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Health and economic outcomes of the detection of *Klebsiella pneumoniae*-produced extended-spectrum β-lactamase (ESBL) in a hospital with high prevalence of this infection

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Summary

Introduction: Klebsiella pneumoniae is of high prevalence in hospital infections, mainly in bloodstream infections (BSI), and some produce extended-spectrum β-lactamase (ESBL). For hospitals with a high prevalence of strains producing this enzyme, there is no reference material to show whether the use of the E-test method for their detection, which can be quite expensive, is actually required.
Objective: To evaluate the cost-benefit of the disk diffusion and E-test methods for the detection of ESBL-producing *K. pneumoniae* strains in hospitals where a high prevalence of this resistance mechanism in BSI is found.
Methods: One hundred and eight patients with *K. pneumoniae* BSI were evaluated retrospectively. ESBL-producing strains were identified by the disk diffusion method and by the E-test method. We estimated the costs of both diagnostic methods based on antimicrobial therapy adequacy.
Results: Fifty-two percent of *K. pneumoniae* infections were due to ESBL-producing strains. The disk diffusion method yielded a positive predictive value (PPV) of 94.7% (95% CI: 88.9—100%) and a negative predictive value (NPV) of 96.1% (CI 95%: 90.8—101.4%) in relation to the E-test. We evaluated cost-effectiveness, i.e., we analyzed the cost of both E-test and disk diffusion methods with carbapenem and cephalosporins, and found that the use of the disk diffusion method accounts for approximately US$3300.

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Conclusions: In hospitals with a high prevalence of ESBL-producing strains, the disk diffusion method can be used to detect ESBL-producing *K. pneumoniae* without compromising the clinical progression of patients with BSI. The E-test showed higher accuracy but this method was more expensive than the disk diffusion method. However, the use of the E-test method was demonstrated to be more cost-effective, as we evaluated cost based on antimicrobial therapy adequacy.

Introduction

Production of β-lactamases in Gram-negative bacteria represents their main mechanism of resistance to β-lactam antimicrobials. With particular regard to *Klebsiella pneumoniae*, greater resistance is mediated by the extended-spectrum β-lactamases (ESBL), so-named because of the number of substrates they reach.[1]

These mutant enzymes provide increased levels of resistance to cefotaxime, ceftazidime, to the further extended-spectrum cephalosporins and to monobactams, such as aztreonam. They show no activity against cephamycins or imipenem.[2]

Prevalence of ESBL-producing *K. pneumoniae* strains in hospitals ranges from 5 to 25% in several parts of the world.[3–6]

The lack of a rapid and effective method of determining resistance mechanisms and no gold standard for the detection of ESBL, have made the rapid administration of suitable antimicrobial treatment difficult. With regard to hospitals having a high prevalence of strains producing this enzyme, there are no studies showing whether the use of the E-test for their detection, which can be quite expensive, is actually required.

The aim of this study was to determine the cost-benefits of the disk diffusion and E-test methods for the detection of ESBL-producing *K. pneumoniae* strains in hospitals where a high prevalence of this resistance mechanism in bloodstream infections (BSI) is found.

Materials and methods

This is a retrospective cohort study, carried out at the Federal University of São Paulo, a 624-bed academic tertiary care hospital, located in São Paulo State, Brazil. The study took place between January 1996 and May 2001. All patients (the majority being adults) whose blood culture results were positive for *K. pneumoniae*, were eligible for inclusion. We excluded patients with false bacteremia by *K. pneumoniae*, defined as the presence of positive blood culture without clinical manifestations of infection, and patients with incomplete data in their medical charts. Each patient was included only once. If multiple blood cultures from the same patient were positive for the above organism, only the first episode was reviewed and recorded.

Antimicrobial adequacy

Inadequate empiric antimicrobial treatment was defined as therapy administered within 24 hours following blood culture, that included the administration of an antimicrobial agent to which the *K. pneumoniae* isolate was resistant. Antimicrobial agents were considered adequate if the organism was susceptible, except when cephalosporins were used for the treatment of ESBL infections.[4] A correction was defined when the antimicrobial agent was changed according to the antimicrobial susceptibility test results within 48 hours after the blood culture had been drawn.

