Prevalence of multidrug-resistant *Klebsiella pneumoniae* strains isolated from patients with cardiovascular disease

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ABSTRACT

Klebsiella spp. are common Gram-negative pathogens, frequently encountered in nosocomial infections. The production of antibiotic-inactivating enzymes, such as ESBL (extended-spectrum beta-lactamases) and carbapenemases associated with multidrug-resistant (MDR) phenotypes are increasingly reported in the last decade, leading to very limited therapeutic options of the produced infections. The purpose of this study was to highlight the antibiotic profile of *Klebsiella spp.* strains isolated from clinical samples in patients with cardiovascular disease.

Materials and Methods: A number of 256 *Klebsiella* strains isolated from different clinical specimens were analysed for the antimicrobial susceptibility profiles testing by disk - diffusion and Vitek2 automated system. ESBL production was confirmed by the double disk test and carbapenemases producing by the modified Hodge test and E-tests.

Results: Out of the 256 isolated strains, multi-drug resistance (cephalosporins/aminoglycosides/quinolones) was encountered in 149 (58%) strains. A number of 53 (21%) strains were ESBL producers and 96(38%) were resistant to carbapenems, out of which, 41 (43%) were carbapenemase positive.

Conclusion: Due to the extremely low therapeutic alternatives in case of infections produced by MDR strains rapid identification of colonized or infected patients and screening of carriers, together with the routine detection of ESBL and carbapenemases by standard methods is highly recommended to be available in every clinical laboratory, to achieve effective antibiotic resistance management policies.

Key words: antibiotic susceptibility profile, ESBL, MDR, carbapenemases, double disk test, Modified Hodge test, E-test

INTRODUCTION

Klebsiella is one of the most common Gram-negative pathogens, frequently encountered in nosocomial infections (1-4). *Klebsiella spp.* are known as a cause of community-acquired pneumonia, in particular associated with chronic

alcoholism, with a high mortality rate, especially in the absence of adequate treatment (5). As an opportunistic pathogen, *Klebsiella spp.* occurs in patients with diabetes mellitus or chronic obstructive pulmonary disease.

MDR strains are a major problem in nosocomial infections management (6), and moreover, similar resistance profiles were found in *Klebsiella spp.* strains isolated from community infections (7). One of the universally recognized major reasons involved in the development of MDR is the widely misused antibiotic therapy (8). The prevalence of resistance due to ESBL production was also recently reported, in very rapid growth (9). ESBLs are enzymes that mediate resistance to third and fourth generation cephalosporins (GIII, GIV) and monobactams but do not affect susceptibility to cefamicine and carbapenems. ESBLs belong to the 2b of the 2 class after Bush, Jacoby and Medeiros classification schema, and to class A in Ambler molecular classification (10). ESBL production is plasmid mediated, and could easily be transferred in Enterobacteriaceae group, leading to the accumulation of resistance genes in strains containing multidrug-resistance plasmids (15-19). ESBL-producing microorganisms often contain determinants of resistance to aminoglycosides and fluoroquinolones and therefore treatment options are drastically limited (20-26).

Carbapenemases are beta-lactamases capable of hydrolyzing penicillins, cephalosporins, carbapenems and monobactam, being included in A, Band D of the Ambler molecular classification. Such enzymes producing bacteria cause severe infections in which beta-lactams are ineffective (11-14). The purpose of this study was to detect the prevalence of ESBL and

carbapenemases producing *Klebsiella spp.* strains in patients of cardiology clinics.

MATERIALS AND METHODS

There were studied 256 *Klebsiella spp.* strains collected from different biological specimens i.e.: urine, blood, respiratory tract secretions, wound and soft tissue secretions and prosthetic devices, over a ten-month period (from January 2014 to November 2014). Out of the 256 strains, 86 were isolated from urine, 68 from wounds, 72 from respiratory tract secretions, 21 from blood and 9 from prosthetic devices infections. Identification to species level was based on biochemical characteristics using chromogenic media: Chromatic Mueller Hinton (MH Chromatic), chromID ESBL, chromID Carba, Api20E and Vitek2 Compact System. Antibiotic susceptibility testing was done by the Kirby - Bauer - disc diffusion method and automatic Vitek II system (MIC), according to CLSI standard 2014 (27-37). Isolates resistant to cefotaxime or ceftazidime, were further subjected to confirmatory test by double disk method and/or E-test, for ESBL production. We used the following discs: cefotaxime (30 µg), piperacillin-tazobactam (100 µg /10 µg), ceftazidime (30 µg), and ceftazidime-clavulanic acid (30 µg /10 µg). After incubating the plates at 37° C, for 16-20 h in an aerobic atmosphere, there were observed and measured the diameters of growth inhibition zones around the disks. An increase of inhibition zone diameter with ≥ 5 mm for each antimicrobial agent tested in combination with clavulanic acid, versus inhibition agent zone test alone, confirmed the ESBL production in the studied strains (figure 1).

Figure 1 - ESBL production using double disk (left) and E-test (right)

Figure 2 - Carbapenemases production using Chromatic MH: Modified Hodge test (left), E-test MBL (right)

Phenotypic detection of carbapenemases producing strains was made for all isolates showing a decreased susceptibility to carbapenems revealed by disk-diffusion method. Modified Hodge test revealed that the strains with low susceptibility to carbapenems are producing carbapenemases. E-test was used for detection of MBL-producing strains teston Chromatic MH medium (figure 2).

