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Original Article

Antibiogram, ESBL production and carbapenemase detection of Klebsiella spp. in hospital-acquired infection

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ABSTRACT

Background: Infections with ESBL *Klebsiella pneumoniae* are increasing, particularly among patients in ICUs. This pathogen is usually multidrug-resistant and there are limited treatment options available. Active surveillance for ESBL-producing pathogens in high-risk populations should be performed using appropriate antimicrobial techniques. The carbapenems, that is, imipenem and meropenem, are safe and effective antibiotics for the treatment of severe ESBL-producing *K. pneumoniae* infection in preterm infants.

Purpose: To study the antibiotic sensitivity, detection of ESBL production and detection of carbapenemase of *Klebsiella* spp. in hospital-acquired infection.

Methods: Clinical specimens were collected in aseptic conditions and were cultured and suspected Gram-negative organisms were identified. Antimicrobial susceptibility was performed by modified Kirby–Bauer sensitivity testing methods. Suspected ESBL-producing isolates were tested further for ESBL production and confirmed. Screening and confirmatory tests were also performed for carbapenemase production.

Result: During the study, 170 patients with *K. pneumoniae* isolates were identified. ESBL-producing *K. pneumoniae* was detected in 30 of 170 patients (17%), AmpC β-lactamase producer in 109 of 170 patients (64%) and carbapenemase producer in 53 of 170 patients (31%). The most frequent sources of infection were blood (18%), pus and wound swab (18%), respiratory (32%) and urinary (29%).

Conclusion: The type of ESBL enzyme produced and the site and severity of infection are important considerations in determining antimicrobial therapy. Therefore, active surveillance for ESBL-producing organisms is critical to describe fully the local epidemiology of a given institution and/or referring centres; currently, the carbapenems, that is, imipenem and meropenem, are the only class of antimicrobials that have consistently been effective against ESBL-producing *K. pneumoniae*.

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1. Introduction

Bacteria belonging to the genus *Klebsiella* frequently cause human nosocomial infections. Amongst these, *Klebsiella pneumoniae* medically accounts for a significant proportion of hospital-acquired urinary tract infections, pneumonia, septicaemias and soft tissue infections.[1]

The principal pathogenic reservoirs for transmission of *Klebsiella* are the gastrointestinal tract and the hands of hospital personnel. Because of their ability to spread rapidly in the hospital environment, these bacteria tend to cause nosocomial outbreaks. Hospital outbreaks of multidrug-resistant *Klebsiella* spp., especially those in neonatal wards, are often caused by new types of strains, the so-called extended-spectrum-β-lactamase (ESBL) producers. The resulting limitations on the therapeutic options demand new measures for the management of *Klebsiella* hospital infections.

Reported carrier rates in hospitalized patients are 77% in the stool, 19% in the pharynx and 42% on the hands of patients.[2] Infections caused by multidrug-resistant Gram-negative bacilli that produce ESBL enzymes have been reported with increasing frequency in intensive care units and are associated with significant morbidity and mortality. Diagnosis is made by culturing appropriate specimen and identifying the isolate by biochemical reaction. Antibiotics sensitivity should invariably be done. Many strains carry plasmids determining multiple drug resistance (Table 1).[3]

2. Materials and methods

(1) Clinical Specimen: Urine, Pus and wound swab, Respiratory and blood specimen.
(2) Culture media: Blood agar, MacConkey agar, Brain heart infusion Broth, Muller Hinton agar.
(3) Biochemical test: Peptone water, Citrate agar, Urease agar, Triple sugar iron agar, Phenol red broth.
(4) Gram stain: Crystal violet, Grams Iodine, Decolourizer and Safranine.
(5) Reagent: Kovac Indole reagent, Phenol red and Sugar Disc
(6) Antibiotics: Ceftazidime, Ceftriaxone, Aztreonam, Cefotaxime, Cefotaxime-clavulanic acid and Meropenem disc.
(7) The ATCC strain used is *Klebsiella pneumoniae* 13883.

2.1. *Clinical specimen collection*[4]

2.1.1. *Urine*

Early morning mid-stream urine was collected taking all aseptic precautions.

2.1.2. *Pus and wound swab*

Pus sample was either collected by sterile aspirate or by sterile swabs; wound swabs were collected by aseptic techniques. Special care was taken to avoid contamination with commensal organisms from the skin.

2.1.3. *Specimen from respiratory tract*

Respiratory specimens included Sputum, Throat swabs and Bronchoalveolar lavage fluid.

2.1.4. *From the bloodstream*

Paired blood culture specimens were collected adhering to proper aseptic techniques.

2.2. *Inoculation of samples*

All samples were routinely cultured on MacConkey and blood agar plates and incubated at 37 °C aerobically. After overnight incubation, they were checked for bacterial growth.

2.3. *Isolation and identification of organisms*[5]

Suspected Gram-negative organisms were identified by colony characteristics, Gram staining, motility, citrate utilization, indole production, MR-VP, Urease production and sugar fermentation reactions. Triple sugar iron agar was used for sugar and H2S production.

2.4. *Antimicrobial susceptibility test by modified Kirby–Bauer sensitivity testing methods*

The Kirby–Bauer method is the most commonly used disc diffusion methods. The method most commonly employed is to use filter paper discs, impregnated with antibiotics. Results

Table 1 – Mechanism of antibiotics resistance.

Sr. no.	Mechanism	Antibiotic group	Example
1	Enzymatic inactivation	B-Lactam	B-lactamase: penicillinases, cephalosporineses, carbapenemase
		Amino glycosides	Aminoglycosides-modifying enzymes of Gram-negative and Gram-positive bacteria
2	Altered receptors	B-Lactams	Altered penicillin binding protein of Gram-positive and Gram-negative bacteria
		DNA gyrase alterations	Quinolones
		Altered bacterial enzyme	Sulfamethoxazole, trimethoprim
3	Altered antibiotic transport	Alteration in outer membrane proteins (porins)	Gram-negative bacteria; decrease influx
		Reduce proton motive force	Aminoglycosides and Gram-negative bacteria; decreased influx
		Active transport from bacterial cell	Tetracycline, erythromycin, active efflux

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