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APPENDICES

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APPENDIX 1

Medias, Chemical Agents ,Materials, Instruments and Identification procedures

Medias

1. Blood agar

Base : yeast extract	0.5 g
Trypticase soy base (Difco, USA)	40 g
Distrilled water	1000 ml.
Supplement : vitamin K- hemin solution	10 ml.
Human or sheep blood	50 ml.

2. Muller Hinton agar medium (Merck, Germany)

Beef, Infusion form	300 g
Bacto casamino acids, Technical	17.5 g
Starch	1.5 g
Bacto agar	17 g
Distrilled water	1000 ml.

pH: 7.3 +/- 0.1 at 25 °C

3. Mac Conkey agar (BBL, USA)

Pancreatic digest of gelatin	17 g
Pancreatic digest of casein	1.5 g

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Peptic digest of animal tissue	1.5 g
Lactose	10 g
Bile salts	1.5 g
Sodium chloride	5 g
Neutral red	0.03 g
Crystal violet	0.001 g
Agar	13.5 g

Media preparation

All of ingredients were dissolved in 1000 ml of distrilled water and heat to boiling to dissolved completely. The medium sterilied by autoclaving at 121 °C, 15 pounds / inch^2 pressure, for 15 minutes. The sterile medium was cooled at 45 °C to 50 °C.

For sterile medium , supplement was added aseptically. The medium was mix and then dispensed into sterile petri dishes.

Chemical agents

Low melting point agarose (Bio Rad, USA)

Ultrapure high melting temperature agarose (BioRad, USA)

Rnase (Bio- Rad, USA)

Phenylethylsulfonyl fluoride (Sigma, USA)

Sodium lauroyl sarcosine (Sigma, USA)

Protease (Sigma , USA)

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Tris base (Sigma, USA)

Sodium choride (Merck , Germany)

HCl (Merck, Germany)

EDTA (Sigma , USA)

Boric acid (Sigma , USA)

Lysozyme (Sigma , USA)

Ethidium bromide (Bio Rad , USA)

Mc Farland No.4 (bio Merieux)

SDS (Bio Rad , USA)

Lambda ladder (Bio Rad, USA)

Restriction enzyme *Spe* I (Bio Rad , USA)

Restriction enzyme *Spe* I buffer (Bio Rad , USA)

Materials

Plug mold (Bio Rad , USA)

Eppendorf microcentrifuge

Micropippet

Tip

Cotton swab

Cylinder

Test tube

Pasture pipet

Gel block

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Instruments

Centrifuge (Tomy Seiko, Japan)

pH meter (Orion, USA)

Microcentrifuge (Tomy Seiko, Japan)

Magnetic stirrer (VELP Scientifica, Italy)

Pulsed -Field Gel Box (Bio Rad , USA)

Pump, Gel molds (Bio Rad, USA)

Cooling system (Bio Rad , USA)

Power supply, Pulse wave switcher (Bio Rad , USA)

Contour-clamped homogenous electric field apparatus (Bio Rad, USA)

UV transilluminator (Spectroline, USA)

Water bath shaker (Yamato, Japan)

Polaroid camera (Polaroid, USA)

Automatic pipette (Brand, Germany)

Rotary shaker (Bellco Glass, USA)

Incubator (Sanyo, Japan)

Refrigerator (Sharp, Japan)

Freezer - 20°C (Sanyo, Japan)

Balance (Mettler, Japan)

Autoclave (Yamato, Japan)

Microwave (Sanyo, Japan)

Vortex mixer (Scientific, USA)

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Identification procedures

1. **Gram staining procedure**

Gram crystal violet solution

Gram iodine solution

Gram safranin solution

95 % ethanol

Staining procedure : The organisms were smeared on a clean slide and allowed to dry. The slide was heated with a flame to fix the smear. Gram crystal violet was dropped on the smear. After minute, the slide was then washed with water and drained. Next, gram iodine solution was dropped on the smear, and washed with the water after 1 minute. The smear was decolorized with 95 % ethanol and then washed with water. Gram safanin solution was next dropped on the smear in order to use as counterstain for 30 seconds. The smear was allowed to dry and then examined by microscope under 100x objective lens over the entire smear.

2. Biochemical tests.

2.1 Indole test

Purpose : Indole broth is used for distinguishing between bacteria based on ability to produce indole from trytophan.

Principle and interpretation : Indole broth contains trytophan - rich peptone and NaCl. The trytophan present in peptone is oxidized by certain bacteria to indole, skatole , and indoleacetic acid. The intracellular enzymes that are responsible for metabolizing tryptophan to these compounds are collectively termed trytophanase.

Indole is detected in broth cultures of bacteria with an alcoholic *p* - dimethylaminobenzaldehyde reagent. Indole reacts with the aldehyde to give a red product in the alcoholic layer of the broth -reagent mixture.

Two reagents may be used to detect indole : Kovacs and Ehrlich. Ehrlich reagent is believed to be more sensitive than Kovacs and is recommended for detection of indole production by anaerobic bacteria and nonfermentative gram negative organisms. Kovacs reagent was used initially to classify members of the family *Enterobacteriaceae* and should be used with these organisms.

Ingredients and preparation : Mix the ingredients, heat to boiling, dispense into tubes, and sterilize at 121 °C for 15 minutes.

Indole broth:

Pancreatic digest of casein , *USP*	20	g
NaCl	5	g
Distrilled water	1000	ml

Final pH 7.2

Reagents

Kovacs indole reagent. Dissolve the aldehyde in the alcohol and slowly add acid to the mixture

Alcohol, amyl or isoamyl	150	ml
P - Dimethylaminobenzaldehyde	10	g
Hydrocholic acid, concentrated	50	ml

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Procedure: Inoculate the test organism into indole broth, incubate at 35 °C for 18 to 24 hours, and test as follows.

Indole test: Add five drops of Kovacs reagent directly to the broth culture, shake gently, and observe for development of a red color in the upper alcohol layer.

2.2 Methyl red - Voges- Proskauer broth

Purpose: Methyl red- Voges - Proskauer (MR- VP) broth is useful for distinguish between members of the family *Enterobacteriaceae* based on their ability to produce acetylmethylcarbinol (acetoin) and strong acids from fermentation of glucose. The broth, which contains protein, glucose, and phosphate buffer, is used for the MR test and the VP test.

Principle and interpretation: Members of the family *Enterobacteriaceae* may be divided metabolically into two groups: the mixed acid producers and the butylene glycol producers. The mixed and producers such as *Escherichia coli* produce large amounts of organic acids including lactic, acetic, formic, and succinic. Butylene glycol producers such as *Klebsiella* and *Enterobacter* spp. produce smaller amounts of organic acids and large amounts of neutral products, especially 2,3- butanediol.

The MR test is used to distinguish the mixed acid producers. In this test a methyl red indicator is added to the MR -VP test broth after incubation. At a pH of 4.4 the indicator remains red, and at the pH of 6.0 it become yellow. The MR- positive organisms are those that produce large amounts of acid and a red color, whereas the MR- negative organisms produce a yellow color.

The VP test detects the presence of acetoin, or acetylmethylcarbinol, an intermediate in the production of butylene glycol. In this test two reagents, α - naphthol and 40 % KOH , are added to the test broth after appropriate incubation. The broth- reagent mixture is then mixed throughly to expose the medium atmospheric oxygen. If acetoin is present, it is oxidized in the presence of air and KOH to diacetyl. Diacetyl then reacts with the guanidine components of peptone, in the presence of α -naphthol, to form a red color (α -naphthol serves as a catalyst and acts as a color intensifier). Development of a red color is a positive VP test result.

Ingredients and preparation: Mix the ingredients, heat to boiling, dispense into tubes (1 ml per tube), and sterilize at 121°C for 15 minutes.

Pancreatic digest of casein and peptic Digest of animal tissue, *USP*	7 g
D - glucose	5 g
Dipotassium phosphate	5 g
Distrilled water	1000 ml

Final pH 6.9

Reagents:

Methyl red reagent: Dissolve the methyl red in alcohol and add the distrilled water. Store at room or refrigerator temperature.

