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Research Article

Antibiogram, ESBL Production and Carbapenemase Detection of Klebsiella Spp. in Hospital Acquired Infection

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Abstract

Infections with ESBL *K. 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. Disease progression has occurred while on treatment with antibiotics to which there is *in vitro* susceptibility. The carbapenems, that is, imipenem and Meropenam, are safe and effective antibiotics for the treatment of severe ESBL-producing *K. pneumoniae* infection in preterm infants.

Keywords: *K. Pneumonia,* patients, infection, carbapenems, imipenem, Meropenam

Introduction

Bacteria belonging to the genus *Klebsiella* frequently cause human nosocomial infections. Among these *Klebsiella pneumoniae* is medically accounts for a significant proportion of hospital-acquired urinary tract infections, pneumonia, septicaemias, and soft tissue infections (Poddschun and Ullmann 1998).

The term hospital infection, hospital acquired infection or nosocomial infections are applied to infections developing in hospitalised patients, not present or in incubation at the time of their admission. Such infections may become evident during their stay in hospital or sometimes, only after their discharge. The genus *Klebsiella* consists of nonmotile, capsulated gram negative rods that grow well on ordinary media forming large, dome-shaped, mucoid colonies of varying degree of stickiness. 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 incidence of

ESBL-producing strains among clinical *Klebsiella* isolates has been steadily increasing over the past years. The resulting limitations on the therapeutic options demand new measures for the management of *Klebsiella* hospital infections. While the different typing methods are useful epidemiological tools for infection control (Ananthanarayan and Panikar 2009).

Reported carrier rates in hospitalized patients are 77% in the stool, 19% in the pharynx, and 42% on the hands of patients (Cook *et.al* 1979, Devis *et.al* 1974).The high rate of nosocomial *Klebsiella* colonization appears to be associated with the use of antibiotics rather than with factors connected with delivery of care in the hospital (Pollack *et.al* 1972, Rose and Schreierl 1968). Apart from medical equipment (contaminated due to faulty hygienic procedures) and blood products (Goetz *et.al* 1995, Jumaa 1992) the principal reservoirs for transmission of *Klebsiella* in the hospital setting are the gastrointestinal tract of patients and the hands of hospital personnel. Infections caused by multidrug-resistant Gram negative bacilli that produce extended-spectrum ß-lactamase (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 (Ananthanarayan and Panikar 2009).

1.1 Virulence Factors

Numerous virulence factors have been described in *Klebsiella* spp. Extracellular capsules are essential to virulence; (Cryz *et.al* 1984, Domenico *et.al* 1982)the capsular material forms thick bundles of fibrillous structures that cover the bacterial surface in massive layers. This protects the bacterium from phagocytosis by polymorphonuclear granulocytes and prevents killing by bactericidal serum factors via the complement-mediated cascade.

1.2 Molecular Mechanisms of Resistance

Resistance to antimicrobials is a natural biological phenomenon. The introduction of every antimicrobial agent into clinical practice has been followed by the detection in the laboratory of strains of microorganisms that are resistant, i.e. able to multiply in the presence of drug concentrations higher than the concentrations in humans receiving therapeutic doses (Koneman *et.al* 1997).

Table1. Mechanism of antibiotics resistant

S. No.	Mechanism	Antibiotic group	Example
1.	Enzymatic inactivation	B-Lactam	B-lactamase: penicillinases Cephalosporineses, carbapenemase
		Aminoglycosides	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

1.3 B-Lactams

1.3a - Extended-Spectrum B-Lactamase (ESBL producer)

ESBLs are inhibitor- susceptibility enzyme in classes A and D that arise by mutations in genes for common plasmid – encoded B-Lactamas, such as TEM-1, SHV-1, and OXA-10. ESBLS may confer resistant to penicillins, cephalosporins and aztreonam in clinical isolates of *K. pneumoniae, K. oxytoca, E. coli, P. mirabilis,* and other genera of the family Enterobacteriaceae (CLSI 2012).

1.3b- AmpC Enzymes

The AmpC B-lactamases are chromosomal or plasmid-encoded enzymes. Bacteria expressing AmpC enzyme test as resistant to Cefoxitin, penicillins, cephalosporins and aztreonam (CLSI 2012).

