CHAPTER V

RESULTS

1. Lactobacilli

1.1 Primary antimicrobial screening

In the present study, 172 isolates of lactobacilli were recovered. Among these isolates, most of them demonstrated antimicrobial activities against 4 reference bacterial indicators including *S. aureus* ATCC 25923, *Sarcina lutea* ATCC 9341, *E. coli* ATCC 25922 and *Bacillus subtilis* ATCC 6633. Twelve isolates, namely F7/22, L541, LSS, B14, F8/22, L1034, G2/22, B305, B280, B21/8, B39/4 and B4/22, which exhibited the greatest inhibition zone against target strains, were selected for the further study (Table 9).

1.2 Secondary antimicrobial screening

Twelve of these lactobacilli were then called as the potent antimicrobial isolates. Of these isolates, L541 and LSS which exhibited the potent activity against 2 reference straiuns and 3 clinical isolates and were selected for the further study (Table 10).

Table 9 Primary screening of 12 selected lactobacilli against 4 standard strains

Lactobacillus	Average diameter of inhibition zone (mm) against			
	S. aureus ATCC 25923	*S. lutea* ATCC 9341	*E. coli* ATCC 25922	*B. subtilis* ATCC 6633
F7/22	16.0	19.0	22.0	17.0
L541	23.0	20.0	19.5	17.0
LSS	19.5	17.0	16.0	17.0
B14	15.5	18.5	17.0	18.0
F8/22	17.0	13.5	17.0	18.5
L1034	22.0	20.0	19.0	17.0
G2/22	22.0	17.5	18.0	18.0
B305	19.5	19.0	19.0	18.0
B280	18.5	17.0	18.0	17.0
B21/8	20.0	18.5	18.0	17.5
B39/4	21.0	18.5	17.0	16.5
B4/22	18.0	18.0	18.0	16.0

Table 10 Antimicrobial screening of 2 potent lactobacilli against 2 standard strains and 3 laboratory strains

Isolates	Average diameter of inhibition zone (mm) against	
	L541	LSS
P. aeruginosa ATCC 27853	15.5	15.0
E. feacalis ATCC 29213	18.5	15.5
A. baumannii u46	14.0	14.0
P. vulgaris u201	13.5	16.5
S. agalactiae u304	16.5	13.0

1.3 Identification of the potent antimicrobial isolates

Two potent isolates were gram positive rods and could not produce endospore. Using API 50 - CHL biochemical kits, they were identified to belong to *Lactobacillus plantarum*1 and *Lactobacillus pentosus* (Table 11). An API 50 - CHL biochemical identification was also shown in Figure 10.

Table 11 Identification of the potent antimicrobial lactobacilli

Strains	Interpretations	% Identity
L541	*Lactobacillus plantarum*1	91.1
LSS	*Lactobacillus pentosus*	99.9

The species identification was acceptable at the levels of 80% identity.

For the control strains, *L. casei* TISTR 330 and *L. rhamnosus* TISTR 108 gave the percent identity at 99.9 and 99.5, respectively.

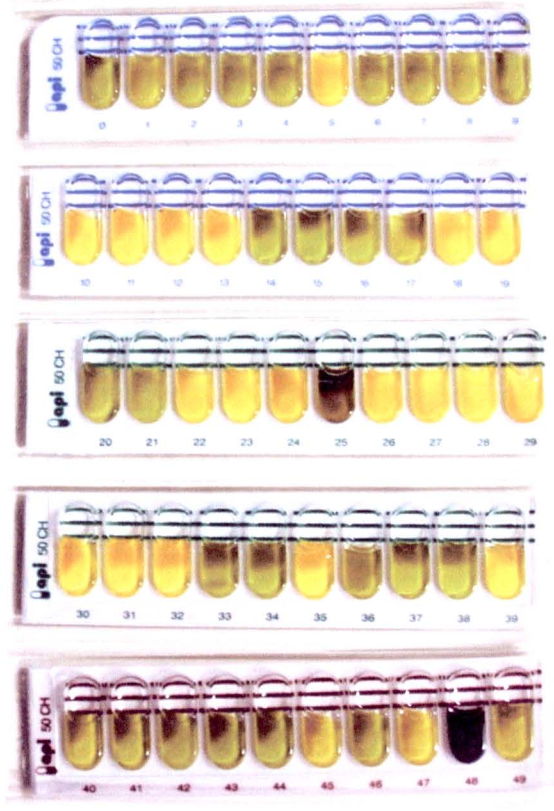


Figure 10. The example of biochemical reactions according to API - 50 CHL kit after incubated at 37°C for 24 hrs

1.4 Characterization of cell - free supernatants

Concentrations of the hydrogen peroxide, lactic acid and total protein were higher in 2 cell - free supernatants (Table 12) than these substances in MRS broth were observed.

Table 12 Concentration of each active substance of the tested isolates

Lactobacillus	Concentration of		
	H_2O_2 (μM)	**Lactic acid (mM)**	**Total protein (mg/ml)**
L541	6.01	6.49	1.53
LSS	5.64	8.96	1.85
MRS broth	1.57	1.48	1.46

Three uropathogens and *E. coli* ATCC 25922 were selected to test the cut - off concentration, the minimal concentration of the standard hydrogen peroxide which could inhibit 2 tested strains, *E. coli* strain u1104 and u1249, and *E. coli* ATCC 25922. They were 4, 2 and 1 mM, respectively. While the cut - off concentration of *E. coli* strain u874 was less than 1 mM. However, the cut - off concentration of each tested strains was higher than the hydrogen peroxide concentration produced by *L. plantarum*1 strain L541 and *L. pentosus* strain LSS.

The cut - off concentration of lactic acid to inhibit the 2 tested pathogenic isolates, *E. coli* strain u1249 and u874, and *E. coli* ATCC 25922 was 50 mM. While the cut - off concentration of lactic acid to inhibit *E. coli* strain u1104 was 100 mM. These concentrations were higher than the concentration of lactic acid produced by *L. plantarum*1 strain L541 and *L. pentosus* strain LSS.

Therefore, the concentration of hydrogen peroxide and lactic acid produced by *L. plantarum* strain L541 and *L. pentosus* strain LSS may be not the major antimicrobial substance to inhibit these tested strains.

1.5 Characterization of crude bacteriocins

After ammonium sulphate precipitation at 40% saturation, the total protein concentration of each crude protein was determined as shown in Table 13.

The protein extracted from *L. plantarum* strain L541, *L. pentosus* strain LSS, and *L. crispatus* strain B120 could be detected by using the silver stain. The detectable protein bands were shown in Figure 11.

Table 13 Comparison of total protein concentrations between cell - free supernatant and crude extract of the potent lactobacillus

Lactobacillus	**Total protein concentration (mg/ml)**		**Protein yield (folds)**
	Cell - free supernatant	**Crude extract**	
L541	1.53	4.05	2.65
LSS	1.85	6.86	3.71
B120 (control strain)	2.00	7.55	3.78
MRS broth	1.46	3.72	2.55

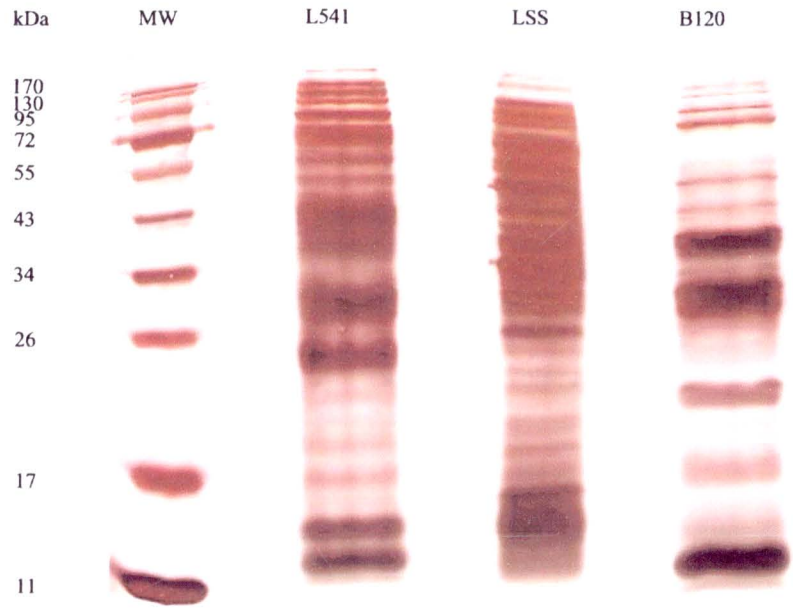


Figure 11 The proteins patterns of each crude bacteriocins from *Lactobacillus* strains using ammonium sulphate precipitation; lane MW, molecular weight marker (Page Ruler™ Prestained Protein Ladder; Fermentas INC, Burlington, ON, Canada)

2. ESBL - producing uropathogenic enterobacteriaceae and their β - lactamases

2.1 Collection and antimicrobial susceptibility test

One hundred and forty nine isolates of recovered uropathogenic bacteria in the family Enterobacteriaceae were collected and tested the antimicrobial susceptibility. The results were shown in Table 14.