Outcome

Mortality related to bacteremia was considered to be present when a patient died within fifteen days of the start of treatment and the death could not be directly attributed to any other cause.

Microbiological methods

Blood cultures (consisting of two blood culture bottles including aerobic and anaerobic resin-containing media) obtained from patients were processed using the BACTEC 9240 blood culture system (Beckton Dickinson, USA). Growing isolates were identified according to routine bacteriological procedures. For every *K. pneumoniae* sample, a bacterial suspension in Muller–Hinton broth was performed, to achieve 0.5 equivalent turbidity on McFarland's scale. After homogenization of the suspension, it was spread on two 15×150 mm Muller–Hinton agar plates and then the following antimicrobial disks were placed on the plates: amikacin 30 μg, aztreonam 30 μg, cephalothin 30 μg, cefepime 30 μg, cephoperazone 75 μg, cefotaxime 30 μg, cefoxitin 30 μg, ceftazidime 30 μg, ceftriaxone

30 μg, cefuroxime 30 μg, ciprofloxacin 5 μg, gentamicin 10 μg, imipenem 10 μg and meropenem 10 μg.

The plates were incubated at 35 °C for 18–24 hours, and the inhibition zones were then read. Antimicrobial susceptibility testing was performed by the disk diffusion method, according to the Clinical and Laboratory Standards Institute (CLSI, formerly National Committee for Clinical Laboratory Standards – NCCLS).[7,8]

ESBL detection

The *K. pneumoniae* strain was considered to have an ESBL phenotype if it demonstrated a reduced inhibition zone diameter for ceftazidime (22 mm) or ceftriaxone (≤25 mm) or aztreonam (≤27 mm).

ESBL production was confirmed with the E-test ESBL strips (AB BIODISK®).[9] The E-test ESBL strip is an inert plastic strip that has an antimicrobial concentration gradient on both ends of one of its surfaces. One of the ends has ceftazidime concentrations ranging from 0.5 μg/mL to 32 μg/mL and the other end, ceftazidime with a concentration gradient ranging from 0.064 μg/mL to 4 μg/mL, combined with clavulanic acid in a fixed concentration of 4 μg/mL. E-test ESBL strips containing cefotaxime were also used. Like the ceftazidime strip, the strip containing cefotaxime has a drug concentration gradient on both ends. On one of the ends, the cefotaxime concentration ranges from 0.25 μg/mL to 16 μg/mL and on the other end, an increasing concentration of cefotaxime ranging from 0.016 μg/mL to 1 μg/mL combined with the fixed 4 μg/mL concentration of clavulanic acid. The E-test was considered the gold standard method for the detection of ESBL-producing strains. Disk diffusion results achieved by both lab methods were in agreement.

Costs

To detect antimicrobial susceptibility using the disk diffusion screening method (twelve disks per plate), US$0.60 was spent per *K. pneumoniae* strain. To detect the ESBL-producing *K. pneumoniae* strains, two E-test strips were used at a cost of US$10.60.

For antimicrobial treatment of BSI caused by an ESBL-producing strain we used for calculation purposes, the standard adult dose for normal renal function which is 2.0 g imipenem per day for 14 days, corresponding to US$1679.92 per patient treated. Likewise, we used the same calculation basis for the antimicrobial treatment of BSI caused by a non-ESBL producing strain using 2.0 g ceftriaxone daily for 14 days, corresponding to US$295.96.[10] Patients deemed false negative for the production of ESBL, who were given cephalosporins, we considered as having been given five days of ceftriaxone and then 14 days of imipenem, following the assumptions of Paterson et al.,[5] of antimicrobial therapy failure or death for the patients with BSI caused by the ESBL-producing strain and not treated with cephalosporins. We estimated the costs of the E-test and disk diffusion methods based on antimicrobial therapy adequacy (calculations below) and then the cost difference between the two methods within the population studied, comparing them to hospitals with 2, 10, 25 and 52% prevalence of ESBL strains as shown in our study.