Methyl red	50 mg
Ethyl alcohol, 95 %	150 ml
Distilled water	100 ml

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Voges -Proskauer reagents:

VP -1

α - naphthol	5	g
Ethyl alcohol, absolute	100	ml

VP - 2

Potassium hydroxide	40	g
Distilled water	100	ml

Procedure: Inoculate the test organism to two tubes of MR-VP broth,each containing 1 ml, and incubate for 1 to 3 days at 35°C.

Methyl red test : Add five drops of methyl red reagent to one broth culture and observe for development of a red color. This is a positive MR test, which is indicative of mixed acid fermentation.

Voges- Proskauer test : Add 0.6 ml of VP -1 reagent to another broth culture, shake the tube, and add 0.2 ml of VP-2 reagent. The reagents must be added in the preceding sequence. Shake the tube gently. Allow the tube to stand for at least 15 minutes and observe for formation of a red color. This is a positive VP test and indicates butylene glycol fermentation. Hold tubesin which results are negative for an additional 45 minutes, since maximum color development occurs within 1 hour after the reagent is added. Ignore a copper color of the medium , which occurs after 1 hour's incubation. This color is due to reaction between α-naphthol and KOH.

2.3 Citrate agar , Simmons

Purpose : Simmons citrate agar is used to distinguish gram negative bacteria based on their ability to utilize as a sole source of carbon.

Principle and interpretation : Organisms that metabolize citrate as a sole of carbon cleave citrate to oxaloacetate and acetate via the citritase enzyme. Another enzyme, oxaloacetate decarboxylase , then coverts oxaloacetate to pyruvate and CO_2. CO_2 combines with sodium and water to form Na_2CO_3 , an alkaline compound. As a result, the pH of the medium rises and the indicator (bromthymol blue) changes from green to Prussian blue. Presence of the blue color constitutes a positive finding for citrate utilization.

Ingredients and Preparation : Mix the following ingredients , heat to boiling, dispense into test tubes, and sterilize at 121°C for 15 minutes. Cool each tube of medium in a slanted position.

Sodium citrate	2 g
NaCl	5 g
$MgSO_4$	0.2 g
Ammonium dihydrogen phosphate	1 g
Dipotassium phosphate	1 g
Bromthymol blue	80 mg
Agar	15 g
Distrilled water	1000 ml

Final pH 6.9

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Procedure: Lightly incubate the test organism to the surface of citrate medium, incubate at 35°C for 24 to 48 hours, and observe for a Prussian blue color change.

2.4 Urea agar

Purpose: Urea agar are used for distinguishing between species of aerobic bacteria based on ability to hydrolyze urea.

Principle and interpretation: A variety of media are used to test for ability to hydrolyze urea. The hydrolysis of urea by urease to ammonia is accompanied by a rise in pH of the medium and a concomitant change in the color of the indicator from yellow to red.

Ingredients and preparation: Mix urea basal ingredients, sterilize by filtration, and add sterile agar solution (50 °C). Mix and dispense into tubes, and allow tubes of medium to cool in a slanted position.

Urea base:

Pancreatic digest of gelatin, *USP*	1 g
NaCl	5 g
Monopotassium phosphate	2 g
D - Glucose	1 g
Urea	20 g
Phenol red	12 mg
Distrilled water	100 ml

Final pH 6.8

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Agar solution:

Agar	15	g
Distrilled water	900	ml

Urea agar:

Urea base	100	ml
Agar solution	900	ml

Procedure: Inoculate the organism to the urea agar, incubate for 24 to 48 hours at 35 °C, and observe for a red color change in the medium.

2.5 Triple sugar iron agar

Purpose: Triple sugar iron (TSI) agar is a screening medium used to identify gram negative bacilli based on ability to ferment the carbohydrates glucose, sucrose, and lactose to produce H_2S gas.

Principle and interpretation: TSI agar contain protein, NaCl, lactose, sucrose, dextrose, a sulfur source, an H_2S indicator, a pH indicator, and agar. The medium includes ten times as much lactose and sucrose as glucose. Bacteria that ferment glucose produce a variety of acids, turning the color of the medium from red to yellow. Larger amounts of acids are produced in the butt of the tube (fermentation) that in the slant of the tube (respiration). Organisms growing on TSI also from alkaline products from the oxidative decarboxylation of peptone. These alkaline

products from the oxidative decarboxylation of peptone. These alkaline products neutralize the small amounts of acids present in the butt. Thus, the appearance of an alkaline (red) slant and an acid (yellow) butt after 24 hours incubation indicates that the organism is a glucose fermenter but is unable to ferment lactose and sucrose.

Bacteria that ferment lactose or sucrose (or broth), in addition to glucose, reduce such large amounts of acid that the oxidative deamination of protein that may occur in the slant does not yield enough alkaline products to cause a reversion of pH in that region. Thus, these bacteria produce an acid slant and acid butt. It is impossible to determine from the TSI reaction whether both lactose and sucrose are being fermented or only one of these carbohydrates is being fermented, individual carbohydrate fermentation tests are required to make this assessment.

Gas production (CO_2 and hydrogen) is detected by the presence of cracks or bubbles in the medium. These are formed when the accumulated gas escapes.

H_2S gas is produced as a results of the reduction of thiosulfate. H_2S is a colorless gas and can be detected only in the presence of an indicator, in this case ferric ammonium sulfate. H_2S combines with the ferric ions of ferric ammonium sulfate to produce the insoluble black precipitate ferrous sulfide. Reduction of thiolsulfate proceeds only in an acid environment, and blackening usually occurs in the butt of the tube. Although the black precipitate may frequently obscure the color of the butt, it can be assumed that the organism is a glucose fermenter because of the requirement for an acid environment. The reactions can summarized as follow:

Alkaline slant/ acid butt : glucose only fermented

Acid slant / acid butt: glucose and sucrose fermented or glucose and lactose fermented or glucose, lactose, and sucrose fermented

Bubbles or cracks present : gas produced

Black precipitate present : H_2S gas produced

Ingredients and preparation : Mix the ingredients, heat to boiling, dispense into tubes, and sterilize at 121 °C for 15 minutes, and allow tubes of medium to cool in a slanted position.

Pancreatic digest of casein, *USP*	10 g
Peptic digest of animal tissue, *USP*	10 g
NaCl	1 g
Lactose	10 g
Sucrose	10 g
D - Glucose	1 g
Ferric ammonium sulfate	0.2 g
Sodium thiosulfate	0.2 g
Phenol red	25 mg
Agar	13 g
Distrilled water	1000 ml

Final pH 7.3-7.4

Procedure: Inoculate test cultures to TSI agar by first touching a sterile bacteriologic needle to a colony and then stabbing the needle into the deep agar region of the medium. Hen withdrawing the needle, move it from side to side over the

surface of the medium. Incubate cultures at 35°C for 18 to 24 hours. Examine cultures for color of the slant, butt , gas cracks, and blackening caused by H_2S.

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APPENDIX II

Reagent for molecular analysis

Stock reagents and buffer for PFGE

1. 20 mg/ml Protease

Protease (Sigma, USA)	100	mg
Sterile deionized water	5	ml

The stock reagent was prepared by dissolved 100 mg of protease in 5 ml of sterile deionized water. The stock reagent was stored at - 20 °C

2. 0.5 Tris HCl (pH 8.0)

Tris base	60.55	g
Deionized water	1000	ml

This stock reagent was prepared by dissolved 60.55 g of Tris base in 700 ml of deionized water , then the pH was adjusted to 8.0 with HCl (conc.). The final volume was bought up to 1000 ml with deionized water. The stock reagent sterilized by autoclaving at 121 °C, 15 ponds/inch^2 pressure, for 15 minutes. The stock reagent was store at room temperature.

3. 0.5M EDTA

Ethylene diaminetetraacetic acid	93.05	g
Deionized water	500	ml

6. 2M Tris HCl (pH 7.6)

Tris base	12.11	g
Deionized water	50	ml

This stock reagent was prepared by dissolved 12.11 g Tris base in 35 ml of deionized water , then the pH was adjusted to 7.6 with HCl (conc.). The final volume was bought up to 50 ml with deionized water. The stock reagent sterilized by autoclaving at 121 °C, 15 ponds/inch^2 pressure, for 15 minutes. The stock reagent was store at room temperature.