RESULTS

Out of the 256 *Klebsiella spp.* strains, 252 were *Klebsiella pneumoniae* subsp. *pneumoniae* and 4 were *Klebsiella oxytoca*.

Antibiotic susceptibility profile: 43 strains were susceptible to all tested antibiotics excepting ampicillin, which belongs to the natural resistance phenotypes of these species. The prevalence of antibiotic resistance

was: cefazolin - 85%, cefuroxime - 82%, cefotaxime - 73%, cefepime - 70% gentamicin - 60%, amikacin - 25%, ciprofloxacin - 74%, nitrofurantoin - 48%, trimethorim / sulphametoxazole - 78%, piperacillin-tazobactam - 70%, ertapenem - 56%, imipenem - 49%, and meropenem - 49% (figure 3).

ESBL production was highlighted in 53 strains (21%), out of which, 14 strains (26%) were isolated from urine, 14 (26%) from respiratory tract secretions, 20(38%) from wound secretions, 3 (6%) from blood and 2 (4%) from prosthetic devices associated infections (figure 4).

Carbapenemases production was highlighted in 96 strains (38%) showing carbapenems resistance phenotypes to imipenem and meropenem. All 96 MDR strains were tested for carbapenemases production using the modified Hodge test. From these, 41 strains (16%) were Hodge positive, of which 15 were isolated collected from urine, 11 from respiratory tract secretions, 10

Figure 3 - Prevalence of antibiotic resistance phenotypes in *Klebsiella spp.* strains

Figure 4 - Prevalence of ESBL phenotypes in *Klebsiella spp.* strains

Figure 5 - Prevalence of carbapenemases producing *K. pneumoniae* strains established by the modified Hodge test

from wound secretions, 4 from blood and 1 from prosthetic devices associated infections (figure 5).

DISCUSSIONS

Klebsiella pneumoniae is the most common pathogen isolated from ICU and the most common species of carbapenem producing *Enterobacteriaceae*. The type of carbapenemase – producing *Enterobacteriaceae* depends of the country and may be associated with historical/cultural relationships and exchange of populations with other countries with high prevalence. The future trend of these carbapenemase – producing *Enterobacteriaceae* epidemics will be an increasing one, as the reservoir of cabapenemase

producers are growing worldwide. Cross- border transfer of patients, travel, medical tourism and refugees might also play an important role. Another important aspect of the genus *Klebsiella* is the rapid emergence of MDR strains that often leads to treatment failure, with dramatic consequences especially for immuno-suppressed patients.

Empiric antibacterial therapy without a secure background provided by laboratory assays, often leads not only to the emergence of antibiotic resistance but also to an increasing incidence of the mortality rate. Therefore, routine laboratory detection of ESBL and carbapenemases producing strains by standard methods is highly recommended to be available in every clinical lab, because carbapenemases production

in *Enterobacteriaceae* are mostly plasmid-encoded, which largely explain their common association with other resistance markers and their multidrug resistance patterns.

The increasing prevalence of infections with *Klebsiella spp.* was observed in patients aged between 20 and 65 years. Only 43 strains (17%) showed susceptibility to all tested antibiotics. Among the resistant strains, the highest resistance rates were recorded for the first generation cephalosporins (cefazolin - 85%) and second generation cephalosporins (cefuroxime - 82%), followed by third generation cephalosporins (cefotaxime - 73%) and piperacillin-tazobactam (70%). An increased rate of resistance was also observed for aminoglycosides (60%) and quinolones (74%). The strains exhibiting the ESBL phenotype were isolated with the highest rates in wound secretions (38%), followed by urine (26%), respiratory tract secretions (26%), blood (6%) and prosthetic devices associated infections (4%).

In this study, 10 patients were infected with MDR *Klebsiella spp.* strains, which were sensitive only to colistin and polymyxin B. These strains were identified as *Klebsiella pneumoniae subsp. pneumoniae* with one exception, i.e. *K. oxytoca* isolated from an urinary tract infection of acatheterized diabetic patient. These patients were treated with colistin (iv), and supportive therapy, but 6 of the 10 patients died. The dead patients were aged between 40 and 80 years and harboured polymicrobial infections with multiresistant strains and sepsis before exitus. Other bacterial species isolated from these patients except *Klebsiella pneumoniae* were *Staphylococcus aureus*, *Serratia marcescens* and *Pseudomonas aeruginosa*. The cause of death may be multifactorial, because none of these patients had *Klebsiella pneumoniae* bacteremia.

CONCLUSIONS

Although important nosocomial outbreaks with carbapenemase – producing *Enterobacteriaceae* have been often reported in our country, many cases were imported from other hospital units from the country and from abroad. Although dissemination of these strains mainly occurs among hospitalized patients, community acquisition is also increasing. The present study highlights the high prevalence of MDR *Klebsiella spp.* strains (38%), mainly *Klebsiella pneumoniae subsp. pneumoniae* in our hospital setting, being associated with limited therapeutic options and a high mortality rate.

These results underline the necessity for readily

available methods for the rapid identification of colonized or infected patients and screening of carriers must in every laboratory, contributing to a better surveillance of resistance transfer and spreading and to the optimization of the therapeutic strategies.

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