Percentage of antibiotic susceptibility towards the multidrug resistance uropathogenic bacteria was shown in Figure 12. From this figure, the different manners of antibacterial susceptibility were shown. Firstly, the highest resistance

agents, the prevalence of susceptible tested isolates were lower than 5%. They were ampicillin and carbenicillin. Secondary, the resistance agents, prevalence of susceptible tested isolates were in range of 5 - 20%. They were cephalothin, piperacillin and tetracycline. Thirdly, the moderate resistance agents, prevalence of susceptible tested isolates were in range of 20 - 50%. They were gentamicin, amoxicillin/clavulanic acid, cefotaxime, ciprofloxacin, sulfamethoxazole/ trimethoprim, cefamandole, kanamycin, chloramphenicol, tobramycin and norfloxacin. Forthly, the moderate susceptible agents, prevalence of susceptible tested isolates were between 50% to 89%. They were cefepime, cefoxitin, ceftazidime, trimethoprim and nitrofurantoin. Fifthly, the highest susceptible agents, 90% of tested isolates were susceptible to amikacin, netilmicin and all isolates were susceptible to imipenem.

Table 14 Antimicrobial susceptibility of 149 bacterial isolates according to the numbers of resistant drugs.

Isolates	Interpretation of susceptibility of each isolate toward																				
	Group A*			Group B**										Group C***						Group U****			
	AM	CF	GM	AK	AMC	CIP	CTX	FEP	FOX	IPM	MA	PRL	STX	C	CAZ	K	NET	TE	TOB	CA	F/M	NOR	TMP
u1/27	R	R	R	S	R	R	R	R	R	S	R	R	R	R	R	R	R	R	R	R	R	R	R
u2/3	R	R	R	I	R	R	R	R	R	S	R	R	R	R	R	R	S	R	R	R	R	R	R
u8/18	R	R	R	S	R	R	R	R	S	S	R	R	R	R	R	R	I	R	R	R	R	R	R
u1/23	R	R	R	S	R	R	R	R	S	S	R	R	S	R	R	R	R	R	R	R	R	R	S
u6/8	R	R	R	S	R	R	R	R	S	S	R	R	R	R	R	R	S	R	R	R	S	R	R
u2/2	R	R	R	I	R	R	R	R	S	S	R	R	R	S	R	R	R	R	R	R	I	R	R
u3/14	R	R	R	I	I	R	R	R	S	S	R	R	R	R	R	R	R	S	R	R	R	R	R
u5/7	R	R	R	S	R	R	R	R	I	S	R	R	R	R	I	R	R	R	R	R	S	R	R
u5/23	R	R	R	S	R	R	R	R	I	S	R	R	R	I	R	R	I	R	R	R	R	R	R
u3/13	R	R	S	S	R	R	R	R	S	S	R	R	R	R	R	R	S	R	R	R	S	R	R
u7/7	R	R	R	S	R	R	R	R	S	S	R	R	R	S	R	R	S	R	R	R	S	R	R
u1/25	R	R	R	S	R	R	R	R	S	S	R	R	S	R	R	R	I	R	R	R	R	R	S
u1/26	R	R	R	S	R	R	R	I	S	S	R	R	R	S	R	R	S	R	R	R	R	R	R
u1/4	R	R	R	S	R	R	R	S	R	S	R	R	R	R	R	R	S	R	I	R	S	R	R
u6/9	R	R	R	S	S	R	R	R	S	S	R	R	R	R	R	R	S	R	R	R	I	R	R
u8/16	R	R	R	S	R	R	R	R	S	S	R	R	R	S	R	R	S	R	R	R	I	R	R
u1/24	R	R	R	S	R	R	R	R	S	S	R	R	R	S	R	R	I	R	I	R	R	R	R
u1/30	R	R	R	R	S	R	R	I	R	S	R	R	R	S	R	R	R	I	R	R	R	R	S
u3/9	R	R	R	S	S	R	R	R	S	S	R	R	R	R	I	R	R	R	R	R	I	R	R
u4/10	R	I	R	I	R	R	R	R	S	S	R	R	R	R	R	R	S	R	R	R	S	R	R

Table 14 (continued)

Isolates	Interpretation of susceptibility of each isolate toward																				
	Group A*			Group B**										Group C***						Group U****			
	AM	CF	GM	AK	AMC	CIP	CTX	FEP	FOX	IPM	MA	PRL	STX	C	CAZ	K	NET	TE	TOB	CA	F/M	NOR	TMP
u5/3	R	R	R	S	R	I	R	R	S	S	R	R	R	R	R	R	S	R	R	R	I	R	R
u6/13	R	R	R	S	I	R	R	R	S	S	R	R	R	R	R	R	S	R	I	R	R	R	R
u1/7	R	R	R	S	I	R	R	R	R	S	R	R	R	R	R	R	I	R	I	R	S	R	R
u7/9	R	R	R	I	I	R	R	R	I	S	R	R	R	R	R	R	I	R	R	R	I	R	R
u2/4	R	R	R	S	S	R	R	S	R	S	R	R	R	S	R	R	S	R	R	R	S	R	R
u6/4	R	R	R	S	S	R	R	S	S	S	R	R	R	R	R	R	S	R	R	R	S	R	R
u7/17	R	R	S	S	R	R	R	S	R	S	R	R	S	S	R	R	S	R	R	R	R	R	R
u7/13	R	R	R	S	I	R	R	R	S	S	R	R	R	R	R	R	S	S	R	R	S	R	R
u8/7	R	R	S	S	R	R	I	S	R	S	R	R	R	R	R	R	S	R	R	R	S	R	R
u2/7	R	R	R	S	R	R	I	S	R	S	R	R	R	R	I	R	S	R	R	R	S	R	R
u3/15	R	R	R	S	R	R	R	I	S	S	R	R	R	S	S	R	I	R	R	R	R	R	R
u4/18	R	I	S	S	R	R	R	S	R	S	R	R	R	R	R	R	S	R	R	R	I	R	R
u5/20	R	R	R	S	I	R	R	R	S	S	R	R	R	R	R	R	S	R	I	R	S	R	R
u6/7	R	R	R	S	I	R	R	R	S	S	R	R	R	R	R	I	S	R	R	R	S	R	R
u7/10	R	R	R	S	I	R	R	I	S	S	R	R	R	R	R	R	S	R	R	R	S	R	R
u7/6	R	R	R	S	R	R	R	R	S	S	R	R	R	R	S	I	I	R	R	R	S	R	R
u5/11	R	R	R	S	I	R	R	R	I	S	R	R	R	R	R	R	S	R	I	R	S	R	R
u5/22	R	R	R	S	I	I	R	R	S	S	R	R	R	R	R	R	I	R	R	R	R	S	R
u7/2	R	R	R	I	I	R	R	R	S	S	R	R	R	S	I	R	R	R	R	R	S	R	R
u1/22	R	R	R	S	I	R	R	R	S	S	R	R	I	R	R	R	I	R	R	R	R	R	I
u7/16	R	R	R	S	I	R	R	I	S	S	R	R	R	R	R	R	I	R	R	R	R	R	I

Table 14 (continued)

Isolates	Interpretation of susceptibility of each isolate toward																					
	Group A*			Group B**										Group C***						Group U****			
	AM	CF	GM	AK	AMC	CIP	CTX	FEP	FOX	IPM	MA	PRL	STX	C	CAZ	K	NET	TE	TOB	CA	F/M	NOR	TMP
u4/17	R	R	S	R	I	S	R	R	S	S	R	R	R	R	R	R	R	S	R	R	S	S	R
u5/5	R	R	R	S	R	R	S	I	R	S	R	R	R	S	R	R	S	S	R	R	S	R	R
u6/10	R	R	R	S	I	R	R	R	S	S	R	R	R	S	R	R	S	R	S	R	S	R	R
u7/5	R	R	R	S	R	S	R	R	R	S	R	R	R	R	R	S	S	R	S	R	I	S	R
u4/14	R	R	R	S	I	R	R	R	I	S	R	R	R	S	R	R	S	R	R	R	S	R	S
u7/11	R	R	R	S	S	R	R	R	S	S	R	R	R	R	R	I	S	R	I	R	S	R	R
u8/10	R	R	R	S	I	R	R	S	S	S	R	R	R	R	I	R	S	R	R	R	S	R	R
u1/20	R	R	R	S	I	R	R	S	I	S	R	R	R	R	S	R	I	R	R	R	S	R	R
u2/8	R	R	R	S	S	R	R	R	S	S	R	R	R	R	I	I	I	R	R	R	S	R	R
u3/1	R	R	R	I	I	R	R	S	I	S	R	R	R	R	S	R	S	R	R	R	S	R	R
u3/5	R	R	R	S	I	R	R	S	I	S	R	R	R	R	S	R	I	R	R	R	S	R	R
u4/4	R	R	S	I	R	R	I	S	R	S	R	R	R	S	R	R	I	R	R	R	S	R	R
u4/5	R	R	R	I	I	R	R	S	S	S	R	R	R	R	S	R	S	R	R	R	I	R	R
u8/19	R	R	R	I	I	R	S	S	S	S	R	R	R	I	S	R	R	R	R	R	R	R	R
u7/14	R	R	R	S	R	I	I	S	R	S	R	R	R	R	R	I	S	R	I	R	R	R	R
u8/4	R	R	R	S	I	R	R	R	I	I	R	R	R	S	S	R	S	R	R	R	I	R	R
u1/16	R	R	R	S	S	R	R	I	S	S	R	R	R	R	S	R	S	R	S	R	S	R	R
u1/2	R	R	S	S	I	R	R	S	R	S	R	R	R	R	S	R	S	R	S	R	S	R	R
u7/18	R	R	R	S	R	R	R	R	R	S	R	R	R	S	R	S	S	S	S	R	I	R	S
u1/17	R	R	R	S	I	R	R	I	S	S	R	R	R	S	S	R	S	R	R	R	S	R	R
u1/19	R	R	R	S	I	R	R	S	S	S	R	R	R	S	R	I	S	R	R	R	S	R	R

