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# Epidemiology of carbapenem-resistant *Klebsiella pneumoniae* colonization: a surveillance study at a Turkish university hospital from 2009 to 2013☆

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ABSTRACT

Between June 2009 and December 2013, 4105 patients were screened for carbapenem-resistant *Klebsiella pneumoniae* (CR-Kp) colonization in a tertiary care university hospital. The antimicrobial susceptibility and resistance determinants of 279 (6.8%) CR-Kp isolates from single patients were investigated. Additional analysis was performed to evaluate the characteristics and various risk factors for infection in patients with colonization. Of the 279 isolates, 270 harboured OXA-48-like enzymes, and a single isolate harboured IMP-type carbapenemase. A high proportion of isolates were susceptible to carbapenems – except ertapenem. All isolates were susceptible to amikacin and most (94%) were susceptible to colistin and fosfomycin. There was consistent high-level resistance for all isolates to temocillin, piperacillin-tazobactam, amoxicillin-clavulanate and ticarcillin-clavulanate. When colonized and infected patients were compared, only prior carbapenem administration ($P = 0.003$), was found to be significantly associated with patients with CR-Kp infection.



## 1. Introduction

Carbapenem-resistant *Klebsiella pneumoniae* (CR-Kp) is a challenging nosocomial pathogen expressing multi-drug resistance phenotypes and causing infections with high mortality rates. Rapid spread of CR-Kp is being reported worldwide in several health care facilities during the last decade. The epidemiology of CR-Kp is diverse across countries and regions. Hence, local surveillance data for antimicrobial resistance and identification of risk factors for colonization and infection are of extreme importance (Cantón et al., 2012; Munoz-Price and Quinn, 2009; Nordmann, 2014).

The aims of this study were to determine the antimicrobial susceptibility and resistance determinants of CR-Kp isolates recovered from colonized patients during an active surveillance program. Additional analysis was provided to investigate the frequency for CR-Kp colonization along with the characteristics and various risk factors in patients with colonization and/or infection.

## 2. Materials and methods

### 2.1. Study population

An active surveillance program for screening of CR-Kp colonization was performed between June 2009 and December 2013 in Hacettepe University Adult and Oncology Hospitals, an 800- and a 100-bed tertiary care facilities, respectively, in the same campus. All neutropenic patients in various medical wards and the patients in medical/surgical intensive care units (ICUs), solid/bone marrow transplantation units and burn unit were included.

Perirectal swabs were collected from adult patients (>18 years) by the nurses of the Infection Control Committee. All patients hospitalized in any of the selected wards were screened once weekly for the entire duration of their hospitalization. Whenever CR-Kp colonization was reported, colonized patients were isolated and further screened until discharge or death or until after three consecutive negative perirectal swab cultures were obtained; whichever occurred earlier. If a patient was found to be not-colonized at the first screening culture, no further follow-up was undertaken, thus these patients were not included in the study.

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Data were collected from hospital charts for demographics, medical history and other potential risk factors for CR-Kp colonization and/or infections, retrospectively.

### 2.2. Isolation and identification of CR-Kp

In accordance with recommendations from the US Centers for Disease Control and Prevention (CDC), perirectal swabs were placed into universals containing 5 ml tryptic soy broth (Oxoid, UK) with an ertapenem 10 μg disk (ERT, BBL, USA) and sent to the Infectious Disease Research Laboratory for overnight incubation at 37 °C. The broth cultures were then inoculated onto MacConkey agar (Oxoid, UK), and lactose-fermenting colonies were identified with API20E (bioMérieux, France) and confirmed by MALDI Biotyper CA System (Bruker, Daltonics, Bremen, Germany). Carbapenem resistance was determined phenotypically with ERT Etest (bioMérieux, France) and the isolates with a MIC value of >0.5 mg/L were reported as CR-Kp (Cohen Stuart et al., 2010). Only the first CR-Kp isolate of each patient was included in the study.

### 2.3. Determination of carbapenem resistance genes

All 279 CR-Kp isolates obtained during the data collection period were screened for the presence of the 5 most prevalent carbapenemase genes (OXA-48-like, IMP, KPC, NDM-1, VIM).

DNA isolation method: Two colonies from an overnight culture were transferred into 300 μL of sterile milli-Q water containing 5–10% Chelex 100 (Sigma-Aldrich, CA) in a microcentrifuge tube and mixed thoroughly. The cell suspension was centrifuged at 13,000×*g* for 5 minutes. Two microlitres of the resulting supernatant were used in the multiplex PCR reaction.

Multiplex PCR: The presence of all 5 genes was tested in a multiplex PCR against all 279 CR-Kp isolates, plus controls. Each PCR reaction (25 μL) contained 12.5 μL of Taq PCR master mix (Qiagen); 5.5 μL sterile- RNase free water; 0.5 μL of each primer (100 μM, final concentration 2 μM); 2 μL of DNA template.