1.3c- Carbapenemase (Carbapenem-resistant)

Carbapenemase activity in clinical isolates of *Enterobacteriaceae* occurs as a result of B-lactamase enzyme in Classes A, B, and D. KPC-type enzymes within Classes A, NDM-type enzymes within Class B, and OXA-type enzymes within class D represent major families of clinical importance. The presence of KPC- type enzymes can be confirmed using the modified Hodge test (CLSI 2012).

1.3e - NDM-1 (New Delhi metallo-beta-lactamase)

Originally described from New Delhi in 2009, this gene is now widespread in *Escherichia coli* and *Klebsiella pneumoniae* from India and Pakistan. As of mid-2010, NDM-1 carrying bacteria have been introduced to other countries (including the USA and UK), presumably by medical tourists undergoing surgery in India. Inhibitor- Resistant beta-Lactamases.
NDM-type and other metallo-B-lactamase enzymes require zinc for activity and are inhibited by substance such as ethylenediaminetetraacetic acid (EDTA), which binds zinc (CLSI 2010).

2. Risk group

(a) Patients at high-risk for ESBL and Carbapenemase include:
1. Neutropenic patients
2. Transplant recipients
3. Premature neonates
4. Elderly persons
5. Prolonged/extensive antibiotic use (e.g., cephalosporins)
6. Post-gastrointestinal surgery

(b) Consider screening of all admissions to high risk units. high risk unit include
1. Intensive care units
2. Haematology/oncology units
3. Transplantation units
4. Long term/ chronic care facility

3. Measures to control infection

- An inspection should be made daily of all individuals reporting for duty in operating rooms, nurseries, surgical ward, obstetric services, paediatric wards, central supply and food handling areas.
- No person who has overt disease should be allowed on duty, especially those with infected lesion of a superficial nature on the hands or other exposed areas.
- Personal should be conversant with measures for the maintenance of appropriate personal hygiene they must understand the necessity for using aseptic technique in all procedures and the importance of avoiding transmission of microorganism.
- Hand washing is one of the most critical means of preventing person to person transmission of infection

3.1 Infection Control Strategies

To control the spread of ESBL-producing pathogens, appropriate infection control interventions should be implemented for all

patients who are infected or colonized with ESBL-producing bacteria.

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 Meropenam disc.

1. Clinical Specimen collection:(Bagley et.al, 1978)

Urine:- Patients included in the study were given a sterile, dry, container and request for 10-20 ml specimen. The first urine passed by the patient at the beginning of the day was collected for examination (clean catch, mid stream).

Pus and wound swab:- A sterile technique was applied to aspirate or collect pus or wound swab from abscess or wound infection, either by disposable syringe or by sterile swab stick. Specimen was collected in a sterile container before an antiseptic dressing is applied. Special care was taken to avoid contamination with commensal organisms from the skin.

Specimen from respiratory tract:- Oral cavity: swab are rubbed firmly over ulcerated or patch like lesions.

- Throat: the mouth is held wide open and the tongue depressed. Swab is firmly rubbed over the tonsils and pharyngeal mucosa.
- Nasopharynx: A pernasal swab is used this is made from fine and fairly flexible wire which is bent at one end. The swab is carefully passed through the nasal cavity till it impinges on the nasopharynx, and then firmly rubbed over this area.
- Sputum: This should be coughed up from far down the bronchial tree and expectorated immediately and should not be mixed with saliva or oropharyngeal secretion.

From the Bloodstream:- Blood is collected by a strict aseptic technique. The skin is cleaned with 70% alcohol and the specimen is collected using a sterile syringe in Blood culture bottle.

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

3. Isolation and identification of organisms (Mena 2006)
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, .

4. Antimicrobial susceptibility test by modified Kirby - bauer sensitivity testing methods
The Kirby-bauer method are the most commonly used disc diffusion methods. The method most commonly employed is to use filter paper discs, impregnated with antibiotics. Results are reported as susceptible, intermediately susceptible or resistant to the different drugs.