Table 14 (continued)

Isolates	Interpretation of susceptibility of each isolate toward																					
	Group A*			Group B**										Group C***						Group U****			
	AM	CF	GM	AK	AMC	CIP	CTX	FEP	FOX	IPM	MA	PRL	STX	C	CAZ	K	NET	TE	TOB	CA	F/M	NOR	TMP
u8/14	R	R	R	S	R	R	R	R	I	S	R	R	R	S	I	S	S	R	S	R	S	R	R
u8/9	R	R	S	S	R	S	I	S	R	S	R	R	R	R	S	R	I	R	R	R	S	R	R
u2/1	R	R	R	S	I	R	S	S	S	S	I	R	R	R	S	R	R	I	R	R	R	R	R
u8/13	R	R	S	S	I	R	S	S	S	S	R	R	R	R	S	R	R	S	R	R	S	R	R
u4/12	R	R	R	S	I	R	S	S	I	S	I	R	R	R	S	R	S	R	R	R	S	R	R
u4/6	R	R	R	S	I	R	R	R	I	S	R	R	S	S	R	R	S	R	I	R	I	R	S
u7/15	R	R	I	S	S	R	I	S	S	S	R	R	R	R	R	I	S	R	I	R	R	R	R
u1/8	R	R	R	S	I	R	R	I	S	S	R	R	R	S	I	I	I	R	R	R	S	R	R
u2/6	R	R	R	S	S	R	R	R	S	S	R	R	R	I	I	I	I	R	I	R	S	R	R
u1/12	R	R	S	S	S	R	S	R	R	S	R	R	S	R	S	R	S	R	S	R	S	R	S
u7/3	R	R	S	S	S	R	R	I	S	S	R	R	S	R	R	S	S	R	S	R	R	R	S
u1/9	R	R	S	S	I	R	R	I	S	S	R	R	R	R	S	S	S	R	S	R	S	R	R
u3/8	R	R	R	S	S	R	I	S	S	S	R	R	R	S	S	I	S	R	R	R	S	R	R
u5/1	R	R	S	I	R	R	I	S	S	S	R	R	R	S	S	R	S	S	R	R	S	R	R
u7/4	R	R	S	S	S	R	R	I	I	S	R	R	R	R	S	S	S	R	S	R	S	R	R
u1/1	R	R	S	S	I	R	R	S	S	S	R	R	R	S	S	S	S	R	S	R	S	R	R
u3/4	R	R	S	S	I	R	R	R	S	S	R	R	S	S	R	S	S	R	S	R	S	R	S
u4/9	R	R	R	S	S	R	S	S	S	S	S	R	R	R	S	I	S	R	S	R	S	R	R
u8/3	R	R	R	S	I	R	R	S	S	S	R	R	R	S	S	S	S	R	S	R	S	S	R
u5/9	R	I	R	S	S	R	S	S	S	S	S	R	R	R	S	R	S	R	I	R	S	R	R
u8/12	R	R	S	S	I	R	S	S	I	S	R	R	R	R	S	S	S	R	S	R	S	R	R

Table 14 (continued)

Isolates	Interpretation of susceptibility of each isolate toward																					
	Group A*			Group B**											Group C***						Group U****			
	AM	CF	GM	AK	AMC	CIP	CTX	FEP	FOX	IPM	MA	PRL	STX	C	CAZ	K	NET	TE	TOB	CA	F/M	NOR	TMP
u1/11	R	R	R	S	S	R	S	S	S	S	S	R	R	R	S	I	I	R	I	R	S	R	R
u1/13	R	R	R	S	I	S	R	S	S	S	R	R	R	R	S	I	S	R	I	R	S	S	R
u8/6	R	R	R	I	I	R	S	S	R	S	R	R	I	S	S	S	S	R	S	R	S	R	R
u3/3	R	I	R	I	S	R	S	S	S	S	S	R	R	R	S	I	I	R	R	R	S	R	R
u6/11	R	R	R	S	I	S	I	S	S	S	R	R	R	R	S	I	S	R	R	R	I	S	R
u2/5	R	R	I	S	I	R	I	I	S	S	R	I	R	S	R	S	R	I	R	R	S	R	R
u7/8	R	R	S	S	S	R	R	R	S	S	R	R	S	S	R	S	S	S	S	R	S	R	S
u8/17	R	R	S	S	S	R	S	S	S	S	R	R	S	R	S	S	S	R	S	R	S	R	R
u4/11	R	I	R	S	S	R	S	S	S	S	S	R	R	S	S	R	S	R	S	R	S	R	R
u6/6	R	R	S	S	S	R	S	S	S	S	S	R	R	R	I	S	S	R	S	R	S	R	R
u7/12	R	R	S	S	S	R	R	R	S	S	R	R	S	I	S	S	S	R	S	R	S	R	S
u7/19	I	R	S	S	R	S	S	S	R	S	S	S	S	R	R	R	S	R	S	R	S	R	R
u8/5	R	R	S	S	I	R	S	S	S	S	S	R	R	R	S	S	S	R	S	R	S	R	R
u5/16	R	I	S	I	S	R	S	S	S	S	R	R	R	R	S	S	S	R	S	R	S	R	R
u3/12	R	R	R	S	I	S	S	S	S	S	I	R	R	R	S	I	S	R	R	R	S	S	R
u3/6	R	I	R	S	S	R	S	S	S	S	S	R	R	S	S	I	I	R	R	R	S	R	R
u6/2	R	I	R	S	S	R	S	S	S	S	I	R	R	S	S	I	S	R	R	R	S	R	R
u5/12	R	I	R	I	S	R	S	S	S	S	S	R	R	S	S	I	I	R	R	R	S	R	R
u5/18	R	R	S	S	I	R	R	I	I	S	R	R	R	S	I	I	S	R	R	S	S	S	R
u5/18	R	R	S	S	I	R	R	I	I	S	R	R	R	S	I	I	S	R	R	S	S	S	R
u4/16	R	S	R	S	S	R	S	S	S	S	S	I	R	S	S	S	S	R	S	R	R	R	R

Table 14 (continued)

Isolates	Interpretation of susceptibility of each isolate toward																					
	Group A*			Group B**											Group C***						Group U****			
	AM	CF	GM	AK	AMC	CIP	CTX	FEP	FOX	IPM	MA	PRL	STX	C	CAZ	K	NET	TE	TOB	CA	F/M	NOR	TMP
u3/2	R	R	S	S	I	R	R	S	S	S	R	R	S	S	S	S	S	R	S	R	S	R	S
u3/7	R	I	S	S	S	R	S	S	S	S	S	R	R	R	S	S	S	R	S	R	S	R	R
u8/15	R	R	R	S	S	R	I	S	S	S	R	R	S	S	S	S	S	R	S	R	S	R	S
u6/12	R	R	S	S	S	S	I	S	S	S	R	R	R	R	S	S	S	R	S	R	I	S	R
u7/1	R	R	S	S	I	R	S	S	R	S	R	R	S	I	S	S	S	R	S	R	S	R	S
u7/20	R	R	S	S	R	S	R	R	R	S	R	R	S	S	S	S	S	R	S	I	I	S	S
u8/11	R	R	S	S	I	R	S	S	S	S	I	R	R	S	S	S	S	R	S	R	S	R	R
u1/28	R	R	S	S	R	S	I	S	R	S	I	R	R	R	S	S	S	S	S	R	I	S	R
u4/13	R	I	R	S	S	R	S	S	S	S	S	R	R	R	S	S	S	R	I	R	S	R	R
u4/2	R	R	R	S	S	R	S	S	S	S	S	R	I	R	S	I	S	R	I	R	I	R	S
u6/3	R	R	S	S	I	R	R	I	S	S	R	R	R	S	S	I	S	S	S	R	I	S	R
u5/8	R	I	S	S	S	R	S	S	S	S	S	R	R	S	S	S	S	R	S	R	S	R	R
u5/10	R	I	S	I	S	R	S	S	S	S	S	R	R	S	S	S	S	R	S	R	S	R	R
u5/2	R	R	S	S	S	S	S	S	I	S	S	I	R	R	S	S	S	R	S	R	R	S	R
u1/5	R	R	S	S	S	S	S	S	S	S	S	R	R	S	S	S	S	R	S	R	S	S	R
u1/10	R	R	R	S	S	I	S	S	S	S	S	R	S	S	S	R	S	S	S	R	S	R	S
u1/14	R	S	S	S	S	R	S	S	S	S	S	I	R	S	S	S	S	R	S	R	S	R	R
u4/8	S	I	R	S	S	R	S	S	S	S	S	S	R	S	S	S	S	R	S	S	R	R	R
u5/13	R	S	S	S	S	R	S	S	S	S	S	I	R	S	S	S	S	R	S	R	S	R	R
u3/10	R	R	S	S	I	S	S	S	S	S	I	R	R	S	S	S	S	R	S	R	S	S	R
u3/11	R	R	R	S	S	R	S	S	S	S	S	R	S	S	S	I	S	S	I	R	S	R	S

Table 14 (continued)