Buffer

1. TES

0.5 M Tris HCl	100	ml
0.5 M EDTA	10	ml
2M NaCl	2.92	ml
Sterile deionized water up to	1000	m

2. Lysis solution I

SDS	0.03	g
10mM Tris - HCl pH 7.6, 10mM EDTA	3	ml
Lysozyme (10 mg/ml)	300	µl
Rnase (25 mg/ml)	2.4	µl

This buffer was freshly prepared before used

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This stock reagent was prepared by dissolved 93.05 g of ethylene diaminetetraacetic acid in 400 ml of deionized water, then the pH was adjusted to 8.0 with NaOH (pellets). The final volume was bought up to 500 ml with deionized water. The stock reagent steriled by 121 °C, 15 ponds/inch^2 pressure, for 15 minutes. The stock reagent was store at room temperature.

4. 5X TBE

Tris base	54	g
Boric acid	27.5	g
0.5 M EDTA pH 8.0	20	ml
Deionized water	1000	ml

This stock reagent was prepared by dissolved all ingredients in 1000 ml of deionized water. The stock reagent steriled by autoclaving at 121 °C, 15 ponds/inch^2 pressure, for 15 minutes. The stock reagent was store at room temperature.

5. 2 M NaCl

NaCl	2.92	g
Deionized water	500	ml

To prepared this stock reagent, 2.922 g of NaCl was dissolved in 500 ml of deionized water. The stock reagent steriled by autoclaving at 121 °C, 15 ponds/inch^2 pressure, for 15 minutes. The stock reagent was store at room temperature.

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3. Lysis solution II

Sarkocyl	0.03	g
0.5 M EDTA	2.7	ml
20 mg/ml protease	0.3	ml

4. Phenyle thylsulfonyl fluoride (PMSF)

Phenyle thylsulfonyl fluoride	0.087	g
1X TE	50	ml

This stock reagent was prepared by dissolved 0.087 g of PMSF in 50 ml of 1X TE. The stock reagent was stored at -20°C.

Working PMSF

This working was prepared by mixing 2 ml of PMSF with 18 ml of 1 X TE. This buffer was freshly prepared before use.

5. 1X TE

0.5 M Tris HCl	20	ml
0.5 M EDTA	2	ml
Sterile deionized water up to	1000	ml

This buffer was prepared by mixing these stock solution in 1000 ml of sterile deionized water .

6. 10mM Tris - HCl + 10mM EDTA

2 M Tris - HCl	5	ml
0.5 M EDTA	20	ml
Sterile deionized water up to	1000	ml

This buffer was prepared by mixing these stock solution in 1000 ml of sterile deionized water.

7. 1.6 % Low melting point agarose

Low melting point agarose	0.16	g
TES	10	ml

This low melting point agarose was prepared by suspending 0.16g of low melting point agarose in 10 ml of TES. The agarose was melted by microwave or placing the flask into a beaker of boiling water.

8. 1% Ultrapure high melting temperature agarose

Ultrapure high melting temperature agarose	1	g
0.5 X TBE	100	ml

This ultrapure high melting temperature agarose was prepared by suspending 1 g of ultrapure high melting temperature agarose in 100 ml of 0.5x TBE. The agarose was melted by microwave or placing the flask into a beaker of boiling water.

APPENDIX III

Table a III 1 Inhibition zone sizes from the initial screen test of *K. pneumoniae* isolated from blood and control strains

Organism	Inhibition zone sizes (mm.)		
	CTX ≤ **27**	**CAZ** ≤ **22**	**CRO** ≤ **25**
KB001	24	27	27
KB004	31	27	27
KB005	24	26	18
KB007	31	26	25
KB008	26	25	25
KB010	26	25	28
KB002	18	16	16
KB003	20	6	17
KB006	15	6	17
KB009	15	6	14
KB011	13	6	10
KB012	23	10	21
KB013	24	10	24
KB014	24	8	20
KB015	14	6	11
KB016	15	6	12
KB017	13	6	12
KB018	20	6	21
KB019	19	10	18
KB020	21	6	18

Table a III 1 (cont.)

Organism	Inhibition zone sizes (mm.)		
	CTX ≤ **27**	**CAZ** ≤ **22**	**CRO** ≤ **25**
E.coli ATCC 32518	34	30	32
K.pneumoniae ATCC 700603	23	15	20
K.high	20	10	18
K.low	28	26	28
K.non	28	27	28

* CTX = cefotaxime, CAZ= ceftazidime, CRO = ceftriaxone.

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Table aIII 2 Inhibition zone sizes test of *K. pneumoniae* isolated from blood and control strains from the phenotypic confirmatory test (combination disk method)

Organism	Inhibition zone sizes (mm.)					
	CTX	CTXL	CTXL/ CTX >5	CAZ	CAZL	CAZL/ CAZ >5
KB001	34.29	34.80	-	29.67	30.20	-
KB004	33.85	34.41	-	30.25	30.52	-
KB005	32.08	34.05	-	30.11	30.54	-
KB007	33.05	32.33	-	30.04	30.38	-
KB008	33.89	33.92	-	29.27	30.82	-
KB010	33.83	34.03	-	31.64	31.82	-
KB002	19.53	30.54	+	17.06	24.59	+
KB003	17.77	31.79	+	6	27.36	+
KB006	19.21	31.81	+	6	27.58	+
KB009	23.14	30.78	+	6	24.50	+
KB011	14.56	31.87	+	6	24.83	+
KB012	22.57	32.39	+	6	29.03	+
KB013	22.16	31.14	+	6	27.92	+
KB014	20.96	31.29	+	6	25.75	+
KB015	13.32	23.23	+	6	24.83	+
KB016	17.07	30.86	+	6	24.44	+
KB017	14.55	29.41	+	8.92	25.10	+
KB018	19.69	33.16	+	6	28.21	+
KB019	17.20	31.68	+	8.78	28.21	+
KB020	20.58	32.50	+	6	25.41	+

Table a III 2 (cont.)

Organism	Inhibition zone sizes (mm.)					
	CTX	**CTXL**	**CTXL/ CTX ≥ 5**	**CAZ**	**CAZL**	**CAZL/ CAZ ≥ 5**
E.coli ATCC 32518	38.28	39.09	-	33.13	34.21	-
K.pneumon ATCC 700603	20.85	27.83	+	11.38	21.66	+
K.high	19.24	31.34	+	6	23.82	+
K.low	30.20	31.28	-	27.27	28.33	-
K.non	34.40	35.16	-	30.52	31.62	-

*CTX= cefotaxime, CTXL= cefotaxime + clvulanic acid

CAZ = ceftazidime . CAZL = ceftazidime + clavulanic acid

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Table a III 3 MIC of *K. pneumoniae* isolated from blood and control strains detected by E –test ESBL test

Organism	MIC					
	CTX	**CTXL**	**CTX/ CTXL ≥ 8**	**CAZ**	**CAZL**	**CAZ/ CAZL ≥ 8**
KB001	< 0.25	0.094	-	< 0.5	0.19	-
KB004	< 0.25	0.094	-	< 0.5	0.19	-
KB005	<0.25	0.064	-	< 0.5	0.25	-
KB007	< 0.25	0.094	-	< 0.5	0.25	-
KB008	0.25	0.064	-	< 0.5	0.38	-
KB010	< 0.25	0.094	-	< 0.5	0.125	-
KB002	> 16	0.125	+	24	2	+
KB003	> 16	0.25	+	> 32	0.25	+
KB006	> 16	0.094	+	> 32	0.5	+
KB009	> 16	0.094	+	> 32	0.125	+
KB011	> 16	0.19	+	> 32	0.5	+
KB012	> 16	0.19	+	> 32	0.5	+
KB013	> 16	0.25	+	> 32	0.5	+
KB014	> 16	0.25	+	> 32	0.38	+
KB015	> 16	0.094	+	>32	0.075	+
KB016	> 16	0.094	+	> 32	0.5	+
KB017	> 16	0.125	+	> 32	1.5	+
KB018	3	0.047	+	>32	0.25	+
KB019	4	0.125	+	>32	0.38	+
KB020	3	0.125	+	>32	2	+

Table aIII 3 (cont.)

