Significance of Screening Tests and the Incidence of New Delhi Metallo-beta-lactamase-Producing Gram-negative Bacilli in the Surgery and Transplantation Wards of a Warsaw Medical Center During the Period From April 2014 to May 2017

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ABSTRACT

Background. The first New Delhi metallo-beta-lactamase (NDM)-producing bacteria were isolated in 2008 in the world, and in 2011 in Poland. Due to the high clonal diversity (17 types) of their *bla*NDM gene, encoded on (Tn125-like) mobile genetic elements, these strains usually exhibit resistance to nearly all available antibiotics, which is particularly dangerous for organ transplant recipients.

Purpose. To assess of the prevalence of Gram-negative NDM-positive bacilli in surgery/transplantation wards of a teaching hospital in Warsaw and to ascertain the significance of screening tests on the rates and nature of colonization.

Materials and Methods. The evaluated strains were isolated from 30 patients (between April 2014 and May 2017). The species were identified with VITEK-MS, antibiotic susceptibility was determined with VITEK 2, disk-diffusion, and/or E-test methods, according to EUCAST guidelines. The presence of the *bla*NDM-1 gene was confirmed using the polymerase chain reaction technique.

Results and Conclusions. There were 77 *bla*NDM-1-positive *Klebsiella pneumoniae* strains isolated from 30 patients. Cultures from individual patients, mainly from rectal swabs (53.9%) and urine samples (39.8%), yielded 1–11 isolates. Fifteen patients were already colonized on admission, and the other 15 developed a symptomatic infection. In total, 24 (80%) patients were carriers, and their colonizations persisted for <1–20 months. Most isolates were susceptible only to colistin, gentamicin, amikacin, tigecycline, and/or sulfamethoxazole/trimethoprim. Gastrointestinal-tract-colonizing *K pneumoniae* are the main reservoir of the *bla*NDM-1 gene. Following the introduction of on-admission mandatory screening for carbapenem-resistant strains, the rates of NDM-producing *K pneumoniae* isolation increased (7.5-fold), while the rates of isolation from patients with symptomatic infections considerably decreased (2.8-fold).

UNTIL recently, carbapenems were considered to be antibiotics of last resort, effective in treating severe Gram-negative bacterial infections. The emergence of carbapenem-resistant bacterial strains, especially those exhibiting a plasmid-mediated enzymatic mechanism of resistance, caused serious therapeutic and epidemiological

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problems, particularly in inpatient settings. The carbapenemase-producing *Enterobacteriaceae*, which can produce the Ambler class B1 New Delhi metallo-beta-lactamase (NDM), are considered to be particularly dangerous. NDM-positive strains contain the *bla*NDM gene, responsible for their high level of resistance to all beta-lactam antibiotics approved in Poland, including all penicillin derivatives, first- to fifth-generation cephalosporins, and carbapenems; these strains are also resistant to beta-lactamase inhibitors [1].

*bla*NDM genes (17 genetic variants) are encoded on transposons Tn125-like, derived from *Acinetobacter baumanii* (bracketed by the ISAba125 insertion sequences) [2]. Tn125-like are most commonly transferred via highly mobilizable conjugative plasmids (eg, IncR, IncFII, IncA/C, IncL/M, IncN, IncX) and are characterized by a broad bacterial host spectrum, which allows them to be easily transferred via various mechanisms of horizontal gene transfer. This includes the possibility of transfer between Gram-negative bacilli belonging to different species and genera. Apart from *bla*NDM, these large and diversely sized plasmids (50–180 kb) may transfer gene-encoding virulence factors, those responsible for resistance to other classes of antibiotics (such as *aad*A1 or *rmt* [gentamicin, amikacin, tobramycin], *cml*A7 [chloramphenicol], and *qnr* [ciprofloxacin]), and those responsible for additional mechanisms of resistance to beta-lactam antibiotics (*amp*C, *bla*CTX-M, *bla*CMY-4, *bla*IMP, *bla*VIM, *bla*TEM, *bla*SHV, *oxa*48, and *kpc*). Moreover, there have been reports of *Escherichia coli* strains NDM-5 and NDM-9, which also encode the *mcr*-1 gene responsible for plasmid-mediated resistance to colistin [3,4].