Isolates	Interpretation of susceptibility of each isolate toward																					
	Group A*			Group B**										Group C***						Group U****			
	AM	CF	GM	AK	AMC	CIP	CTX	FEP	FOX	IPM	MA	PRL	STX	C	CAZ	K	NET	TE	TOB	CA	F/M	NOR	TMP
u4/7	R	R	S	S	S	I	R	R	S	S	R	R	S	S	I	S	S	S	S	R	S	S	S
u8/2	R	S	S	S	I	S	S	S	S	S	S	R	R	R	S	I	S	R	S	R	S	S	R
u1/6	R	R	S	S	S	R	I	S	S	S	I	R	I	S	S	S	S	R	S	R	S	R	S
u5/4	R	R	S	S	I	S	S	I	S	S	R	R	S	I	I	R	S	R	S	R	I	S	S
u1/21	R	I	S	S	S	R	S	S	S	S	S	R	S	S	S	S	S	R	S	R	S	R	S
u1/15	R	I	S	S	S	R	S	S	S	S	I	R	S	S	S	S	S	R	S	R	S	R	S
u6/5	R	R	S	S	I	I	S	S	S	S	R	R	S	S	S	S	S	R	S	R	S	S	S
u8/1	R	R	S	I	S	S	S	S	S	S	I	R	R	S	R	S	S	S	S	R	S	S	S
u4/15	R	I	S	S	I	S	S	S	S	S	S	R	R	S	S	R	S	R	S	R	I	S	S
u5/19	R	R	S	S	I	S	S	S	S	S	R	R	S	S	S	I	S	S	S	R	S	S	S
u1/29	I	R	S	S	R	S	S	S	R	S	S	I	S	S	S	S	S	S	S	R	R	S	S
u5/17	R	R	S	R	I	S	S	S	S	S	I	R	S	S	S	I	S	S	S	R	S	S	S
u1/3	R	I	S	S	I	S	S	S	S	S	I	R	R	S	S	I	S	S	S	R	S	S	R
u5/14	R	R	S	S	S	R	S	S	I	S	S	S	S	R	S	I	S	I	S	I	S	R	S
u5/15	R	I	S	S	I	S	S	S	S	S	I	R	I	R	S	S	S	S	S	R	S	S	R
u4/1	R	I	S	S	S	S	S	S	S	S	S	R	S	S	S	S	S	R	S	R	S	S	S
u1/18	R	I	S	S	S	S	S	S	S	S	S	I	R	S	S	R	S	S	S	R	S	S	R
u5/21	R	I	R	S	S	S	S	S	S	S	S	R	S	S	S	I	S	S	I	R	S	S	S
u9/3	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	R	S	S
u6/1	I	R	S	S	S	R	S	S	S	S	S	S	S	S	S	I	S	S	S	S	S	R	S

Table 14 (continued)

Isolates	Interpretation of susceptibility of each isolate toward																					
	Group A*			**Group B****										**Group C*****						**Group U******			
	AM	**CF**	**GM**	**AK**	**AMC**	**CIP**	**CTX**	**FEP**	**FOX**	**IPM**	**MA**	**PRL**	**STX**	**C**	**CAZ**	**K**	**NET**	**TE**	**TOB**	**CA**	**F/M**	**NOR**	**TMP**
u5/6	R	S	S	I	S	S	R	S	S	S	S	S	S	S	S	I	S	S	S	R	S	S	S
u8/8	S	R	R	I	S	S	S	S	S	S	S	S	I	I	S	I	S	R	S	R	S	R	R
u9/1	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	I	S	S
u9/2	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	I	S	S

Bold numbers, Selected isolates for further study; R, resistant; I, intermediate susceptible; S, susceptible; *Group A antibiotic, Primary test and Report. **Group B antibiotic, Primary test Report selectively. ***Group C antibiotic, Supplementaly report selectively. ****Group U antibiotic, Supplementary for urine only.

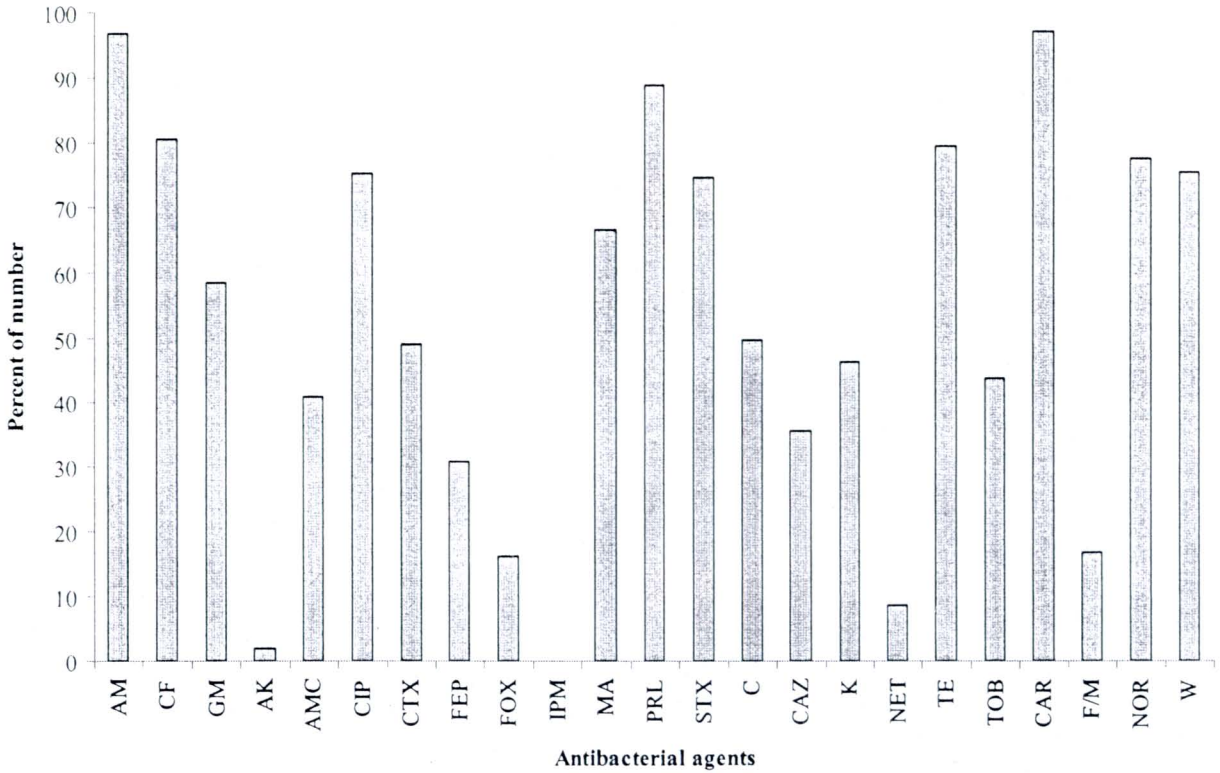


Figure 12 Percentage of antibacterial susceptibility towards the multidrug resistance uropathogenic bacteria; Data were shown as % numbers of resistance isolates (n = 149).

2.2 Identification of the bacterial isolates

The uropathogenic bacteria that resist more than 10 agents were then identified. Examples of biochemical test were demonstrated in Figure 13. All of these belong to gram negative rod. Among 101 isolates, 75 isolates were identified to *E. coli* (74.26%), 21 isolates were identified to *K. pneumoniae* (20.79%) and 5 isolates were identified to *E. cloacae* (4.95%). Interpretations of these experiments were shown in Table 15.

(a) (b) (c)

Figure 13 Four biochemical tests namely, TSI (left slant tube), MIL (stab tube), Urea (middle slant tube), Citrate (right slant tube). The results were identify as (a), *E. coli*; (b), *K. pneumoniae*; (c), *E. cloacae*

2.3 Classification of the uropathogenic ESBL - producing bacteria

The resistance types according to the characteristics of β - lactamase production were determined. After determined the ESBL screening test, 76 of 101 (75.25%) selected isolates were positive. Of these positive isolates, there were 62 (81.58%) isolates demonstrated the activity of ESBL on the confirmation test.

