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Clinical Impact of a PCR Assay for Rapid Identification of *Klebsiella pneumoniae* in Blood Cultures▽

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The clinical impact of a rapid PCR identification assay for *Klebsiella pneumoniae* in positive blood cultures was prospectively evaluated. Multivariate analysis identified the rapid PCR assay as the only significant factor in decreasing the time lapse preceding the initiation of appropriate antimicrobial therapy (hazards ratio, 3.03; confidence interval, 1.62 to 5.68; *P*, 0.001).

The incidence of sepsis and the number of sepsis-related deaths are increasing (8). Inappropriate antimicrobial therapy for the microorganisms causing sepsis has been associated with a significantly higher mortality rate than the use of an appropriate initial regimen (4, 5, 14).

In our institution, *Klebsiella pneumoniae* is the second most frequent organism grown in blood cultures of patients with bacteremia caused by gram-negative organisms. Though the incidence of extended-spectrum β-lactamase (ESBL) production by *K. pneumoniae* is in general high, it varies widely geographically (2, 15). The prevalence of ESBL-producing *K. pneumoniae* in Israel has been reported to be 32% (1). At the Rambam Healthcare Campus, a tertiary medical care center, the prevalence of ESBL production by *K. pneumoniae* in blood cultures is about 50% (Laboratory of Clinical Microbiology, Rambam Medical Center, Haifa, Israel, unpublished data). ESBL production confers resistance to commonly used antibacterial agents, such as penicillins, cephalosporins, and monobactams (13). Coresistance to other classes of antibiotics, like aminoglycosides and fluoroquinolones, is common (6, 7). A delay in the administration of appropriate antibacterial therapy increases mortality in patients with ESBL-producing *K. pneumoniae* (9).

A rapid identification assay for *K. pneumoniae* was developed at our institution. Identification of *K. pneumoniae* is made possible within 3 to 4 h after initial growth in blood culture bottles. The assay identifies a unique genetic sequence of the *K. pneumoniae* hemolysin gene by PCR and was found to be both 100% sensitive and 100% specific (T. Kotlovsky, L. Austin, R. Shalginov, and H. Sprecher, unpublished data). We hypothesized that earlier detection of *K. pneumoniae* in blood cultures would lead to a more rapid administration of appropriate antimicrobial agents.

In this single-center, prospective study, *K. pneumoniae* bacteremia was identified either by the rapid identification assay or by conventional microbiological identification. A comparison of the time schedules and rates of administration of appropriate antimicrobial therapy for the two groups was performed. In addition, the lengths of hospitalization, total mortalities, and mortalities related directly to the infection were compared.

The study was conducted prospectively in the microbiological laboratory of a tertiary care medical center in Haifa, Israel, within a period of 9 months in 2006. All growth-positive blood cultures taken from adult patients with gram-negative rods on the initial Gram stain were included. The etiological agent was further identified either by the rapid identification assay or by conventional microbiological methods. For practical reasons, cultures examined during weekdays before 11 a.m. were examined by the rapid assay, while cultures examined over the weekends or after 11 a.m. were examined by the conventional method.

The study group included patients with *K. pneumoniae* bacteremia diagnosed using the rapid identification assay. The control group included patients with *K. pneumoniae* bacteremia identified by conventional methods.

Exclusion criteria included either identification of more than one type of bacteria in the initial Gram stain or death of the patient before the initial results of the Gram stain were available. The study was approved by the institution's ethics committee.

Blood cultures were performed using the automated Bactec 9240 system (Becton Dickinson, Franklin Lakes, NJ). Bacterial isolates were identified to the genus level using conventional biochemical methods. The PCR assay was performed as described elsewhere (Kotlovsky et al., unpublished). DNA was extracted from positive blood culture bottles using the benzyl-alcohol-guanidine hydrochloride method and was PCR amplified using a pair of primer oligonucleotides encompassing the *K. pneumoniae* hemolysin gene (EMBL accession no. AF293352) and ReddyMix PCR master mix (ABgene) according to the manufacturer's instructions. Cycling conditions were 94°C for 3 min, followed by 30 cycles consisting of 94°C for 20 s, 60°C for 30 s, and 72°C for 30 s. Every sample was also assayed using primer pairs specific to the conserved 5′-end of the 16S rRNA gene (inhibition control). All cases of *K. pneumoniae*

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bacteremia diagnosed by the rapid identification assay were further confirmed by conventional methods as mentioned above. Antimicrobial susceptibility, including ESBL production, was determined by disk diffusion according to the Clinical and Laboratory Standards Institute guidelines (3, 12).

For both groups of patients, the attending physicians were notified by telephone of positive blood cultures with gram-negative rods according to the institution's standard procedure. The PCR assay was then performed for the study group. If the assay was positive for *K. pneumoniae*, the attending physician was notified (at the end of the working day, 3 to 4 p.m.) that *K. pneumoniae* had been detected and was reminded of the high prevalence of ESBL production by *K. pneumoniae* at the institution. Carbapenems were suggested as therapy until antimicrobial susceptibility tests became available. For the control group, no intervention was made. Because of an outbreak of imipenem-resistant *K. pneumoniae* in the general intensive care unit, some of the patients found to have *K. pneumoniae* bacteremia in that unit were treated empirically with colistin before susceptibility results were available.

Demographic and medical data were prospectively obtained from medical charts and from the institution's computerized medical information system.

Continuous variables were described by mean ± standard deviation and median. Univariate comparisons between cases and controls were done with the Mann-Whitney test as appropriate. Survival curves, prepared by means of the Kaplan-Meier method, described the time lapse between the drawing of relevant blood culture and the administration of appropriate antimicrobial treatment.