4.1 Inoculation of isolated bacteria and placement of discs
Modified Kirby-Bauer sensitivity testing method was used for this purpose. Muller Hinton agar media was used, which has PH 7.2-7.4. Media was transfer in to 90 mm diameter sterile Petri dishes to a depth of 4 (four) mm. The surface was lightly and uniformly inoculated by cotton swab in three

directions rotating the plate approximately 60⁰, to ensure even distribution. Prior to inoculation, the swab stick was dipped into bacterial suspension having visually equivalent turbidity to 0.5 McFarland standards.

4.2 Screening tests for ESBLs production

Isolates is screen for ESBL production by using disc Diffusion of cefotaxime , ceftazidime , ceftriaxone and Aztreonam placed on inoculated plates containing Muller Hinton agar according to the CLSI recommendations. Isolates showing inhibition zone size of ≤ 22 mm with ceftazidime (30 μg), ≤ 25 mm with ceftriaxone (30 μg), ≤27 mm with cefotaxime (30 μg) , ≤ 27 mm with Aztreonam (30 μg) were suspected for ESBL production. E*. coli* ATCC 25922 was used as a control.

4.3 Confirmatory test for ESBLs production

In this test a disc of ceftazidime (30 μg), cefotaxime (30μg) alone and a disc of ceftazidime and cefotaxime in combination with clavulanic acid (30/10 μg) were used for each isolates. Both the discs were placed 25 mm apart, centre to centre, on a lawn culture of the test isolate on Muller Hinton agar plate and incubated overnight at 370 C. A ≥5 mm increase in zone diameter for either antimicrobial agent tested in combination with clavulanic acid versus its zone when tested alone was designated as ESBL positive.

4.4 Screening test for Carbapenemase production

Isolates are screen for Carbapenemase production by using disc Diffusion of Meropenem placed on inoculated plates containing Muller Hinton agar according to the CLSI recommendations. Isolates showing inhibition zone size of 16-21 mm with Meropenam (10 μg) were suspected for Carbapenemase production. E*. coli* ATCC 25922 was used as a control.

4.5 Confirmatory test for Carbapenemase production

Carbapenemase production is detected by the modified Hodge test when the test isolate produces the enzyme and allows growth of a carbapenem susceptible strain (*E. coli* 25922) towards a carbapenem disk. The result is a characteristics cloverleaf- like indication.

1. Streak a lawn of the1:10 dilution (0.5 McFarland dilution) of E.coli ATCC 25922 to a Mueller Hinton agar plate and allow to dry 3-5 minutes.
2. Place a 10ug Meropenam susceptibility disk in the centre of the test area.
3. In a straight line streak test organism from the disk to the edge of the disk to the edge of the plate. Up to four organisms can be tested on the same plate with one drug.
4. Incubate overnight at 37°C in ambient air for 16-24 hours.
5. After 16-24 hours of incubation, examine the plate for a clover leaf-type indentation at the intersection of the test organism and the *E. coli* 25922, within the zone of inhibition of the carbapenem susceptibility disk.

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 Betalactamase producer 109 in 170 patients (64%) and carbapenemase producer 53 in 170 patients (31%). The most frequent sources of infection were blood (18%), pus and wound swab (18 %), respiratory (32%) and urinary (29%).

(A) SCREENING OF BACTERIAL ISOLATES FROM DIFFERENT SPECIMEN

Table No.2. Total No. of *Klebsiella* spp. Isolated from different specimen

Sr. No.	Specimen	Specimen Identification No.	Total No. of *Klebsiella* spp. isolated	ESBL producer	Amp C Beta-lactamase producer	Carbapenemase producer
1.	Blood	B-01 to 32	32	08	15	8
2.	Pus	P-01 to 32	32	01	30	10
3.	Respiratory	R-01 to 56	56	09	45	22
4.	Urine	U- 01 to 50	50	12	19	13
	Total No.		170	30	109	53

(B) CULTURE CHARACTERISTICS OF BACTERIAL ISOLATES.

Table. 3. Colony characteristics of Bacterial strains

S. No.	MacConkey Agar	5% Sheep Blood Agar	Gram staining
1.	Large Mucoid Pink (Lactose Fermenting) colonies	Non haemolytic white colonies	Gram negative bacilli

Fig:1 After overnight incubation in
in MacConkey Agar Plate

Fig:2 After overnight incubation
Blood Agar Plate

(C) BIOCHEMICAL REACTION FOR IDENTIFICATION OF BACTERIAL STRAIN.