Table 15 Biochemical identifications of 101 bacterial isolates

Isolates	Gram Stain	Biochemical identifications					
		LF	TSI	MIL	Urea	Citrate	Identification
u1/1	GNB	+	A/AG	+/+/+/-	-	-	*E. coli*
u1/2	GNB	+	A/A	+/+/+/-	-	-	*E. coli*
u1/4	GNB	+	A/AG	+/+/+/-	-	-	*E. coli*
u1/7	GNB	+	A/A	+/+/+/-	-	-	*E. coli*
u1/8	GNB	+	A/AG	+/+/+/-	-	-	*E. coli*
u1/9	GNB	+	A/A	+/+/+/-	-	-	*E. coli*
u1/11	GNB	+	A/AG	+/+/+/-	-	-	*E. coli*
u1/12	GNB	+	A/AG	+/+/+/-	-	-	*E. coli*
u1/13	GNB	+	A/A	+/+/+/-	-	-	*E. coli*
u1/16	GNB	+	A/AG	+/+/+/-	-	-	*E. coli*
u1/17	GNB	+	A/A	+/+/+/-	-	-	*E. coli*
u1/19	GNB	+	A/AG	+/+/-/-	-	-	*E. coli*
u1/20	GNB	+	A/AG	+/+/+/-	-	-	*E. coli*
u1/22	GNB	PT	A/AG	-/-/+/-	+	+	*K. pneumoniae*
u1/23	GNB	PT	A/AG	-/-/+/-	+	+	*K. pneumoniae*
u1/24	GNB	PT	A/A	-/-/+/-	+	+	*K. pneumoniae*
u1/25	GNB	PT	A/AG	-/-/+/-	+	+	*K. pneumoniae*
u1/26	GNB	PT	A/A	-/-/+/-	+	+	*K. pneumoniae*
u1/27	GNB	+	A/A	+/-/-/-	-	+	*E. cloacae*
u1/30	GNB	+	A/AG	+/-/-/-	-	+	*E. cloacae*
u2/1	GNB	+	A/AG	+/+/-/-	-	-	*E. coli*
u2/2	GNB	+	A/A	+/+/+/-	-	-	*E. coli*
u2/3	GNB	+	A/AG	+/+/+/-	-	-	*E. coli*
u2/4	GNB	+	A/AG	+/+/+/-	-	-	*E. coli*
u2/6	GNB	+	A/A	+/+/+/-	-	-	*E. coli*
u2/7	GNB	+	A/AG	+/+/+/-	-	-	*E. coli*
u2/8	GNB	+	A/A	+/+/+/-	-	-	*E. coli*
u3/1	GNB	+	A/AG	+/+/+/-	-	-	*E. coli*
u3/3	GNB	+	A/AG	+/+/+/-	-	-	*E. coli*
u3/5	GNB	+	A/AG	+/+/+/-	-	-	*E. coli*
u3/6	GNB	+	A/AG	+/+/+/-	-	-	*E. coli*
u3/8	GNB	+	A/AG	+/+/+/-	-	-	*E. coli*
u3/9	GNB	+	A/AG	+/+/+/-	-	-	*E. coli*
u3/12	GNB	+	A/AG	+/+/+/-	-	-	*E. coli*
u3/13	GNB	PT	A/AG	-/-/+/-	+	+	*K. pneumoniae*
u3/14	GNB	PT	A/AG	-/-/+/-	+	+	*K. pneumoniae*
u3/15	GNB	PT	A/A	-/-/+/-	+	+	*K. pneumoniae*
u4/4	GNB	+	A/AG	+/+/+/-	-	-	*E. coli*
u4/5	GNB	+	A/AG	+/+/+/-	-	-	*E. coli*
u4/6	GNB	+	A/AG	+/+/-/-	-	-	*E. coli*
u4/10	GNB	+	A/A	+/+/+/-	-	-	*E. coli*
u4/11	GNB	+	A/AG	+/+/+/-	-	-	*E. coli*
u4/12	GNB	+	A/A	+/+/+/-	-	-	*E. coli*
u4/13	GNB	+	A/A	+/+/+/-	-	-	*E. coli*

Table 15 (continued)

Isolates	Gram Stain	Biochemical identifications					
		LF	TSI	MIL	Urea	Citrate	Identification
u4/14	GNB	+	A/AG	+/+/+/-	-	-	*E. coli*
u4/17	GNB	PT	A/A	-/-/+/-	+	+	*K. pneumoniae*
u4/18	GNB	+	A/AG	+/-/-/-	-	+	*E. cloacae*
u5/1	GNB	+	A/AG	+/+/+/-	-	-	*E. coli*
u5/3	GNB	PT	A/AG	-/-/+/-	+	+	*K. pneumoniae*
u5/5	GNB	+	A/A	+/+/+/-	-	-	*E. coli*
u5/7	GNB	+	A/AG	+/+/-/-	-	-	*E. coli*
u5/9	GNB	+	A/A	+/+/+/-	-	-	*E. coli*
u5/11	GNB	+	A/AG	+/+/+/-	-	-	*E. coli*
u5/12	GNB	+	A/AG	+/+/+/-	-	-	*E. coli*
u5/16	GNB	+	A/A	+/+/+/-	-	-	*E. coli*
u5/18	GNB	+	A/AG	+/+/+/-	-	-	*E. coli*
u5/20	GNB	+	A/AG	+/+/+/-	-	-	*E. coli*
u5/22	GNB	PT	A/A	-/-/+/-	+	+	*K. pneumoniae*
u5/23	GNB	PT	A/A	-/-/+/-	+	+	*K. pneumoniae*
u6/2	GNB	+	A/AG	+/+/+/-	-	-	*E. coli*
u6/4	GNB	+	A/AG	+/+/+/-	-	-	*E. coli*
u6/6	GNB	+	K/A	+/+/-/-	-	-	*E. coli*
u6/7	GNB	+	A/A	+/+/+/-	-	-	*E. coli*
u6/8	GNB	+	A/A	+/+/+/-	-	-	*E. coli*
u6/9	GNB	+	A/AG	+/+/+/-	-	-	*E. coli*
u6/10	GNB	+	A/AG	+/+/+/-	-	-	*E. coli*
u6/11	GNB	PT	A/A	-/-/+/-	+	+	*K. pneumoniae*
u6/13	GNB	PT	A/AG	-/-/+/-	+	+	*K. pneumoniae*
u7/1	GNB	+	A/AG	+/+/+/-	-	-	*E. coli*
u7/2	GNB	+	A/AG	+/+/+/-	-	-	*E. coli*
u7/3	GNB	+	A/A	+/+/+/-	-	-	*E. coli*
u7/4	GNB	+	A/AG	+/+/+/-	-	-	*E. coli*
u7/5	GNB	+	A/AG	+/+/+/-	-	-	*E. coli*
u7/6	GNB	+	K/A	+/+/+/-	-	-	*E. coli*
u7/7	GNB	+	K/A	+/+/+/-	-	-	*E. coli*
u7/8	GNB	+	A/A	+/+/+/-	-	-	*E. coli*
u7/9	GNB	+	A/A	+/+/+/-	-	-	*E. coli*
u7/10	GNB	+	A/AG	+/+/+/-	-	-	*E. coli*
u7/11	GNB	+	A/AG	+/+/+/-	-	-	*E. coli*
u7/12	GNB	+	A/A	+/+/+/-	-	-	*E. coli*
u7/13	GNB	+	A/AG	+/+/+/-	-	-	*E. coli*
u7/14	GNB	PT	A/A	-/-/+/-	+	+	*K. pneumoniae*
u7/15	GNB	PT	A/AG	-/-/+/-	+	+	*K. pneumoniae*
u7/16	GNB	PT	A/AG	-/-/+/-	+	+	*K. pneumoniae*
u7/17	GNB	PT	A/AG	-/-/+/-	+	+	*K. pneumoniae*
u7/18	GNB	+	A/A	+/-/-/-	-	+	*E. cloacae*
u7/20	GNB	+	A/AG	+/+/+/-	-	-	*E. coli*
u8/3	GNB	+	A/A	+/+/+/-	-	-	*E. coli*
u8/4	GNB	+	A/A	+/+/+/-	-	-	*E. coli*

Table 15 (continued)

Isolates	Gram Stain	Biochemical identifications					
		LF	TSI	MIL	Urea	Citrate	Identification
u8/5	GNB	+	A/A	+/+/+/-	-	-	*E. coli*
u8/6	GNB	+	A/A	+/+/+/-	-	-	*E. coli*
u8/7	GNB	+	A/AG	+/+/+/-	-	-	*E. coli*
u8/10	GNB	+	A/AG	+/+/+/	-	-	*E. coli*
u8/11	GNB	+	A/AG	+/+/+/	-	-	*E. coli*
u8/12	GNB	+	A/AG	+/+/+/	-	-	*E. coli*
u8/13	GNB	+	A/AG	+/+/+/	-	-	*E. coli*
u8/14	GNB	+	A/A	+/+/+/	-	-	*E. coli*
u8/16	GNB	PT	A/A	-/-/+/-	+	+	*K. pneumoniae*
u8/17	GNB	+	A/AG	+/-/-/-	-	+	*E. cloacae*
u8/18	GNB	PT	A/AG	-/-/+/-	+	+	*K. pneumoniae*
u8/19	GNB	PT	A/AG	-/-/+/-	+	+	*K. pneumoniae*

GNB, gram negative bacilli; PT, partial lactose fermenter; +, positive; -, negative; A, acid; G, gas

There was no the AmpC producer because the blunted zone of cefoxitin - cefotaxime antagonist test was not detected. According to the β - lactamase production, 6 isolates were classified as the derepressed AmpC producers, group 1. There was no isolate which classified as the partially derepressed AmpC producers, group 2. There were 20 isolates which belong to the derepressed AmpC with ESBL producers, group 3. Forty one tested bacteria were the inducible AmpC with ESBL producers, group 4. There was no isolate which belonged to the inducible AmpC producers, group 5. However, 34 isolates could not be classified into 5 groups. Results of the tested isolates in the different antibiogram were shown in Table 16 - 19.

Table 16 Interpretation of susceptibility of group 1, the derepressed AmpC producers

Isolates	Interpretation of susceptibility toward					
	AMC	CAZ	CPD	CTX	FEP	FOX
u1/2	I	S	R	R	I	R
u1/20	I	S	R	**R**	I	R
u2/7	R	I	R	R	S	R
u3/1	I	S	R	R	**S**	R
u3/5	I	S	**R**	**R**	**S**	R
u8/7	R	I	R	I	S	R

Bold letter, keyhole phenomenon; Italic letter, the synergist activity; R, resistant; I, intermediate susceptible; S, susceptible.

Table 17 Interpretation of susceptibility of group 3, the derepressed AmpC with ESBL producers

Isolates	Interpretation of susceptibility toward					
	AMC	CAZ	CPD	CTX	FEP	FOX
u1/4	R	R	R	R	**I**	R
u1/7	I	**R**	R	**R**	**R**	R
u1/27	R	R	R	R	R	R
u1/30	S	**R**	R	**R**	**S**	R
u2/3	I	**R**	R	**R**	**R**	R
u2/4	I	R	R	R	I	R
u4/4	R	I	R	R	**S**	R
u4/6	S	**R**	R	**R**	**R**	R
u4/14	I	**R**	R	**R**	R	R
u4/18	R	R	R	R	*I*	R
u5/5	R	R	R	R	**S**	R
u5/7	I	I	R	R	R	R
u5/23	R	R	R	R	R	R
u7/5	S	R	R	**R**	**R**	R
u7/9	R	R	R	R	R	R
u7/17	R	R	R	R	I	R
u7/18	R	R	R	R	R	R
u7/20	R	R	R	R	I	R
u8/11	S	**I**	R	**R**	**I**	R
u8/14	R	**R**	R	R	**R**	R

Bold letter, keyhole phenomenon; Italic letter, the synergist activity; R, resistant; I, intermediate susceptible; S, susceptible.