Calculations

Cost of E-test/antimicrobial therapy adequacy = (cost of E-test × total patients) + (number of ESBL strains positive × cost of treatment with imipenem) + (number of ESBL strains negative × cost of treatment with ceftriaxone).

Cost of disk diffusion/antimicrobial adequacy = (cost of disk diffusion × total patients) + (number of strains suggestive of ESBL positive × cost of treatment with imipenem) + (number of strains suggestive of ESBL negative × cost of treatment with ceftriaxone) + (number of false negative cases × cost of ceftriaxone for 5 days) + (number of false positive cases × cost of treatment with imipenem).

Results

During the study period, 115 patients with *K. pneumoniae* BSI isolates were identified, 108 of these met criteria for inclusion. ESBL production by

Table 1 Disk diffusion and E-test methods ratio for detection of ESBL-producing *K. pneumoniae* samples.

		E-Test		
		E-test +	E-Test –	Total
		n (%)	*n* (%)	*n* (%)
Disk-diffusion	ESBL-suggestive strain	54 (96.4)	3 (5.8)	57 (52.8)
	ESBL-non-suggestive strain	2 (3.6)	49 (94.2)	51 (47.2)
	Total	56 (100)	52 (100)	108 (100)

n = number of cases; (%) = percentage.

Table 2 Disk diffusion with positive and negative predictive values according to ESBL prevalence.

ESBL Prevalence	2%	10%	25%	52%
PPV (%)	25.4	65.1	85.7	94.7
NPV (%)	99.9	99.6	98.8	96.1

PPV, positive predictive value; NPV, negative predictive value. Sensitivity = 96.4%, Specificity = 94.2%.

K. pneumoniae was present in 51.9% (56/108) of patients.

The concordance between the disk diffusion and E-test methods is shown in Table 1 and corresponds to 95.4% (103/108 cases).

When disk diffusion susceptibility test data were used to establish the antimicrobial treatment, as few as 1.9% (2/108) of false negative cases failed to be adequately treated. Thus, disk-diffusion showed a positive predictive value (PPV) of 94.7% (95% CI: 88.9—100%) and negative predictive value (NPV) of 96.1% (95% CI: 90.8%—101.4%).

By using different ESBL prevalence values we were able to find different PPV and similar NPV, as seen in Table 2.

Antimicrobial therapy was considered adequate in 63.9% (69/108) of cases, inadequate in 27.8% (30/108) and adjusted in 8.3% (9/108). The adequacy of antimicrobial therapy was similar when comparing ESBL and non-ESBL producing strains of *K. pneumoniae*. Mortality related to *K. pneumoniae* up to 15 days after the BSI, occurred in 26/108 (24.1%) patients. In 18 patients (16.7%), death was not related to *K. pneumoniae* BSI. The overall mortality rate if analyzing the full hospitalization period was 40.8%.

Of the five patients presenting with different results from the disk diffusion and E-test, three had false positive results, two of which progressed to death. One of those patients had liver disease and was given inadequate antibiotic therapy (cephalosporin) despite susceptibility test data suggestive of the ESBL-producing strain; the patient's death was related to blood stream infection. The other patient was a newborn with an insulin hypersecreting abdominal tumor, hospitalized for eight months who progressed to non-infection-related death.

Although disk diffusion showed a high positive predictive value (94.7%), in an institution with a high prevalence of the ESBL-producing *K. pneumoniae* strain (52%), we calculated the costs of the diagnostic methods used for the detection of this resistance mechanism, i.e., E-test and disk diffusion, by correlating diagnostic costs with the use of adequate antimicrobial therapy and the prevalence of this enzyme in other hospitals (Table 3).