Organism	MIC					
	CTX	**CTXL**	**CTX/ CTXL ≥ 8**	**CAZ**	**CAZL**	**CAZ/ CAZL ≥ 8**
E.coli ATCC 32518	< 0.25	0.032	-	< 0.5	0.064	-
K.pneumon ATCC 700603	3	> 1	+	>32	0.5	+
K.high	> 16	0.25	+	> 32	0.5	+
K.low	< 0.25	0.064	-	< 0.5	0.25	-
K.non	< 0.25	0.094	-	< 0.5	0.38	-

*CTX= cefotaxime, CTXL= cefotaxime + clvulanic acid

CAZ = ceftazidime . CAZL = ceftazidime + clavulanic acid

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Table aIII 4 Inhibition zone sizes of initial screen test of *K. pneumoniae* isolated from sputum .

Organism	Inhibition zone sizes (mm.)		
	CTX ≤ 27	CAZ ≤ 22	CRO ≤ 25
KS004	30	22	28
KS007	30	24	24
KS009	29	24	25
KS015	30	27	25
KS027	32	20	29
KS003	27	25	25
KS006	27	25	25
KS013	27	24	25
KS014	25	24	25
KS016	17	24	18
KS025	25	22	27
KS001	18	11	16
KS002	19	10	18
KS005	15	6	12
KS008	18	14	12
KS010	20	8	20
KS011	13	6	12
KS012	22	7	20
KS017	17	9	16
KS018	15	21	13
KS019	16	6	13
KS020	21	10	19

Table a III 4 (cont.)

Organism	Inhibition zone sizes (mm.)		
	CTX ≤ 27	CAZ ≤ 22	CRO ≤ 25
KS021	21	11	21
KS022	18	6	15
KS023	12	6	13
KS024	13	6	11
KS.26	15	6	13
KS028	24	9	20
KS029	21	9	18
KS030	18	10	13
KS031	15	10	13
KS032	18	10	18
KS033	23	22	22
KS034	23	6	19
KS035	20	10	18
KS036	18	10	16
KS037	20	13	19
KS038	19	12	18
KS039	13	6	14
KS040	19	9	19
KS041	16	6	15
KS042	21	11	20
KS043	6	6	6
KS044	10	6	10
KS045	16	11	17
KS046	17	10	16

Table a III 4 (cont.)

Organism	Inhibition zone sizes (mm.)		
	CTX ≤ **27**	**CAZ** ≤ **22**	**CRO** ≤ **25**
KS047	23	11	21
KS048	21	14	23
KS049	26	11	25
KS050	24	15	24
KS051	26	14	25
KS052	19	13	20
KS053	21	10	21

* CTX = cefotaxime, CAZ= ceftazidime, CRO = ceftriaxone

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Table aIII 5 Inhibition zone sizes test of *K. pneumoniae* isolated from sputum detected by phenotypic confirmatory test (combination disk method).

Organism	Inhibition zone sizes (mm.)					
	CTX	CTXL	CTXL/ CTX ≥ 5	CAZ	CAZL	CAZL/ CAZ ≥ 5
KS004	30.94	31.80	-	27.79	28.43	-
KS007	31.35	33.40	-	28.92	29.98	-
KS009	34.05	35.50	-	30.38	31.62	-
KS015	31.83	34.27	-	29.32	29.65	-
KS027	30.40	30.66	-	33.91	34.26	-
KS003	30.57	31.76	-	27.79	27.83	-
KS006	31.60	32.09	-	26.11	27.02	-
KS013	33.35	34.18	-	24.11	25.57	-
KS014	33.94	35.46	-	30.68	30.83	-
KS016	32.31	33.22	-	28.78	29.15	-
KS025	29.77	30.34	-	27.56	28.31	-
KS001	13.92	28.72	+	6	24.90	+
KS002	18.61	28.70	+	11.77	26.26	+
KS005	15.39	32.28	+	6	25.29	+
KS008	15.30	31.72	+	15.73	32.49	+
KS010	19.78	33.78	+	6	28.43	+
KS011	14.76	29.87	+	6	25.14	+
KS012	18.27	30.87	+	6	26.41	+
KS017	16.96	32.56	+	9.31	27.04	+
KS018	12.64	28.84	+	21.20	28.07	+

Table a III 5 (cont.)

Organism	Inhibition zone sizes (mm.)					
	CTX	CTXL	CTXL/ CTX ≥ 5	CAZ	CAZL	CAZL/ CAZ ≥ 5
KS019	15.73	30.85	+	6	24.73	+
KS020	19.64	32.02	+	6	27.45	+
KS021	20.22	32.50	+	6	26.95	+
KS022	16.88	31.50	+	6	25.56	+
KS023	11.60	28.22	+	6	21.26	+
KS024	13.39	28.97	+	6	21.24	+
KS026	14.22	27.38	+	6	21.37	+
KS028	19.21	30.85	+	6	22.94	+
KS029	18.15	30.53	+	6	22.29	+
KS030	18.81	33.60	+	11.32	25.09	+
KS031	12.70	27.72	+	6	21.33	+
KS032	17.39	33.04	+	12.27	27.34	+
KS033	21.09	32.85	+	23.08	28.91	+
KS034	19.36	31.24	+	6	26.74	+
KS035	17.75	32.23	+	6	28.53	+
KS036	18.34	31.19	+	10.94	25.68	+
KS037	20.19	29.37	+	13.04	23.36	+
KS038	15.47	33.09	+	13.55	27.84	+
KS039	16.11	32.94	+	6	24.39	+
KS040	18.90	32.92	+	6	27.21	+
KS041	15.86	32.08	+	6	24.80	+
KS042	21.32	31.86	+	6	27.31	+

Table a III 5 (cont.)

Organism	Inhibition zone sizes (mm.)					
	CTX	CTXL	CTXL/ CTX ≥ 5	CAZ	CAZL	CAZL/ CAZ ≥ 5
KS043	6	11.63	+	6	11.33	+
KS044	11.69	32.08	+	6	22.77	+
KS045	17.45	29.35	+	12.07	23.67	+
KS046	16.78	32.53	+	8.69	27.30	+
KS047	19.48	30.14	+	6	26.22	+
KS048	16.92	33.02	+	11.75	26.03	+
KS049	24.40	33.52	+	6	27.37	+
KS050	22.93	32.60	+	6	28.54	+
KS051	24.27	33.64	+	6	29.10	+
KS052	18.80	32.42	+	6	24.18	+
KS053	20.55	31.36	+	6	24.52	+

*CTX= cefotaxime, CTXL= cefotaxime + clvulanic acid

CAZ = ceftazidime . CAZL = ceftazidime + clavulanic acid

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Table aIII 6 MIC of *K. pneumoniae* isolated from sputum detected by E –test ESBL test.

Organism	MIC					
	CTX	CTXL	CTX/ CTXL ≥ 8	CAZ	CAZL	CAZ/ CAZL ≥ 8
KS004	0.25	0.094	-	< 0.5	0.25	-
KS007	0.25	0.125	-	< 0.5	0.25	-
KS009	0.25	0.094	-	< 0.5	0.19	-
KS015	0.25	0.19	-	< 0.5	0.38	-
KS027	<0.25	0.064	-	0.5	0.25	-
KS003	0.25	0.125	-	< 0.5	0.38	-
KS006	0.25	0.25	-	0.5	0.75	-
KS013	0.25	0.25	-	< 0.5	0.75	-
KS014	0.25	0.094	-	< 0.5	0.38	-
KS016	0.25	0.125	-	< 0.5	0.25	-
KS025	<0.25	0.19	-	0.5	0.5	-
KS001	> 16	0.5	+	> 32	0.38	+
KS002	> 16	0.125	+	> 32	0.5	+
KS005	> 16	0.19	+	> 32	1.0	+
KS008	> 16	0.19	+	>32	0.25	+
KS010	> 16	0.094	+	> 32	0.25	+
KS011	> 16	0.094	+	> 32	0.75	+
KS012	> 16	0.25	+	>32	0.75	+
KS017	> 16	0.125	+	> 32	0.5	+
KS018	> 16	0.19	+	2	0.75	-

Table a III 6 (cont.)