Table 18 Interpretation of susceptibility of group 4, the inducible AmpC with ESBL producers

Isolates	Interpretation of susceptibility toward					
	AMC	CAZ	CPD	CTX	FEP	FOX
u1/8	I	**I**	**R**	**R**	**I**	S
u1/16	S	**I**	R	R	**R**	S
u1/19	I	**R**	R	**R**	**S**	S
u1/22	I	R	R	**R**	**R**	S
u1/23	I	**R**	R	**R**	**R**	S
u1/24	I	R	R	**R**	R	S
u1/25	I	R	R	**R**	**R**	S
u1/26	I	R	R	**R**	*R*	S
u2/2	I	**R**	**R**	**R**	**R**	S
u2/6	I	**R**	**R**	**R**	**R**	S
u2/8	S	**I**	R	**R**	**I**	S
u3/9	I	**I**	R	**R**	**R**	S
u3/13	R	**R**	R	**R**	**R**	S
u3/14	R	**R**	R	**R**	R	S
u4/10	I	R	R	**R**	**R**	S
u4/17	I	R	R	**R**	R	S
u5/3	R	R	R	**R**	R	S
u5/11	S	**R**	R	**R**	**R**	S
u5/20	I	**R**	R	R	**R**	S
u5/22	I	**R**	R	**R**	**R**	S
u6/4	I	**R**	**R**	**R**	**S**	S
u6/7	I	**R**	**R**	R	**R**	S
u6/8	R	**R**	**R**	**R**	R	S
u6/9	I	**R**	R	**R**	**R**	S
u6/10	S	**R**	R	**R**	**R**	S
u6/13	I	R	R	**R**	**R**	S
u7/1	I	**R**	R	**R**	**R**	S
u7/2	I	**R**	R	**R**	**I**	S
u7/3	S	R	**R**	R	I	S
u7/6	I	**S**	R	**R**	**R**	S
u7/7	R	R	R	**R**	R	S
u7/8	S	**R**	**R**	**R**	**R**	S
u7/10	I	**I**	**R**	**R**	**I**	S
u7/11	I	**R**	R	**R**	**R**	S
u7/12	I	S	R	**R**	**R**	S
u7/13	I	**R**	**R**	**R**	**R**	S
u7/15	S	**R**	**R**	R	*S*	S
u7/16	R	R	R	**R**	**I**	S
u8/4	I	S	**R**	**R**	**R**	S
u8/16	R	R	R	**R**	**R**	S
u8/18	I	R	R	**R**	**R**	S

Bold letter, keyhole phenomenon; Italic letter, the synergist activity; R, resistant; I, intermediate susceptible; S, susceptible.

Table 19 Interpretation of susceptibility of unclassified group

Isolates	Interpretation of susceptibility toward					
	AMC	CAZ	CPD	CTX	FEP	FOX
u1/1	S	S	R	**R**	S	S
u1/9	I	S	R	**I**	**S**	S
u1/11	S	I	S	S	S	S
u1/12	S	S	S	S	I	I
u1/13	I	S	R	R	I	S
u1/17	I	S	R	R	R	S
u2/1	S	S	S	S	S	S
u3/3	S	S	S	S	S	S
u3/6	S	S	S	S	S	S
u3/8	S	**S**	**R**	**I**	**S**	S
u3/12	I	S	S	S	S	S
u3/15	R	S	R	R	R	S
u4/5	S	**S**	R	**R**	**S**	S
u4/11	S	S	S	S	S	S
u4/12	I	S	I	S	S	S
u4/13	I	S	S	S	S	S
u5/1	I	S	R	*I*	S	S
u5/9	S	S	S	S	S	S
u5/12	S	S	S	S	S	S
u5/16	S	S	S	S	S	S
u5/18	I	S	I	S	S	S
u6/2	S	S	I	S	S	S
u6/6	I	I	S	S	S	S
u6/11	I	*S*	R	**I**	**S**	S
u7/4	S	S	**R**	**I**	**I**	S
u7/14	R	R	R	I	S	S
u8/3	I	S	R	R	**I**	S
u8/5	S	S	I	S	S	S
u8/6	I	S	I	I	S	I
u8/10	I	S	R	**R**	**S**	S
u8/12	S	*S*	R	**R**	**S**	S
u8/13	I	S	S	S	S	S
u8/17	S	S	S	S	S	S
u8/19	S	S	S	S	S	S

Bold letter, keyhole phenomenon; Italic letter, the synergist activity; R, resistant; I, intermediate susceptible; S, susceptible.

In the present study, 62 (61.39%) isolates of ESBL - producing bacteria could be detected, including 39 (68.42%), 15 (26.32%) and 3 (5.26%) isolates for *E. coli, K. pneumoniae* and *E. cloacae*, respectively. Sixty one isolates were also partly

(group 3) or fully (group 4) derepressed AmpC with ESBL producer. Fifty two isolates showed the keyhole phenomenon in the double disc synergy test to at least one of the broad - spectrum cephalosporins surrounding the amoxicillin/clavulanic acid disc. All of 61 isolates were resistant to cefotaxime and 41 (67.21%) of 61 isolates were susceptible to cefoxitin. Example of keyhole phenomenon was demonstrated in Figure 14.

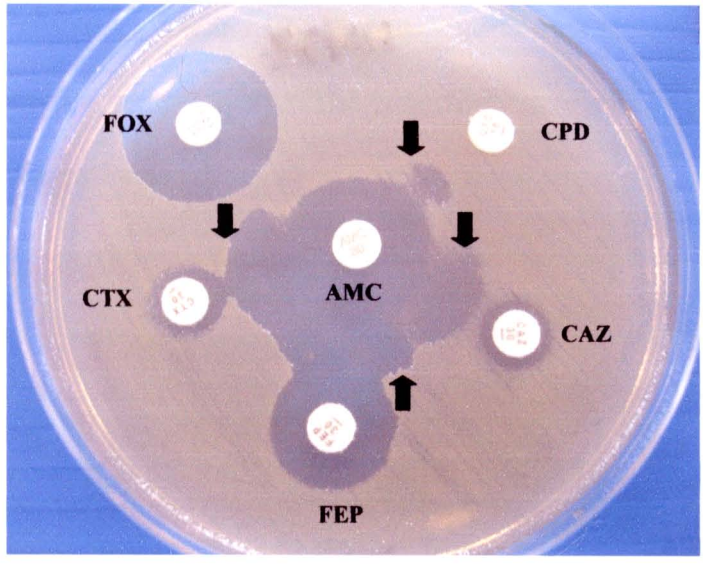


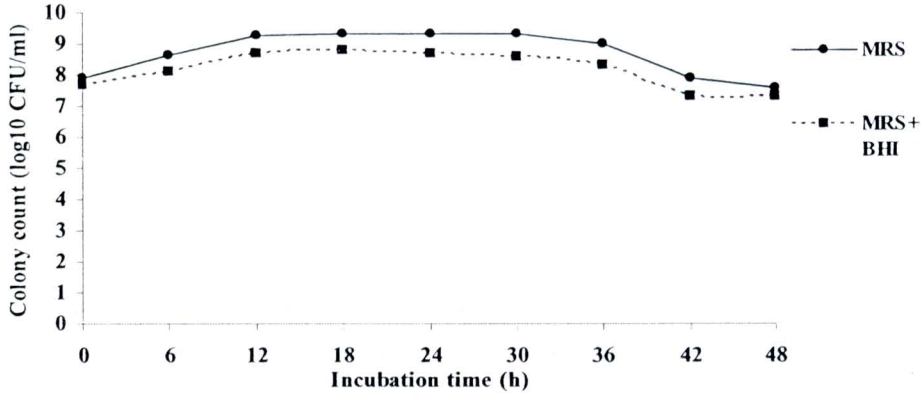
Figure 14 Expanded double disc diffusion synergy test; black arrows indicate keyhole phenomena from the synergist activity of antibiotics (AMC, amoxicillin/ clavulanic acid 20/10 µg/disc; CAZ, ceftazidime 30 µg/disc; CTX, cefotaxime 30 µg/disc; FEP, cefepime 30 µg/disc; CPD, cefpodoxime 10 µg/disc)

Among 4 of 61 isolates, MIC of ceftazidime ≥ 5,000 µg/ml at final concentration, were selected for the further studies. Four isolates, U1/23 and U3/14 strains were identified as *K. pneumoniae* and U6/8 and U2/4 strains were identified as *E. coli*. According to the β - lactamase phenotypes, 3 isolates were classified as group 4 and U2/4 strains was classified as group 3.