Discussion

Most studies identify ESBL-producing *K. pneumoniae* strains by using the E-test ESBL strips.[1,3,4,11,12] In the present study we show a high prevalence (52%) of ESBL-producing strains in BSI. Prevalence of ESBL-producing strains detected by clinical microbiology laboratories using the E-test methodology ranges from 5% in Canada, 8% in the United States, to 23% in Europe and 25% in the Pacific western region.[3]

Paterson et al., in an observational prospective study involving 12 hospitals in the United States, Taiwan, Australia, South Africa, Turkey, Belgium and Argentina, analyzed 455 *K. pneumoniae* bacteremias, detecting 30.8% episodes of nosocomial bacteremia caused by ESBL-producing strains, by the E-test.[5] Resistance detection, by using either ceftazidime or aztreonam as screening for the detection of ESBL-producing strains, failed to establish the diagnosis in 15—20% of cases.[11] However, it should be pointed out that the prevalence of ESBL-producing strains was less than 25% in those studies.[1,2,11]

None of the studies correlated the progression of patients with *K. pneumoniae* infections according to the final susceptibility test data by disk diffusion with the E-test, or evaluated the costs of such diagnostic methods with adequate antimicrobial therapy.

We considered the use of carbapenems in all *K. pneumoniae* strains thought to be suggestive of ESBL by disk diffusion, in accordance with Paterson et al.,[6] and found that the use of carbapenems during the 5-day period after onset of bacteremia due to an ESBL-producing organism, was independently associated with lower mortality.

Table 3 Estimated cost of disk diffusion and E-test methods based on antimicrobial therapy adequacy in 108 patients with *K. pneumoniae* blood stream infections according to ESBL prevalence.

Cost (US$)	ESBL Prevalence			
	2%	10%	25%	52%
E-test	37260.36	48332.04	70475.40	110610.20
Disk-diffusion	44484.12	55555.80	76420.90	113893.50
Cost difference	7223.76	7223.76	5945.50	3283.28

Cost difference = E-test method — disk diffusion method (implicit antimicrobial therapy adequacy).

By using prevalence of ESBL-producing strains with disk diffusion, we found that in institutions with high prevalence of *K. pneumoniae* producing that enzyme, i.e., over 50%, the PPV of the disk diffusion test is high and therefore, the E-test would not be required. In our study the two false-negative patients (1.9%) were inadequately treated using the disk diffusion method. Those two cases did not progress to death, although we used only third-generation cephalosporins. On the other hand, if we evaluate cost-effectiveness, i.e., if we analyze the cost of both E-test and disk diffusion methods with the adequate use of carbapenem and cephalosporins, in this 108-patient population, we find that approximately US$3300 is spent when the disk diffusion method is used. In sites of low prevalence of ESBL-producing *K. pneumoniae*, the disk diffusion test shows a low PPV (Table 2) and even increased cost-effectiveness, approximately US$7200, if disk diffusion is not used (Table 3). This is because the false positive cases are treated with carbapenem agents, with a 14-day treatment cost of approximately US$1680. In our study, we had three cases, i.e., an excess cost of US$5040. If there had been no false positives, these cases would have been treated with cephalosporins (ceftriaxone, for the calculation) and US$888 would have been spent, thus saving US$4152.

The above means that the cost of adequate treatment with carbapenems extends beyond the cost of the diagnostic method for the detection of ESBL-producing strains.

On the other hand, we could have used a confirmed test (with clavulanate disk) for ESBL producing strains.[7] It would be able to detect false-positive cases in the same way as the E-test, however it would not increase the sensitivity of the test. It could be used as another alternative to the E-test and the cost would be lower, but we believe that the sensitivity is higher with the E-test method.

In conclusion, disk diffusion may guide appropriate antimicrobial treatment of blood stream infections caused by ESBL-producing *K. pneumoniae* in hospitals with a high prevalence of this resistance mechanism. However, although the E-test is an expensive laboratory method, when we evaluated cost based on antimicrobial therapy adequacy, cost-effectiveness accounted for US$3300 when patient treatment was guided by this method, as compared to the disk diffusion method. Thus, for adequate treatment of patients with BSI due to *K. pneumoniae*, performance of the E-test screening is justified.

Conflict of interest: None of the authors have commercial associations or financial involvement that might pose a conflict of interest in connection with this article.

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