queenan and Bush, 2005) and thus leaving few therapeutic options for infected patients.

[2] Carbapenem nonsusceptible Klebsiella pneumoniae from Micronet network hospitals, Italy, 2009 to 2012. Euro Surveill. 2012;17(33):pii=20247.
Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20247

In Italy, carbapenem-resistant *K. pneumoniae* has recently been reported as a new, multidrug-resistant nosocomial pathogen in hospitals from different Italian regions. The circulation of such strains in healthcare facilities, however, needs to be clearly measured, since an increased circulation of carbapenem-resistant Enterobacteriaceae has important implications for both the control of both patient-to-patient transmission and hospital-to-hospital transfer of patients (Gaibani et al., 2011).

The purpose of this study is to assess the trend in the proportion of *K. pneumoniae* isolates non-susceptible to selected carbapenems from 2009 to 2012, in a sample of Italian hospitals belonging to Micronet. Micronet is a sentinel laboratory epidemiological surveillance network for infections, which has been established in Italy since 2008. It was created and is managed by the Istituto Superiore di Sanita, the Italian National Public

Health Institute, and CINECA (a supercomputing centre and consortium of Italian universities). It is based on computerised daily collection of data on microbial isolates and of related antibiotic susceptibilities from the laboratory information systems of 27 laboratories nationwide.

2. Methods

2.1 Inclusion criteria

Researchers of the Micronet project collected data on *K. pneumoniae* isolates nonsusceptible to selected carbapenems (imipenem and/or meropenem) from relevant clinical specimens (bronchoalveolar lavages, tracheal aspirates, blood, cerebrospinal fluid, pus, urine) from 1 January 2009 to 30 April 2012 for 14 of the 15 hospitals in which the Micronet interface is fully active and functioning automatically (one hospital was excluded since it had no data for 2009). These are medium or large referral hospitals, located in four Italian regions. Since 2011, they have become part of ARISS, the Italian antimicrobial resistance surveillance system that sends data to the European Antimicrobial Resistance Surveillance Network (EARS-Net), coordinated by the European Centre for Disease Prevention and Control (ECDC).

2.2 Micronet database

Data on *K. pneumoniae* isolates for which antimicrobial susceptibility testing results were available were extracted from the Micronet database, in which the results were described

qualitatively (susceptible, intermediate, resistant). For the purposes of this study, isolates with intermediate and resistant profiles were defined as non-susceptible. Researchers of the Micronet project focused on the following subset of isolates (the data were obtained from the main dataset): isolates of *K. pneumoniae* from all major clinical specimens (blood, bronchoalveolar lavage, tracheal aspirate, cerebrospinal fluid, pus, urine) for which there were antimicrobial susceptibility test data available for the selected carbapenems (imipenem and/or meropenem). Information on susceptibility to ertapenem was not available for many of the participating laboratories for the entire period and was therefore not considered in the proposed analysis.

For patients from whom several isolates had been obtained in the same month, only the first isolate was considered, regardless of the type of clinical specimen from which it was isolated and regardless the result. Multiple isolates from the same patient collected after an interval of 30 days were included. In such instances, researchers of the Micronet project counted only the first isolate of *K. pneumoniae* for which susceptibility to the selected carbapenems was tested, regardless of the result.

An export procedure for the results of microbiological tests (both positive and negative) was developed in each participating laboratory, with the contribution of laboratory information systems managers and clinical microbiologists. Only results validated in the laboratory and used for clinical purposes are sent to a central server located and managed at CINECA, where the data are consolidated. Researchers also used the Micronet database for an analysis stratified by type of clinical specimen.

2.3 Additional data

The participating laboratories were also asked to provide full information on the methods used to identify the organisms (i.e. which automated system for identification was used) as well as which guidelines were used to interpret the results of the antimicrobial susceptibility tests. Furthermore, the laboratories were asked to provide the number of beds and the number of patient days of their respective referral hospitals for January–December 2011, the most recent data available in all the hospitals.

2.4 Data analysis

The proportion of carbapenem non-susceptibility was calculated as the number of carbapenem non- usceptible first isolates of *K. pneumoniae* divided by the total number of

first isolates of *K. pneumoniae*, expressed as percentage. As not all participating laboratories had information on the age of the patients, age was not included in the analysis.

Epi-Info 3.53 (ECDC; 2011)[3] was used to calculate the proportion of isolates that were non-susceptible. OpenEpi 2.3.1 (CDC, 2012)[4] was used to calculate confidence intervals for the proportion (using Fisher's exact test) and also for the extended Mantel–Haenszel chi-square test for linear trend.

3. Results

The mean number of beds of the 14 Micronet hospitals in the study was 631 (median: 516.5; range: 322–1,220). The mean number of patient days was 183,388 (median: 155,084 (range: 84,360–372,646).

Analysis of data from 1 January 2009 to 30 April 2012 from the 14 laboratories revealed a statistically significant increasing trend ($p<0.01$) in the proportion of *K.* *pneumoniae* isolates from clinical specimens that were non-susceptible to the selected carbapenems (See Table 1 of Sisto et al., 2012). The percentage of non-susceptibility was higher for isolates from the respiratory tract, pus and blood. The percentage of *K. pneumoniae* isolates non-susceptible to imipenem and/or meropenem was higher overall in isolates taken from patients in intensive care units and in medicine departments (See Table 2 of Sisto et al., 2012). As well as it has been suggested by Sisto et al. (2012) in Table 3, there are remarkable differences in the percentage of isolates non-susceptible to imipenem and/or meropenem among the 14 laboratories during the study period.

The automated systems used for susceptibility testing and the guidelines adopted by each laboratory are shown in Table 4, as well as it has been proposed in Sisto et al. (2012). Until 2010, all laboratories had adopted the Clinical Laboratory Standards Institute (CLSI) interpretive criteria. Before 2012, 13 of them moved to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) system (See Table 4 of Sisto et al., 2012).

[3] Available: http://ecdc.europa.eu/en/publications/Publications/110913_Risk_assessment_resistant_CPE.pdf
[4] Available: http://wwwn.cdc.gov/epiinfo/html/downloads.htm

Micronet network hospitals:

L. Bertinotti, Ospedale Civile di Legnano, Legnano; E. Bordi, INMI 'Lazzaro Spallanzani' IRCCS, Rome; P. Casella, Ao Ospedale Civile di Vimercate; M.G. Chirillo, Aso S. Luigi, Orbassano; P. Cichero, Ospedale S. Raffaele, Milan; P. Clerici, Ospedale Civile di Legnano, Legnano; S. Cuccurullo, Ospedale Monaldi, Naples; A. De Bernochi , Asl 4 Osp. San Giovanni Bosco, Turiny; G. De Intinis, OIRM S. Anna, Turin; P. A. Dusi, Asl 1 Ospedale di Sanremo, Sanremo; P. Lanzafame, Ospedale S. Chiara, Trento; L Lodolo, Asl 14 Ospedale di Verbania, Verbania; F. Milano, Asl 11 Ospedale S. Andrea, Vercelli; G. Monticone, Asl 19 Ospedali Riuniti di Asti, Asti; D. Morelli, Istituto Nazionale Tumori di Milano, Milan; M. Mori, Ospedale Galliera, Genoa; R. Provenzani, Aso Villa Sofia di Palermo, Palermo; A. Restelli, Fondazione Policlinico Milano, Milan; A. Rocchetti , ASO SS Antonio e Biagio e Cesare Arrigo, Alessandria; P. Sansone, Ospedale Galliera, Genoa; M. Tronci, Azienda Ospedaliera San Camillo Forlanini, Rome.

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