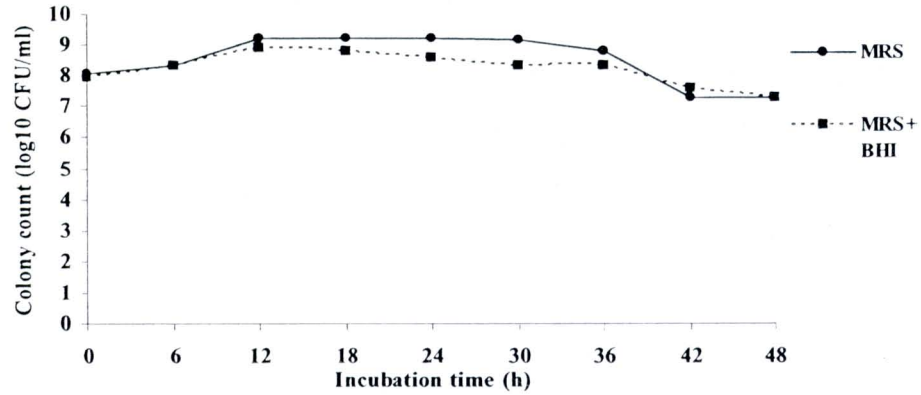
2.4 Characterization of β - lactamase production

2.4.1 Analysis of growth curve and generation time

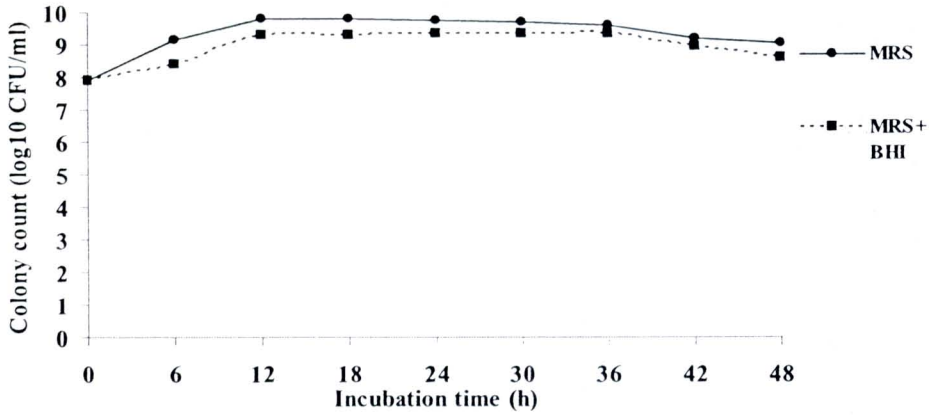
The growth curves of potent antimicrobial producing lactobacilli were plotted from the colony forming unit (CFU/ml) of each collecting time. Lag, log, stationary and declined phases of all isolates were interpreted from their curves. The growth curves of L541, LSS and B120 were shown in Figure 15. The generation times of all isolates were calculated from 6 and 12 hrs of incubation time for early - log phase (B_i) and late log phase (B_f), respectively. The generation time of L541, LSS and B120 isolates were shown in Table 20.



(a)



(b)



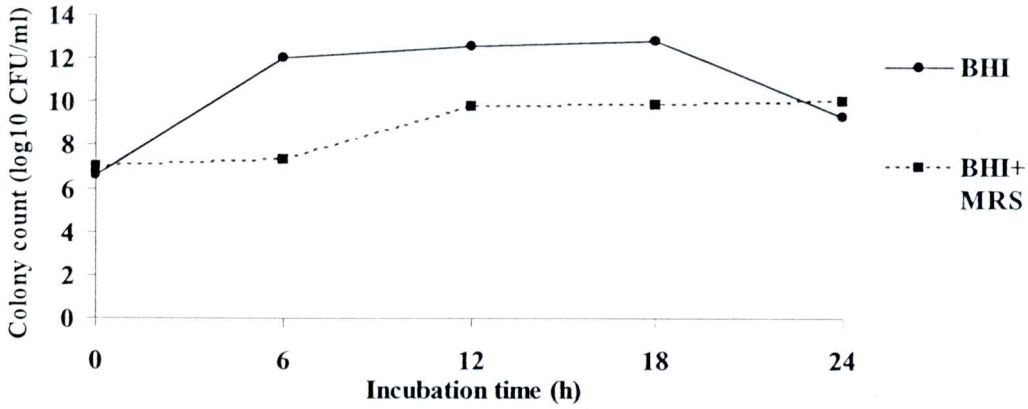
(c)

Figure 15 The growth curves of the potent antimicrobial producing lactobacilli, (a) L541, (b) LSS and (c) B120

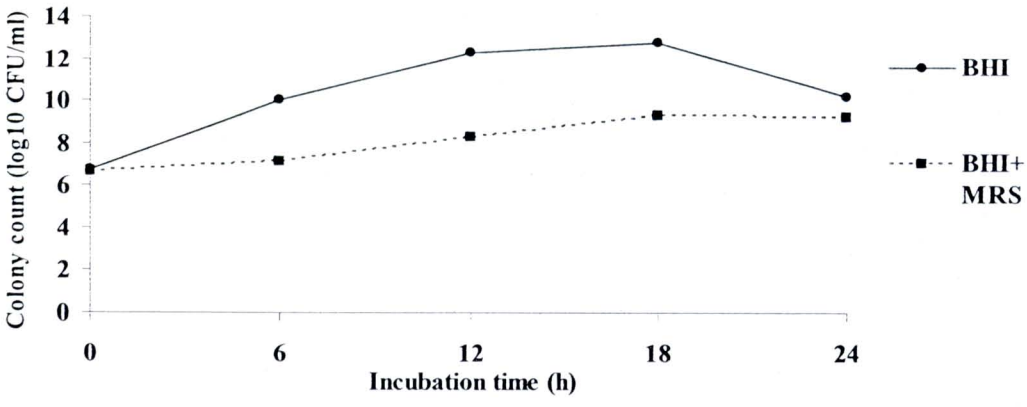
Table 20 The generation time of the potent antimicrobial producing lactobacilli

Isolate	Medium (broth)	Hour of incubation		Time (hr)	Bacterial number (CFU/ml)		Generation time
		B_i	B_f		B_i ($x10^6$)	B_f ($x10^7$)	(hr or min)
L541	MRS	6	12	6	440	186	2.88 or 173
	MRS + BHI	6	12	6	128	49	3.10 or 186
LSS	MRS	6	12	6	200	162	1.98 or 119
	MRS + BHI	6	12	6	220	780	3.27 or 196
B120	MRS	6	12	6	1440	600	2.90 or 174
	MRS + BHI	6	12	6	246	206	1.95 or 117

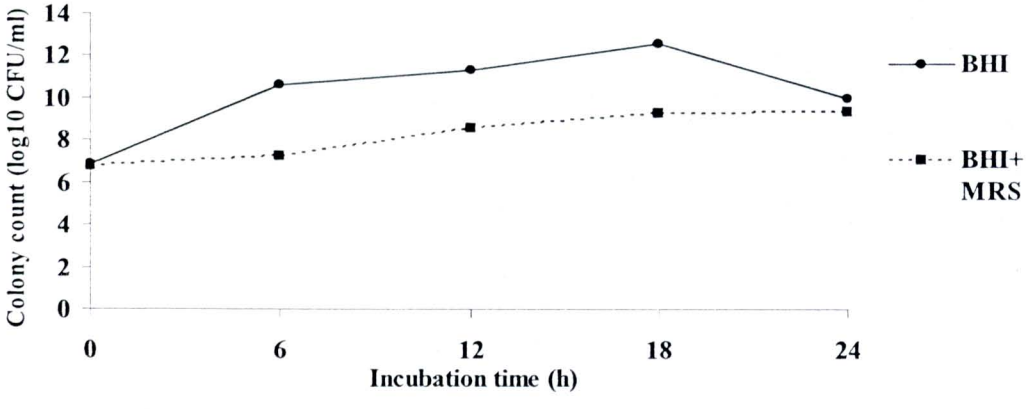
The growth curves of the uropathogenic bacteria were plotted from the colony forming unit (CFU/ml) of each collecting time. Lag, log, stationary and decline phase of all isolates were interpreted from their curves. The growth curves of *E. coli* ATCC 25922, *K. pneumoniae* strain u1/23, *E. coli* strain u2/4, *K. pneumoniae* strain u3/14 and *E. coli* strain u6/8 were shown in Figure 16 and 17. The generation time of the cultured isolates in BHI broth were calculated from 0 and 6 hrs of incubation time. Their generation time of the cultured isolates in BHI supplemented with 50% MRS broth were calculated from 6 and 12 hrs of incubation time for early - log phase (B_i) and late log phase (B_f), respectively. All of these isolates were shown in Table 21.



