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Antimicrobials: Epidemiology of MDR-Gram-negatives

Epidemiology and molecular characterisation of carbapenem-resistant *Klebsiella pneumoniae* isolated over a 13-month period in an Italian hospital

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Objectives

The emergence and spread of carbapenem resistant *Klebsiella pneumoniae* in healthcare facilities has become an important issue, especially those showing decreased susceptibility to colistin and tigecycline. The aim of this study was to investigate the phenotypic/molecular features and the clonal relatedness of *K. pneumoniae* isolates with decreased susceptibility to carbapenems, collected during a 13-month period (September 2012-September 2013) at the INMI Spallanzani hospital in Rome.

Methods

A total of 151 *K. pneumoniae* strains were collected and identified by routine procedures (Vitek-2) and by matrix-assisted laser desorption ionization (Maldi)-time of flight (TOF) mass spectrometry (MS). Antibiotic susceptibility was determined by the microdilution method and phenotypic confirmation of carbapenemase production was performed by boronic acid-meropenem and EDTA-meropenem disc tests. Tigecycline and colistin resistance were confirmed by E-test or Kirby-Bauer methods. Polymerase chain reaction (PCR) analysis followed by sequencing was performed to detect the *bla*KPC gene. Molecular typing was carried out using Multi-Locus-Sequence-Typing (MLST) on the *rpoB, gapA, mdh, pgi, phoE, infB and tonB* genes, as previously described. Sequence types were assigned using the MLST Database of the Pasteur Institute.

Results

Of the *K. pneumoniae* isolates studied, 64/151 (42,4 %) showed multiple resistance to antibiotics, including carbapenems, and were positive for serine-beta-lactamases production in the boronic-acid disk test; 25 of them (39,0 %) were also resistant to colistin, and 15 (23,4 %) to tigecycline. The presence of the *bla*KPC gene was investigated in 11 of those 64 carbapenem-resistant strains, and their clonal relatedness was investigated. Molecular studies showed that all *K. pneumoniae* selected isolates possessed the KPC-3 gene (11/11 strains); of these, 9/11 belonged to the CC258 single clonal complex; in particular 6 strains belonged to the ST512 sequence type and 3 to ST258. The remaining two strains were ST101 and ST307.

Conclusions

The rapid detection of carbapenem-resistant *K. pneumoniae* is important to prevent dissemination of these dangerous strains. Here we describe the intra-hospital spread of the *K. pneumoniae* CC258 clonal complex harbouring the *bla*KPC-3 gene. This is in accordance with the epidemiological data reported for hospitals in central Italy, except for the type ST307 strain, which to our knowledge has not yet been described as harbouring the KPC-3 gene.