Multivariate analysis was performed by stepwise Cox regression analysis; here, hazards ratios (HR) and 95% confidence intervals (CI) are reported.

The Mann-Whitney nonparametric test was used to compare the lengths of stay of the cases and controls. All *P* values were two tailed; a *P* of ≤0.05 was considered statistically significant. All statistical analysis was done with the use of SPSS 14.0 software (SPSS Inc., Chicago, IL).

Over a period of 9 months, 1,359 growth-positive blood cultures from 576 patients with gram-negative rods on the initial stain were detected. For 479 cultures from 272 patients, PCR was performed, and 62 cases (22.8%) of *K. pneumoniae* bacteremia were identified by the PCR assay. A total of 880 blood cultures from 304 patients were analyzed conventionally. *K. pneumoniae* bacteremia was diagnosed by conventional methods in 79 patients (26.0%). Nine patients from the study group and 13 patients from the control group were excluded according to the exclusion criteria. Eleven patients from the study group and 24 patients from the control group were not included in the statistical analysis due to lack of adequate follow-up. The study and control groups eventually consisted of 42 patients each. The sensitivity and specificity of the rapid PCR assay were found to be 100% as assessed against conventional blood culture results.

There was no statistically significant difference between the two groups with regard to age, gender, place of residence (home or long-term-care facility), underlying illnesses, fever and white blood cell count during drawing of the relevant blood culture, time to positivity, percentage of patients receiving appropriate initial antimicrobial therapy, the cause of the patient's admission, or the ward to which he or she was admitted ($P > 0.15$ for all comparisons; data not shown).

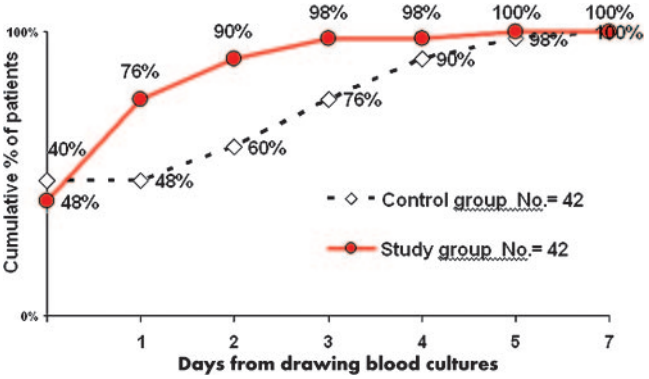

FIG. 1. Cumulative percentage of patients receiving appropriate medical therapy.

The percentages of *K. pneumoniae* producing ESBL were 52.4% for the study group and 47.6% for the control group ($P = 0.83$). It is noteworthy that 15% of the isolates were resistant to imipenem.

Appropriate empirical therapy was administered to 40.5% of patients in the study group and 47.6% of patients in the control group ($P = 0.66$). Patients in the study group whose initial empirical treatment was inappropriate received appropriate antimicrobial therapy sooner than patients in the control group (for the study group, 1.64 ± 1.0 days after the relevant blood culture was obtained, median = 1.0 day; for the control group, 3.5 ± 1.2 days, median = 3.0 days; $P < 0.0001$). This effect was largely accounted for by a "correct" change made 1 day after inappropriate empirical treatment had been administered to 15 patients in the study group. In 11 out of these 15 patients, this change could be traced directly to PCR identification of *K. pneumoniae* on the same day. In the control group, no beneficial changes in antimicrobial treatment were introduced on the day following the initiation of empirical therapy despite a growth-positive signal in cultures from seven patients. In seven patients in the study group, unnecessary carbapenem treatment was administered for 1 to 3 days following the rapid assay diagnosis of *K. pneumoniae* bacteremia. The change in the percentage of patients receiving appropriate antimicrobial therapy over time is demonstrated in Fig. 1.

We performed a multivariate analysis to reveal significant factors influencing the rate of appropriate antimicrobial therapy administration. These variables included patient's age, gender, cause of admission, underlying illnesses, length of hospital stay before bacteremia occurred, percentage of hospital-acquired infections, and the type of hospitalization ward. Implementation of the rapid PCR identification was the only variable with significant influence (HR = 3.03, CI = 1.62 to 5.68, $P = 0.001$). ESBL production was also associated with slower appropriate treatment, but this trend did not reach statistical significance (HR = 2.1, CI = 0.98 to 4.5, $P = 0.054$).

The total length of hospitalization, length of hospitalization after *K. pneumoniae* bacteremia had been diagnosed, and mortality did not differ between the two groups ($P > 0.8$ for all comparisons; data not shown). One patient in the study group

and four patients in the control group died of *Klebsiella* sepsis during the 72 h following the drawing of the blood culture ($P = 0.20$).

Emerging new techniques for faster diagnosis of bloodstream infection have been assessed in recent years (10, 11). These new molecular assays are expected to eventually replace conventional blood cultures (11). Theoretically, *K. pneumoniae* is an important "candidate" for such rapid diagnosis techniques due to rapidly increasing levels of antimicrobial resistance and ESBL production. In this study, the use of such rapid diagnosis is shown to have a meaningful clinical impact, namely, a more rapid administration of appropriate antimicrobial therapy. Although this reduction is statistically nonsignificant, it has been previously demonstrated that earlier appropriate treatment of ESBL-producing *K. pneumoniae* reduces mortality.

This study has the limitations of a small sample size and of a single-center, nonblind, nonrandomized design. More clinical outcome studies are necessary to evaluate both the assay described above and other methods aimed at decreasing the time lapse preceding microbiological diagnosis.

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