Organism	MIC					
	CTX	CTXL	CTX/ CTXL ≥ 8	CAZ	CAZL	CAZ/ CAZL ≥ 8
KS019	> 16	0.19	+	> 32	>4	U
KS020	> 16	0.094	+	> 32	0.5	+
KS021	> 16	0.125	+	> 32	0.38	+
KS022	> 16	0.125	+	> 32	0.38	+
KS023	> 16	0.125	+	>32	> 4	U
KS024	> 16	0.094	+	> 32	1.5	+
KS026	> 16	> 1	U	> 32	> 4	U
KS028	6	0.094	+	> 32	1.5	+
KS029	3	0.094	+	> 32	2	+
KS030	> 16	0.094	+	> 32	0.38	+
KS031	> 16	0.25	+	> 32	>4	U
KS032	> 16	0.064	+	> 32	0.38	+
KS033	1.5	0.064	+	> 32	0.25	+
KS034	4	0.094	+	> 32	0.5	+
KS035	> 16	0.125	+	> 32	0.25	+
KS036	6	0.047	+	> 32	0.25	+
KS037	4	0.094	+	> 32	0.5	+
KS038	> 16	0.064	+	> 32	0.125	+
KS039	> 16	0.38	+	> 32	> 4	U
KS040	3	0.094	+	> 32	0.75	+
KS041	> 16	> 1	U	> 32	0.5	+
KS042	4	0.094	+	> 32	0.19	+

Table a III 6 (cont.)

Organism	MIC					
	CTX	CTXL	CTX/ CTXL ≥ 8	CAZ	CAZL	CAZ/ CAZL ≥ 8
KS043	> 16	> 1	U	> 32	>4	U
KS044	> 16	0.094	+	> 32	> 4	U
KS045	> 16	0.19	+	> 32	0.75	+
KS046	> 16	0.094	+	> 32	0.75	+
KS047	6	0.064	+	> 32	0.5	+
KS048	> 16	0.125	+	> 32	0.38	+
KS049	3	0.125	+	> 32	1.0	+
KS050	3	0.064	+	> 32	0.5	+
KS051	2	0.064	+	> 32	0.38	+
KS052	> 16	0.094	+	> 32	0.75	+
KS053	> 16	0.094	+	> 32	1.5	+

*CTX= cefotaxime, CTXL= cefotaxime + clvulanic acid

CAZ = ceftazidime . CAZL = ceftazidime + clavulanic acid

** U = Undetermined

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Table a III 7 Inhibition zone sizes of initial screen test of *K. pneumoniae* isolated from urine

Organism	Inhibition zone sizes (mm.)		
	CTX ≤ 27	CAZ ≤ 22	CRO ≤ 25
KU005	28	27	25
KU031	28	19	29
KU001	0	17	16
KU002	18	12	16
KU003	6	6	6
KU004	14	6	12
KU006	17	7	9
KU007	16	6	15
KU008	24	14	23
KU009	14	11	17
KU010	18	6	16
KU011	13	6	12
KU012	13	6	12
KU013	11	6	12
KU014	19	6	19
KU015	11	9	14
KU016	13	6	13
KU017	15	6	11
KU018	16	6	16
KU019	18	6	14
KU020	18	6	18
KU021	19	6	17
KU022	13	21	6

Table a III 7 (cont.)

Organism	Inhibition zone sizes (mm.)		
	CTX ≤ 27	CAZ ≤ 22	CRO ≤ 25
KU023	20	10	19
KU024	14	11	12
KU025	12	20	12
KU026	16	6	17
KU027	20	11	20
KU028	21	10	20
KU029	18	9	18
KU030	24	15	25
KU032	18	11	15
KU033	15	6	15
KU034	15	6	16
KU035	17	6	17
KU036	14	6	14
KU037	17	6	13
KU038	12	6	12
KU039	18	6	17
KU040	14	6	17
KU041	11	6	13
KU042	16	6	15
KU043	16	6	13
KU044	20	6	22
KU045	19	6	18
KU046	10	6	9
KU047	16	6	14
KU048	23	6	21

Table a III 7 (cont.)

Organism	Inhibition zone sizes (mm.)		
	CTX ≤ **27**	**CAZ** ≤ **22**	**CRO** ≤ **25**
KU049	21	6	18
KU050	14	6	15

* CTX = cefotaxime, CAZ= ceftazidime, CRO = ceftriaxone

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Table a III 8 Inhibition zone sizes test of *K. pneumoniae* isolated from urine detected by phenotypic confirmatory test (combination disk method).

Organism	Inhibition zone sizes (mm.)					
	CTX	CTXL	CTXL/ CTX ≥ 5	CAZ	CAZL	CAZL/ CAZ ≥ 5
KU005	33.54	34.52	-	31.28	31.65	-
KU031	25.28	25.39	-	18.16	18.47	-
KU001	18.90	31.36	+	6	24.90	+
KU002	18.56	31.76	+	12.19	24.10	+
KU003	6	6	-	6	6	-
KU004	12.40	30.26	+	6	24.02	+
KU006	17.86	28.78	+	6	26.76	+
KU007	15.64	30.25	+	6	28.68	+
KU008	23.14	33.45	+	13.79	30.48	+
KU009	20.59	30.62	+	9.45	24.41	+
KU010	17.04	33.17	+	10.51	27.23	+
KU011	13.19	32.52	+	6	23.63	+
KU012	13.42	32.56	+	6	24.82	+
KU013	13.22	27.56	+	6	26.09	+
KU014	13.80	29.68	+	6	23.74	+
KU015	12.22	25.28	+	6	24.35	+
KU016	16.70	30.78	+	6	24.02	+

Table a III 8 (cont.)

Organism	Inhibition zone sizes (mm.)					
	CTX	CT	CTXL/ CTX ≥ 5	CAZ	CAZL	CAZL/ CAZ ≥ 5
KU017	17.13	34.29	+	6	23.09	+
KU018	17.42	32.48	+	6	25.75	+
KU019	15.11	30.53	+	6	24.15	+
KU020	19.42	32.02	+	6	24.95	+
KU021	25.94	34.74	+	13.64	29.45	+
KU022	8.29	23.12	+	20.21	25.23	+
KU023	16.76	30.75	+	8.92	26.82	+
KU024	12.96	25.43	+	2.09	24.88	+
KU025	12.46	24.09	+	17.98	23.25	+
KU026	6	6	-	6	6	-
KU027	18.50	30.23	+	0	26.13	+
KU028	17.10	30.71	+	19.54	24.57	+
KU029	16.32	33.04	+	6.05	21.60	+
KU030	19.77	32.98	+	13.35	25.95	+
KU032	17.77	31.96	+	10.42	26.35	+
KU033	15.91	27.40	+	6	25.31	+
KU034	13.15	30.23	+	6	24.49	+
KU035	17.62	32.09	+	6	28.29	+
KU036	12.90	32.02	+	6	24.85	+
KU037	18.57	30.02	+	6.33	24.85	+
KU038	12.79	27.06	+	6	22.09	+

Table a III 8 (cont.)

Organism	Inhibition zone sizes (mm.)					
	CTX	CTXL	CTXL/ CTX ≥ 5	CAZ	CAZL	CAZL/ CAZ ≥ 5
KU039	18.57	30.34	+	12.64	26.72	+
KU040	17.99	33.74	+	6	24.36	+
KU041	9.92	32.86	+	6	27.22	+
KU042	19.21	32.90	+	10.91	26.94	+
KU043	15.72	30.32	+	6	26.41	+
KU044	14.73	26.94	+	6	20.82	+
KU045	19.81	32.33	+	6	22.97	+
KU046	12.39	31.68	+	6	23.79	+
KU047	19.17	30.96	+	6	27.96	+
KU048	23.40	32.80	+	6	29.42	+
KU049	20.49	29.76	+	6	25.77	+
KU050	12.03	28.94	+	6	24.01	+

*CTX= cefotaxime, CTXL= cefotaxime + clvulanic acid

CAZ = ceftazidime . CAZL = ceftazidime + clavulanic acid

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Table a III 9 MIC of *K. pneumoniae* isolated from urine detected by E –test ESBL test.