Table- 4. Biochemical Reaction of Bacterial isolates.

Sr. No.	Biochemical test	Result
1.	Catalase	+
2.	Indole production	-
3.	Methyl Red	-
4.	Voges-proskar	+
5.	Citrate utilization	+
6.	Urease production	+
7.	Triple sugar iron agar	Ac/Ac+ gas
8.	Gas in glucose	+
9.	Acid from lactose	+
10.	Motility	-

Note: All bacterial isolates belongs to *Klebsiella pneumoniae* according to biochemical reaction.

(D) ANTIBIOGRAM OF BACTERIAL ISOLATES

Table 5. Zone size Interpretative chart for ESBLs, Amp C-Betalactamase and Carbapenemase production as per CLSI. (Based on Results obtained using Mueller Hinton Agar)

Sr. No.	Antimicrobial agent	Disk content	Zone Diameter interpretive criteria in mm
	For ESBLs screening test		
	Ceftazidime	30 µg	≤ 22 mm
	Aztreonam	30 µg	≤ 27 mm
	Cefotaxime	30 µg	≤ 27 mm
	Ceftriaxone	30 µg	≤ 25 mm
2.	For confirmatory test for ESBL		
	Cefotaxime	30 µg	≤ 27 mm
	Cefotaxime – clavulanic acid	30/10 µg	>5mm increase in a zone diameter
3.	Screening test for Carbapenemase production Meropenam	10 µg	16 – 21 mm

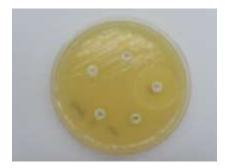Fig:3 ESBL Producing *Klebsiella pneumoniae*

Fig:4 Non-ESBL Producing *Klebsiella pneumoniae*

Fig:5 AMP C Beta lactamase Producing *Klebsiella pneumoniae*

Fig:6 Non AMP C Beta lactamase Producing *Klebsiella pneumoniae*

Fig:7 Screening test for ESBL Producing *Klebsiella pneumoniae*

Fig: 8 Confirmatory test for ESBL Producing *Klebsiella pneumoniae*

Fig:9 Carbapenemase producing *Klebsiella pneumoniae*

Fig:10 Non Carbapenemase producing *Klebsiella pneumoniae*

Fig:11 Screening test for Carbapenemase producing *Klebsiella pneumoniae*

Fig:12 Modified Hodge test (Confirmatory test) Carbapenemase producing *Klebsiella pneumoniae*

Conclusion

Management and treatment of ESBL-producing *K. pneumoniae* infections can be challenging and is evolving. To date, there have been no clinical trials that evaluate the comparative efficacy of antibiotics in the treatment of infections caused by these pathogens. The type of ESBL enzyme produced and the site and severity of infection are important considerations in determining antimicrobial therapy (Sirot 1995 & Rice *et.al,* 1990). 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*. Carbapenems remain stable in the presence of ESBL enzymes and the small compact size of carbapenems allows easy passage through porin into Gram negative bacilli. Thus, carbapenems are often the preferred antimicrobial agent for the treatment of serious infections caused by ESBL-producing organisms. While imipenem was used to treat severe infections during an outbreak of ESBL-producing *Klebsiella* spp, its use was associated with the emergence of imipenem-resistance *Acinetobacter* spp *(*Sirot 1995).

In conclusion, infections with ESBL *K. 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. Disease progression has occurred while on treatment with antibiotics to which there is *in vitro* susceptibility. 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.

To prevent spreading Klebsiella infections between patients, healthcare personnel must follow specific infection control precautions (CDC 2007). These precautions may include strict adherence to hand hygiene and wearing gowns and gloves when they enter rooms where patients with Klebsiella–related illnesses are housed. Healthcare facilities also must follow strict cleaning procedures to prevent the spread of *Klebsiella.*

To prevent the spread of infections, patients also should clean their hands very often, including:

- Before preparing or eating food
- Before touching their eyes, nose, or mouth
- Before and after changing wound dressings or bandages
- After using the restroom
- After blowing their nose, coughing, or sneezing

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