Singleplex PCR: The 5 pairs of primers for $bla_{\text{OXA-48}}$, $bla_{\text{VIM}}$, $bla_{\text{IMP}}$, $bla_{\text{NDM}}$ and $bla_{\text{KPC}}$ genes were also tested using conventional PCR to determine their amplification specificity in all 279 CR-Kp isolates (Ellington et al., 2007; Monteiro et al., 2012; Perry et al., 2011; Poirel et al., 2011). All control isolates were obtained from the National Collection of Type Cultures, London, UK and included the following: *K. pneumoniae* NCTC 13438 for KPC-3, NCTC 13440 for VIM-1, NCTC 13443 for NDM-1, NCTC 13442 for OXA-48, *Escherichia coli* NCTC 13476 for IMP and *E. coli* NCTC 10418 (negative control).

### 2.4. Antimicrobial susceptibility.

Susceptibility testing was performed for 271 isolates producing carbapenemases. Isolates were tested against ertapenem, meropenem, imipenem, doripenem, tigecycline and colistin by agar dilution MIC method using BSAC methodology (Andrews, 2001) and against temocillin, piperacillin-tazobactam, amoxicillin-clavulanate, aztreonam, cefuroxime, ceftazidime, amikacin, ceftriaxone, ticarcillin-clavulanate, fosfomycin, trimethoprim-sulfamethoxazole, ciprofloxacin and gentamicin by disk diffusion method (Matuschek et al., 2013). All results were interpreted according to EUCAST breakpoint criteria, where available (EUCAST, 2015). Since EUCAST zone diameter breakpoints are not available for fosfomycin, breakpoints proposed by Barry et al were used (Barry et al., 1993) *E. coli* ATCC 25922 was included with every batch of susceptibility tests as recommended (EUCAST, 2015).

### 2.5. Statistical analysis

Data were analyzed using SPSS ver.11.5 statistical software package for Windows (SPSS Inc., Chicago, IL, USA). Statistical analysis included frequency and percent distributions. Group comparisons were assessed using chi-square test, Fisher's exact test and Student's *t* test, Mann-Whitney *U* test for categorical and continuous variables, respectively. Statistical significance was assigned to a *P* value of less than 0.05.

## 3. Results

A total of 4105 patients were included in the study; 7014 consecutive perirectal swabs were collected.

### 3.1. Patient characteristics and risk factors for colonization and infection with CR-Kp

Of the 4105 patients screened, 279 patients (6.8%) were detected with CR-Kp colonization. Among colonized patients, 8 (2.9%) had CR-Kp bloodstream infection (BSI). Data from all 279 patients' files indicated that 57.6% were from ICUs, 58.7% were male and mean age was 56.7 years. At least one underlying disease (including, diabetes mellitus, acute/chronic organ failure or malignancy) was identified in 86.3% of the patients. Immunosuppression (including long-term corticosteroid usage, transplantation, neutropenia or anti-cancer chemotherapy) and prior hospitalization in the preceding 6 months were present in 44.3% and 17.6% of patients, respectively. The median length of stay in the hospital was 47 days (ranged from 1 to 524 days). Invasive procedures were identified in 83.4% of patients (57.9% central-, 46.1% urinary-catheterization). Of the patients, 35.8% had undergone surgical procedures (22.1% elective, 14.8% emergency). All patients had been given at least one antibiotic over the preceding 30 days. Patients who have been given carbapenem group of antibiotics either meropenem or imipenem were 47.9%. Death occured in 30.6% of patients during their hospital stay (Table 1).

Of the 8 patients with BSI, half were from ICU, 6 were males and the average age was 59.8 years (SD = 12.4). At least one underlying disease (including diabetes mellitus, acute/chronic organ failure, malignancy) or immunosuppression (long-term steroid usage, transplantation, neutropenia, chemotherapy) was identified in 7 patients. Two patients had been exposed to prior hospitalization in the preceding 6 months. The median length of stay in the hospital was 68 days (min 21 to max 432 days). Invasive procedures were identified in 6 patients (5 central-, 4 urinary-catheterization). Of the patients, 3 had undergone surgical procedures (2 elective, 1 emergency). Antibiotics were administered to all patients over the preceding 30 days and all were given carbapenem group of antibiotics either meropenem or imipenem. All patients were followed up through hospital discharge or death; 4 died due to CRKP infection (Table 1).

Considering 8 patients with BSI as a subgroup of 279 colonized patients; all statistical tests were conducted for comparison of those 8 patients with 271 CR-Kp colonized but noninfected patients (Table 1). All characteristics other than prior carbapenem (meropenem or imipenem) administration, ($P = 0.003$), were not statistically significant.

The distribution of patients included in the surveillance programme and the percentages of CR-Kp colonization by years were as follows: 484, 837, 875, 1316, 593 patients were screened; 43 (8.9%), 49 (5.9%), 34 (3.9%), 84 (6.4%), 69 (11.6%) were found to be colonized each year, respectively. Percentages of colonization was statistically significantly different ($P < 0.0001$) across the years.

The proportion of patients with ICU stay statistically significantly differed ($P < 0.0001$) across the years: ICU stay was recorded in 69.7%, 73.4%, 91.1%, 63.0%, 56.5% of colonized patients over 4-years between 2009–2013. On the contrary, time to colonization was not associated with stay in ICUs ($P = 0.415$) (Fig. 1).

### 3.2. Detection of carbapenem resistance genes

Of the 279 isolates, 8 isolates (all were from colonized but not infected patients) were negative for all carbapenemase genes and were fully susceptible to meropenem. Of the remaining isolates, 270 harboured

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