Organism	MIC					
	CTX	CTXL	CTX/ CTXL ≥ 8	CAZ	CAZL	CAZ/ CAZL ≥ 8
KU005	<0.25	0.047	-	0.5	0.19	-
KU031	2	> 1	-	> 32	>4	U
KU001	> 16	0.25	+	> 32	> 4	U
KU002	8	0.094	+	> 32	0.5	+
KU003	> 16	1.0	+	>32	0.5	+
KU004	> 16	0.094	+	>32	>4	U
KU006	> 16	0.094	+	>32	1.0	+
KU007	> 16	>1	U	>32	> 4	U
KU008	2	0.094	+	>32	1.0	+
KU009	> 16	0.38	+	>32	> 4	U
KU010	> 16	0.094	+	>32	1.0	+
KU011	> 16	0.38	+	> 32	> 4	U
KU012	> 16	0.25	+	> 32	> 4	U
KU013	> 16	1.0	+	> 32	> 4	U
KU014	> 16	1.0	+	>32	> 4	U
KU015	> 16	>1	U	> 32	> 4	U
KU016	> 16	0.125	+	> 32	1.0	+
KU017	> 16	0.125	+	>32	1.0	+
KU018	> 16	0.19	+	> 32	>4	U
KU019	> 16	0.125	+	>32	2	+
KU020	> 16	0.19	+	> 32	1.0	+

Table aIII 9 (cont.)

Organism	MIC					
	CTX	CTXL	CTX/ CTXL ≥ 8	CAZ	CAZL	CAZ/ CAZL ≥ 8
KU021	1.5	0.032	+	> 32	0.064	+
KU022	> 16	> 1	U	> 32	> 4	U
KU023	> 16	0.125	+	> 32	> 4	U
KU024	> 16	0.25	+	>32	> 4	U
KU025	> 16	0.25	+	> 32	> 4	U
KU026	> 16	> 1	U	> 32	1.5	+
KU027	>16	0.094	+	> 32	0.75	+
KU028	8	0.094	+	> 32	1.5	+
KU029	> 16	0.125	+	> 32	> 4	U
KU030	4	0.064	+	> 32	0.5	+
KU032	> 16	0.094	+	> 32	1.0	+
KU033	> 16	> 1	U	> 32	> 4	U
KU034	> 16	0.094	+	> 32	4	+
KU035	> 16	0.047	+	> 32	0.38	+
KU036	> 16	0.5	+	> 32	3	+
KU037	> 16	0.25	+	> 32	>4	U
KU038	> 16	> 1	U	> 32	> 4	U
KU039	4	0.094	+	> 32	1.0	+
KU040	> 16	0.094	+	> 32	1.5	+
KU041	> 16	0.125	+	> 32	0.75	+
KU042	> 16	0.125	+	> 32	0.75	+
KU043	> 16	0.094	+	> 32	>4	U
KU044	> 16	0.125	+	> 32	> 4	U

Table aIII 9 (cont.)

Organism	MIC					
	CTX	**CTXL**	**CTX/ CTXL ≥ 8**	**CAZ**	**CAZL**	**CAZ/ CAZL ≥ 8**
KU045	3	0.094	+	> 32	> 4	U
KU046	> 16	0.094	+	> 32	> 4	U
KU047	> 16	0.125	+	> 32	0.5	+
KU048	2	0.064	+	> 32	1.0	+
KU049	2	0.125	+	> 32	0.38	+
KU050	> 16	0.094	+	> 32	4	+

*CTX= cefotaxime, CTXL= cefotaxime + clvulanic acid

CAZ = ceftazidime . CAZL = ceftazidime + clavulanic acid

** U = Undetermined

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APPENDIX IV

Table aIV 1 Criteria for classifying of phenotypes of extended spectrum β – lactamase (ESBL) producing *K.pneumoniae* according to Livermore and Williams (80)

Suggestive phenotypes of ESBL	Characteristics
Broad type activity	CAZ ≥ 32 ,CTX ≥ 32
Ceftazidimase activity	CAZ ≥ 128, CTX ≤ 4
Marginal activity	CAZ = 4-16, CTX < 4
Undetermined type I activity	CAZ < 32, CTX < 32
Undetermined type II activity	CAZ ≥ 32 , CTX < 32

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Table aIV 2 Inhibition zone sizes and antimicrobial susceptibility pattern of ESBL producing *K. pneumoniae* isolates from blood (14 isolates) and control strains

Inhibition zone sizes of antimicrobial agents (mm.) / Antimicrobial susceptibility pattern										
Organism	**GN**		**AK**		**TOB**		**CIP**		**SXT**	
E.coli ATCC 32518	29.35	S	25.49	S	24.50	S	32.41	S	25.36	S
K.pneumo ATCC 700603	13.88	S	22.27	S	14.37	S	23.34	S	6	R
K.high	13.39	S	16.47	S	10.50	R	30.87	S	6	R
K.low	20.75	S	21.52	S	21.06	S	27.27	S	22.01	S
K.non	21.56	S	22.47	S	21.19	S	26.64	S	6	R
KB002	12.51	S	21.94	S	14.32	S	21.88	S	6	R
KB003	18.91	S	14.65	S	11.10	R	6	R	18.50	S
KB006	17.47	S	14.35	R	9.88	R	6	R	14.60	S
KB009	19.05	S	17.23	S	8.35	R	7.31	R	19.50	S
KB011	14.22	S	12.67	R	12.15	R	6	R	6	R
KB012	13.65	S	12.41	R	12.29	R	6	R	6	R
KB013	14.48	S	20.52	S	13.25	S	6	R	6	R
KB014	20.63	S	18.97	S	8.38	R	10.50	R	6	R
KB015	6	R	12.61	R	12.15	R	27.63	S	27.85	S
KB016	6	R	18.01	S	12.25	R	29.68	S	31.32	S
KB017	6	R	14.83	S	6	R	29.85	S	6	R
KB018	13.28	S	18.38	S	11.64	R	22.42	S	23.20	S

Table a IV 2 (cont.)

Inhibition zone sizes of antimicrobial agents (mm.) / Antimicrobial susceptibility pattern										
Organisms	**GN**		**AK**		**TOB**		**CIP**		**SXT**	
KB019	10.80	R	12.27	R	6	R	21.22	S	6	R
KB020	19.11	S	14.11	R	9.49	R	6	R	6	R

* GN = gentamicin, AK = amikacin, TOB = tobramycin

CIP = ciprofloxacin , SXT = trimethoprim - sulfamethoxazole

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Table a IV 3 MIC of antimicrobial agents and antimicrobial susceptibility of ESBL producing *K. pneumoniae* isolates from blood (14 isolates) and control strains

MIC of antimicrobial agents / Antimicrobial susceptibility												
Organism	**CTX (≥ 64)**		**CAZ (≥ 32)**		**CRO (≥ 64)**		**CXM (≥ 32)**		**FOX (≥ 32)**		**IPM (≥ 16)**	
E.coli ATCC 35218	0.047	S	0.19	S	0.047	S	2	S	2	S	0.19	S
K.pneum ATCC 700603	16	S	32	R	16	S	24	S	16	S	0.125	S
K.high	12	S	> 256	R	12	S	48	R	12	S	0.25	S
K.low	0.125	S	0.5	S	0.094	S	4	S	4	S	0.19	S
K.non	0.094	S	0.38	S	0.094	S	2	S	2	S	0.19	S
KB002	6	S	8	S	12	S	12	S	4	S	0.5	S
KB003	24	S	256	R	24	S	48	R	12	S	0.064	S
KB006	24	S	256	R	24	S	256	R	2	S	0.19	S
KB009	24	S	>256	R	32	S	256	R	4	S	0.38	S
KB011	32	S	64	R	4	S	48	R	3	S	0.19	S
KB012	4	S	48	R	4	S	48	R	3	S	0.19	S
KB013	3	S	128	R	2	S	48	R	3	S	0.19	S
KB014	48	S	256	R	256	R	96	R	4	S	0.19	S
KB015	32	S	256	R	24	S	24	S	2	S	0.25	S
KB016	12	S	> 256	R	12	S	16	S	3	S	0.38	S
KB017	24	S	128	R	12	S	32	R	4	S	0.5	S
KB018	4	S	> 256	R	6	S	256	R	3	S	0.38	S
KB019	4	S	256	R	6	S	24	S	16	S	0.19	S
KB020	6	S	> 256	R	4	S	24	S	12	S	0.094	S

*CTX= cefotaxime, CAZ = ceftazidime , CRO = ceftriaxone

CXM = cefuroxime, FOX = cefoxitin, IPM = imipenem

Table a IV 4 Inhibition zone sizes and antimicrobial susceptibility pattern of ESBL producing *K. pneumoniae* isolates from sputum (42 isolates)