Patients after surgery, organ transplant recipients, patients receiving immunosuppressive therapies, and patients receiving frequent and/or long-term antibiotic therapies are at high risk of infections with multidrug-resistant NDM-producing bacilli. The purpose of this study was to assess the prevalence of Gram-negative *bla*NDM-positive bacilli and evaluate the epidemiological status at surgery and transplantation wards of one of the teaching hospitals in Warsaw during the period from April 2014 to May 2017 (over 3 years). Moreover, the study attempted to verify whether early detection of asymptomatic bacterial colonization can help reduce the incidence of NDM-positive *Enterobacteriaceae* symptomatic infections.

MATERIALS AND METHODS

Bacterial Strains and Patients Tested in the Study

We analyzed strains of Gram-negative, carbapenem-resistant bacilli isolated from 30 patients treated in surgery and transplantation wards, as well as in an outpatient clinic during the period from April 2014 to May 2017. Demographic data (age and sex of tested patients) are presented in Table 1.

Strain Identification

The isolates were identified based on their unique protein profiles, with the use of mass spectrometry technology (VITEK-MS, bioMérieux, Marcy-l'Étoile, France), according to the manufacturer's instructions.

Antibiotic Susceptibility Testing and Strains Qualification

Antibiotic susceptibility testing was performed with VITEK 2 (AST-N330/-N332 cards) (bioMérieux), according to the manufacturer's protocol, as well as with a disk-diffusion method. When the results were inconclusive, minimum inhibitory concentration values were measured with an E-test (bioMérieux), according to EUCAST guidelines. Strains resistant to at least 1 carbapenem were qualified to be subjected to a double-disk susceptibility test, to assess their capability of producing Ambler class B metallo-beta-lactamases, and a Carba-NP test, to assess their capability of producing enzymes that hydrolytically inactivate carbapenem antibiotics.

Testing for Ambler Class B Metallo-beta Lactamase Production

Metallo-beta lactamase production testing was conducted using the ethylenediaminetetraacetic acid disk-diffusion method, according to the EUCAST protocol.

Testing for Carbapenemase Production (Carba-NP Test)

The procedure was conducted according to the Polish National Reference Center for Antimicrobial Susceptibility guidelines. Each bacterial isolate was concurrently evaluated in 2 test tubes: a control and an experimental one. A 0.1 mL aliquot of the B-PER II reagent (Bacterial Protein Extraction Reagent, Thermo Fisher Scientific, Waltham, Mass, United States) for bacterial lysis was added to either test tube, followed by half a loop of bacterial culture grown on Columbia agar with 5% sheep blood (bioMérieux). Then, 0.1 mL of buffer A was added to the control test tube, while 0.1 mL of solution B (12 mg of TIENAM [imipenem and cilastatin sodium; MSD, Warsaw, Poland] in 1 mL of buffer A) were added to the experimental test tube. The samples were incubated up to 2 hours at 37°C. The test result was obtained by comparing the color of the mixture in the 2 (control and experimental) test tubes. The result was positive if the experimental sample turned yellow or orange, while the control sample turned red.

*bla*NDM-1 Gene Detection

Genetic analyses were conducted with genomic DNA matrices, isolated with a commercially available Genomic Mini Kit (A&A Biotechnology, Gdynia, Poland), according to the manufacturer's instructions. The *bla*NDM-1 gene was detected via the polymerase chain reaction technique, according to the procedure described by Mulvey [5]. Amplification products were separated via horizontal gel electrophoresis in 1.5% agarose gels.

RESULTS

During the period from April 2014 to May 2017 a total of 78 isolated strains were found to harbor the *bla*NDM-1 gene (77 strains of *Klebsiella pneumoniae* [KP-NDM] and 1 strain of *Enterobacter cloacae*). In the individual years, that is, April-December 2014, 2015, 2016, and January-May 2017, *bla*NDM-1-positive isolates came from 4, 1, 14, and 11 patients, respectively. Each of these patients yielded 1–11 isolates, which were derived mostly from rectal swabs (53.9%), urine samples (39.8%), and blood samples and wound swabs (2.5% each). The earliest isolates obtained