Inhibition zone sizes of antimicrobial agents (mm.)/ Antimicrobial susceptibility										
Organisms	**GN**		**AK**		**TOB**		**CIP**		**SXT**	
KS001	6	R	24.30	S	9.45	R	13.24	R	6	R
KS002	23.15	S	16.90	S	8.63	R	18.50	S	6	R
KS005	15.20	S	19.43	S	11.38	R	19.64	S	6	R
KS008	16.45	S	21.07	S	13.24	S	22.15	S	6	R
KS010	14.65	S	19.21	S	10.35	R	22.48	S	17.08	S
KS011	6	R	23.85	S	12.17	R	27.05	S	21.56	S
KS012	13.50	S	18.42	S	11.18	R	6	R	6	R
KS017	6	R	18.34	S	6	R	26.52	S	18.04	S
KS018	6	R	21.77	S	14.72	S	13.78	R	6	R
KS019	19.14	S	12.74	R	8.47	R	20.63	S	6	R
KS020	14.17	S	19.52	S	12.63	S	28.94	S	6	R
KS021	12.65	R	19.09	S	11.33	R	20.25	S	20.07	S
KS022	25.36	S	21.80	S	20.45	S	20.16	S	17.40	S
KS023	26.72	S	24.87	S	19.72	S	27.44	S	25.96	S
KS024	23.50	S	22.76	S	20.26	S	25.84	S	23.49	S
KS026	6	R	16.13	S	6	R	6	R	6	R
KS028	6	R	13.98	R	6	R	20.45	S	6	R
KS029	6	R	13.64	R	6	R	19.72	S	6	R
KS030	6	R	18.21	S	6	R	25.64	S	20.28	S
KS031	6	R	12.78	R	6	R	6	R	6	R
KS032	6	R	18.15	S	6	R	27.41	S	18.67	S

Table a IV 4 (cont.)

Inhibition zone sizes of antimicrobial agents (mm.)/ Antimicrobial susceptibility										
Organisms	**GN**		**AK**		**TOB**		**CIP**		**SXT**	
KS033	13.29	S	22.023	S	17.11	S	20.33	S	20.45	S
KS034	12.48	R	16.10	S	10.08	R	21.49	S	19.30	S
KS035	13.35	S	12.23	R	6	R	26.27	S	6	R
KS036	6	R	18.05	S	6	R	26.42	S	20.75	S
KS037	6	R	17.89	S	10.07	R	26.83	S	6	R
KS038	18.55	S	17.48	S	9.98	R	27.98	S	22.53	S
KS039	18.53	S	12.59	R	9.27	R	6	R	6	R
KS040	13.20	S	18.83	S	17.30	S	17.59	S	21.26	S
KS041	18.43	S	11.79	R	8.24	R	6	R	6	R
KS042	13.97	S	17.32	S	11.01	R	25.27	S	22.90	S
KS043	6	R	11.93	R	8.57	R	6	R	6	R
KS044	19.34	S	11.48	R	8.47	R	12.43	R	6	R
KS045	6	R	14.91	S	6	R	27.16	S	6	R
KS046	6	R	17.45	S	6	R	29.96	S	16.49	S
KS047	15.68	S	19.42	S	11.64	R	25.99	S	20.09	S
KS048	6	R	19.48	S	6	R	34.96	S	22.82	S
KS049	6	R	16.41	S	6	R	6	R	6	R
KS050	16.34	S	20.75	S	12.38	R	27.51	S	20.89	S
KS051	16.06	S	20.45	S	6	R	27.15	S	21.69	S
KS052	11.99	R	13.04	R	6	R	22.85	S	6	R
KS053	25.15	S	24.92	S	18.98	S	21.47	S	16.13	S

* GN = gentamicin, AK = amikacin, TOB = tobramycin

CIP = ciprofloxacin , SXT = trimethoprim – sulfamethoxazole

Table aIV 5 MIC of antimicrobial agents and antimicrobial susceptibility of ESBL producing *K. pneumoniae* isolates from sputum (42 isolates)

MIC of antimicrobial agents / Antimicrobial susceptibility												
Organism	**CTX (≥ 64)**		**CAZ (≥ 32)**		**CRO (≥ 64)**		**CXM (≥ 32)**		**FOX (≥ 32)**		**IPM (≥ 16)**	
KS001	256	R	256	R	256	R	256	R	4	S	0.25	S
KS002	16	S	32	R	12	S	24	S	2	S	0.19	S
KS005	96	R	256	R	48	S	128	R	6	S	0.25	S
KS008	32	S	24	S	128	R	256	R	4	S	0.38	S
KS010	6	S	256	R	24	S	64	R	3	S	0.38	S
KS011	256	R	256	R	256	R	256	R	3	S	0.25	S
KS012	16	S	256	R	8	S	256	R	4	S	0.25	S
KS017	32	S	64	R	48	S	16	S	2	S	0.19	S
KS018	32	S	48	R	64	R	256	R	2	S	0.19	S
KS019	48	S	256	R	128	R	256	R	4	S	0.19	S
KS020	4	S	256	R	6	S	48	R	3	S	0.19	S
KS021	8	S	256	R	12	S	64	R	2	S	0.25	S
KS022	32	S	64	R	48	S	24	S	3	S	0.19	S
KS023	96	R	256	R	128	R	128	R	4	S	0.19	S
KS024	96	R	256	R	64	R	128	R	3	S	0.19	S
KS026	16	S	256	R	24	S	48	R	32	R	0.19	S
KS028	8	S	128	R	12	S	12	S	3	S	0.25	S
KS029	6	S	192	R	8	S	12	S	6	S	0.25	S
KS030	16	S	32	R	12	S	16	S	2	S	0.19	S
KS031	32	S	256	R	32	S	48	R	16	S	0.064	S
KS032	8	S	32	R	8	S	12	S	3	S	0.125	S
KS033	4	S	1.5	S	4	S	6	S	3	S	0.25	S

Table aIV 5 (cont.)

MIC of antimicrobial agents / Antimicrobial susceptibility												
Organism	**CTX (≥ 64)**		**CAZ (≥ 32)**		**CRO (≥ 64)**		**CXM (≥ 32)**		**FOX (≥ 32)**		**IPM (≥ 16)**	
KS034	4	S	128	R	6	S	48	R	4	S	0.19	S
KS035	8	S	256	R	8	S	48	R	3	S	0.125	S
KS036	8	S	32	R	12	S	12	S	1.5	S	0.19	S
KS037	4	S	48	R	4	S	12	S	3	S	0.19	S
KS038	12	S	16	S	6	S	16	S	3	S	0.38	S
KS039	12	S	>256	R	24	S	96	R	12	S	0.19	S
KS040	6	S	> 256	R	6	S	>256	R	8	S	0.19	S
KS041	32	S	>256	R	6	S	3	S	16	S	0.125	S
KS042	4	S	>256	R	4	S	64	R	3	S	0.25	S
KS043	>256	R	>256	R	>256	R	>256	R	>256	R	2	S
KS044	48	S	>256	R	64	R	128	R	3	S	0.19	S
KS045	16	S	48	R	8	S	12	S	4	S	0.38	S
KS046	12	S	96	R	8	S	16	S	3	S	0.25	S
KS047	4	S	>256	R	8	S	>256	R	4	S	0.25	S
KS048	16	S	64	R	8	S	24	S	3	S	0.25	S
KS049	3	S	>256	R	3	S	96	R	8	S	0.25	S
KS050	4	S	>256	R	4	S	48	R	3	S	0.25	S
KS051	4	S	>256	R	3	S	32	R	3	S	0.25	S
KS052	8	S	>256	R	8	S	>256	R	3	S	0.25	S
KS053	12	S	>256	R	6	S	128	R	6	S	0.25	S

*CTX= cefotaxime, CAZ = ceftazidime , CRO = ceftriaxone

CXM = cefuroxime, FOX = cefoxitin, IPM = imipenem

Table a IV 6 Inhibition zone sizes and antimicrobial susceptibility pattern of ESBL producing *K. pneumoniae* isolates from urine (44 isolates)

Inhibition zone sizes of antimicrobial agents (mm.) / Antimicrobial susceptibility										
Organisms	**GN**		**AK**		**TOB**		**CIP**		**SXT**	
KU001	6	R	20.29	S	10.05	R	6	R	6	R
KU002	18.47	S	24.87	S	18.36	S	24.72	S	6	R
KU004	8.15	R	14.52	S	8.27	R	6	R	6	R
KU006	9.53	R	18.42	S	8.44	R	6	R	6	R
KU007	12.18	R	18.91	S	11.35	R	25.72	S	18.33	S
KU008	16.31	S	22.24	S	15.74	S	29.97	S	21.61	S
KU009	6	R	21.15	S	11.48	R	6	R	6	R
KU010	6	R	18.54	S	6	R	28.73	S	21.06	S
KU011	21.54	S	15.15	S	11.33	R	22.07	S	6	R
KU012	20.93	S	14.11	R	10.13	R	20.06	S	6	R
KU013	22.48	S	15.12	S	9.54	R	6	R	6	R
KU014	23.15	S	19.04	S	22.74	S	6	R	6	R
KU015	21.57	S	16.16	S	11.62	R	6	R	6	R
KU016	17.33	S	22.54	S	19.08	S	19.53	S	22.96	S
KU017	10.49	R	25.35	S	9.57	R	6	R	6	R
KU018	6	R	14.74	S	6	R	18.66	S	6	R
KU019	6	R	14.84	S	6	R	19.36	S	6	R
KU020	20.69	S	10.14	R	6	R	15.28	R	6	R
KU021	21.05	S	13.33	R	9.68	R	18.70	S	6	R
KU023	6	R	12.85	R	6	R	24.25	S	6	R
KU024	6	R	20.47	S	6	R	6	R	6	R
KU027	6	R	20.92	S	6	R	25.31	S	20.58	S

Table aIV 6 (cont.)

Inhibition zone sizes of antimicrobial agents (mm.) / Antimicrobial susceptibility										
Organisms	**GN**		**AK**		**TOB**		**CIP**		**SXT**	
KU028	6	R	20.47	S	11.95	R	26.84	S	11.17	S
KU029	11.67	R	25.88	S	6	R	6	R	6	R
KU030	22.61	S	17.45	S	10.63	R	31.57	S	29.97	S
KU032	6	R	20.70	S	10.68	R	30.04	S	25.73	S
KU033	6	R	19.04	S	6	R	6	R	6	R
KU034	6	R	22.37	S	6	R	30.66	S	22.84	S
KU035	6	R	23.54	S	6	R	19.22	I	6	R
KU036	14.06	S	12.63	R	6	R	19.68	I	6	R
KU037	6	R	11.59	R	6	R	26.33	S	17.66	S
KU038	10.65	R	23.61	S	6	R	6	R	6	R
KU039	6	R	12.95	R	6	R	28.15	S	6	R
KU040	22.25	S	10.78	R	8.53	R	11.25	R	6	R
KU041	8.56	R	23.77	S	9.47	R	22.36	S	6	R
KU042	18.92	S	12.84	R	9.53	R	28.04	S	29.36	S
KU043	6	R	6	R	6	R	24.95	S	6	R
KU044	12.67	S	18.58	S	6	R	28.22	S	6	R
KU045	6	R	13.22	R	6	R	22.39	S	6	R
KU046	20.31	S	10.49	R	6	R	11.90	R	6	R
KU047	12.48	R	16.32	S	6	R	22.07	S	6	R
KU048	15.10	S	18.79	S	12.79	S	21.75	S	20.15	S
KU049	20.46	S	22.68	S	11.59	R	20.13	S	6	R
KU050	6	R	6	R	6	R	29.29	S	6	R

* GN = gentamicin, AK = amikacin, TOB = tobramycin

CIP = ciprofloxacin , SXT = trimethoprim - sulfamethoxazole

Table a IV 7 MIC of antimicrobial agents and antimicrobial susceptibility of ESBL producing *K.pneumoniae* isolates from urine (44 isolates)

MIC of antimicrobial agents / Antimicrobial susceptibility												
Organism	**CTX (≥ 64)**		**CAZ (≥ 32)**		**CRO (≥ 64)**		**CXM (≥ 32)**		**FOX (≥ 32)**		**IPM (≥ 16)**	
KU001	16	S	256	R	24	S	256	R	12	S	0.38	S
KU002	8	S	64	R	16	S	16	S	3	S	0.19	S
KU004	32	S	256	R	256	R	256	R	4	S	0.25	S
KU006	32	S	256	R	64	R	256	R	64	R	0.19	S
KU007	32	S	256	R	64	R	256	R	64	R	0.19	S
KU008	2	S	64	R	4	S	16	S	2	S	0.5	S
KU009	8	S	96	R	8	S	16	S	6	S	0.125	S
KU010	12	S	48	R	16	S	6	S	8	S	0.19	S
KU011	256	R	256	R	256	R	256	R	8	S	0.25	S
KU012	256	R	256	R	256	R	256	R	12	S	0.25	S
KU013	64	R	256	R	48	S	256	R	256	R	0.19	S
KU014	64	R	256	R	64	R	64	R	32	R	0.125	S
KU015	256	R	256	R	256	R	64	R	32	R	0.19	S
KU016	16	S	96	R	16	S	24	S	4	S	0.38	S
KU017	64	R	256	R	128	R	128	R	8	S	0.19	S
KU018	256	R	256	R	256	R	64	R	32	R	0.19	S
KU019	24	S	256	R	32	S	32	R	4	S	0.25	S
KU020	16	S	256	R	24	S	32	R	3	S	0.25	S
KU021	4	S	32	R	8	S	12	S	4	S	0.25	S
KU023	12	S	256	R	48	S	32	R	4	S	0.19	S
KU024	48	S	64	R	256	R	256	R	6	S	0.25	S
KU027	6	S	256	R	6	S	96	R	6	S	0.5	S
KU028	6	S	8	S	8	S	32	R	4	S	0.5	S

Table aIV 7 (cont.)

MIC of antimicrobial agents / Antimicrobial susceptibility												
Organism	**CTX (≥ 64)**		**CAZ (≥ 32)**		**CRO (≥ 64)**		**CXM (≥ 32)**		**FOX (≥ 32)**		**IPM (≥ 16)**	
KU029	16	S	>256	R	24	S	64	R	6	S	0.25	S
KU030	4	S	48	R	4	S	12	S	4	S	0.38	S
KU032	16	S	>256	R	24	S	48	R	6	S	0.38	S
KU033	12	S	>256	R	12	S	48	R	>256	R	0.064	S
KU034	48	S	>256	R	32	S	128	R	4	S	0.25	S
KU035	12	S	>256	R	12	S	>256	R	4	S	0.25	S
KU036	32	S	>256	R	24	S	>256	R	12	S	0.25	S
KU037	6	S	>256	R	8	S	24	S	8	S	0.19	S
KU038	24	S	>256	R	64	R	>256	R	64	R	0.19	S
KU039	6	S	64	R	6	S	12	S	4	S	0.38	S
KU040	8	S	>256	R	12	S	24	S	4	S	0.19	S
KU041	48	S	>256	R	48	S	>256	R	6	S	0.25	S
KU042	6	S	32	R	4	S	16	S	6	S	0.25	S
KU043	8	S	>256	R	24	S	32	R	2	S	0.38	S
KU044	16	S	>256	R	16	S	>256	R	8	S	0.38	S
KU045	4	S	128	R	4	S	12	S	4	S	0.38	S
KU046	32	S	>256	R	32	S	128	R	8	S	0.25	S
KU047	3	S	>256	R	4	S	96	R	4	S	0.25	S
KU048	1.5	S	64	R	2	S	32	R	3	S	0.25	S
KU049	1.5	S	>256	R	2	S	32	R	3	S	0.094	S
KU050	12	S	64	R	6	S	16	S	3	S	0.19	S

*CTX= cefotaxime, CAZ = ceftazidime , CRO = ceftriaxone

CXM = cefuroxime, FOX = cefoxitin, IPM = imipenem

BIOGRAPHY

Miss Julintorn Pongtongkam was born on October 31, 1977 in Bangkok, Thailand. She graduated with the Bachelor degree of Science in General Science from Faculty of Science, Kasetsart University, in 1988

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