(a)

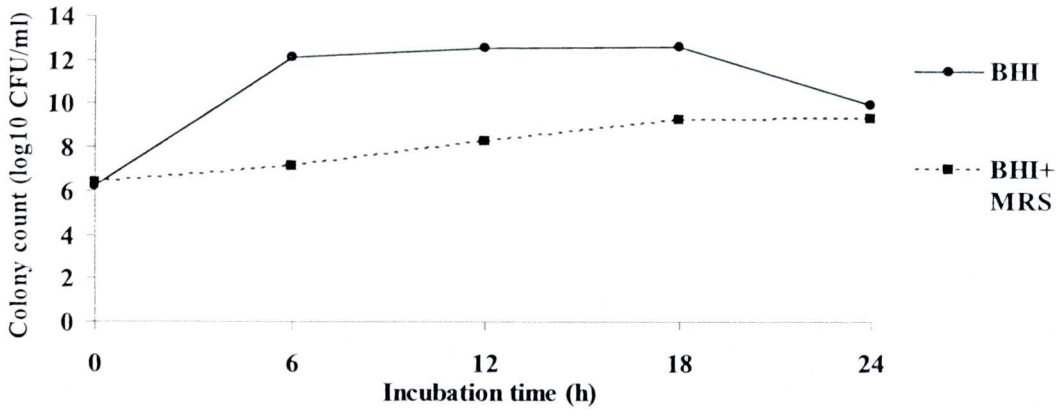


(b)

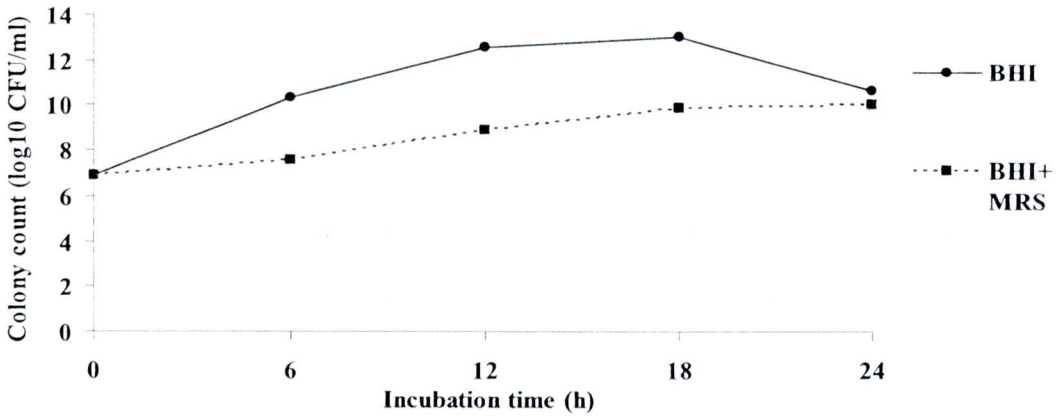


(c)

Figure 16 The growth curves of *E. coli*, (a) *E. coli* ATCC 25922, (b) u2/4, (c) u6/8



(a)



(b)

Figure 17 The growth curves of *K. pneumoniae*, (a) u1/23 and (b) u3/14

Table 21 The generation time of the uropathogens and standard strain

Isolate	Medium (broth)	Hour of incubation		Time (hr)	Bacterial number (CFU/ml)		Generation time
		B_i	B_f		B_i ($x10^6$)	B_f ($x10^8$)	(hr or min)
E. coli							
ATCC 25922	BHI	0	6	6	4.2	10000	0.33 or 20
	BHI + MRS	6	12	6	20	60	0.73 or 44
u2/4	BHI	0	6	6	5.0	100	0.55 or 33
	BHI + MRS	6	12	6	12	2	1.47 or 88
u6/8	BHI	0	6	6	7.6	400	0.48 or 29
	BHI + MRS	6	12	6	18	4	1.33 or 80
K. pneumoniae							
u1/23	BHI	0	6	6	1.6	14000	0.30 or 18
	BHI + MRS	6	12	6	15	2	1.60 or 96
u3/14	BHI	0	6	6	7.4	200	0.51 or 31
	BHI + MRS	6	12	6	34	8	1.32 or 79

2.4.2 Extraction of periplasmic proteins and determination of the protein concentration

The total protein concentrations of periplasmic proteins extracted from five bacterial cultures were determined by Qubit® Fluorometer. Total protein concentrations of each isolate were not significant different in each induced condition. Except, the total protein concentrations of *E. coli* strain u2/4 which induced by sub - MIC concentration of CAZ were 1.5 folds higher than its MIC condition. On the other hand, the total protein concentrations of *K. pneumoniae* strain u3/14 which induced by sub - MIC concentration of AM were 0.66 folds lower than its MIC condition. These results were shown in Table 22.

Table 22 Protein concentration of crude β - lactamase extracted from periplasm

Isolates	Protein concentrations (mg/ml)				
	Non induced	AM induced at		CAZ induced at	
		Sub MIC	MIC	Sub MIC	MIC
E. coli					
ATCC 25922	1.57	1.47	1.57	1.38	1.33
u2/4	1.59	1.19	1.57	2.00	1.37
u6/8	1.90	1.99	1.63	2.00	1.88
K. pneumoniae					
u1/23	0.60	0.53	0.79	0.97	0.91
u3/14	0.92	0.67	0.96	1.06	1.15

AM, Ampicillin; CAZ, Ceftazidime

2.4.3 One - dimensional polyacrylamide gel electrophoresis

Between the non - induced and AM induced conditions at MIC of these isolates, the protein patterns in SDS - PAGE of the extracted periplasmic proteins could be detected by the silver stain and were shown in Figure 18, 20 and 22. Detected bands were analyzed by the program of OriginPro version 7.5 (OriginLab®) and were shown in Figure 19, 21 and 22. For *E. coli* ATCC 25922, the peak comparison of the periplasmic proteins between the non - induced and AM induced conditions at MIC demonstrated that there were 5 different intensities of the peaks (peak 1 - 5; 72.0, 53.0, 47.5, 30.0 and 26 kDa) which significantly increased in the AM induced conditions. However, there was one of different intensity of the peak (peak 6; 16.3 kDa) which significantly increased in non - induced conditions. They were shown in Figure 19. For *E. coli* strain u2/4, the peak comparison of the periplasmic proteins between the non - induced and AM induced conditions at MIC demonstrated that there were 4 different intensities of the peaks (peak 1 - 4; 63.5,

49.0, 28.0 and 24.5 kDa) which significantly increased in the AM induced conditions. However, there were 2 different intensities of the peak (peak 5 - 6; 43.0 and 19.3 kDa) which significantly increased in non - induced condition. They were shown in Figure 21. For *K. pneumoniae* strain u3/14, the peak comparison of the periplasmic proteins between the non - induced and AM induced conditions at MIC demonstrated that there was one of different intensity of the peaks (peak 1; 40.8 kDa) which significantly increased in the AM induced conditions. However, there was one of different intensity of the peak (peak 2; 21.9 kDa) which significantly increased in non - induced conditions. They were shown in Figure 21.

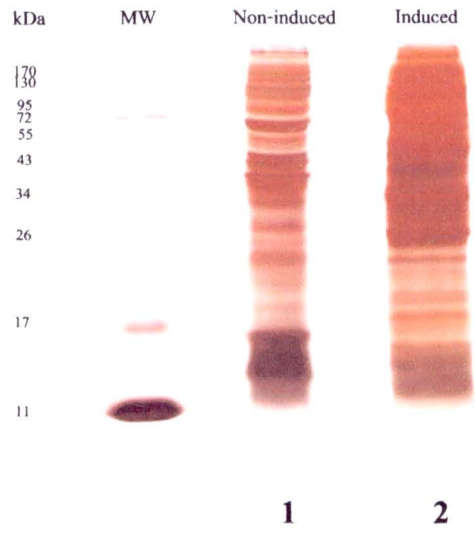


Figure 18 Periplasmic protein of *E. coli* ATCC 25922 extracted using PeriPreps™ Periplasting kit; lane MW, molecular weight marker (Page Ruler™; Fermentus INC); lane 1, non induced; 2, AM induced at MIC.

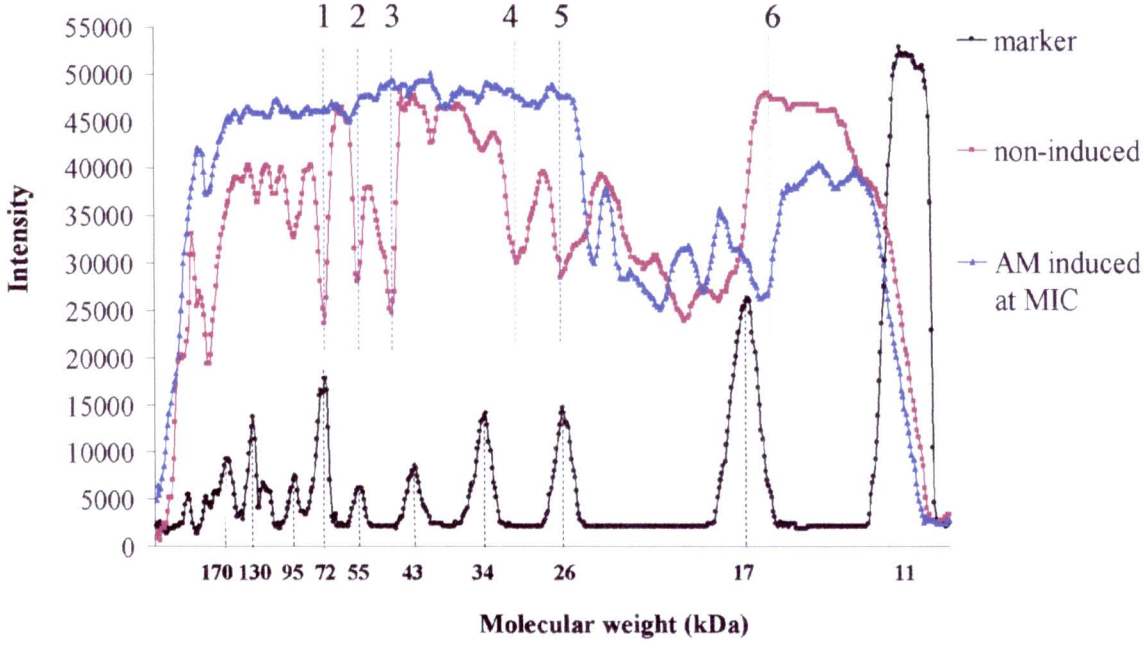


Figure 19 Data analysis of protein bands of *E. coli* ATCC 25922 created using OriginPro 7.5 (OriginLab®)

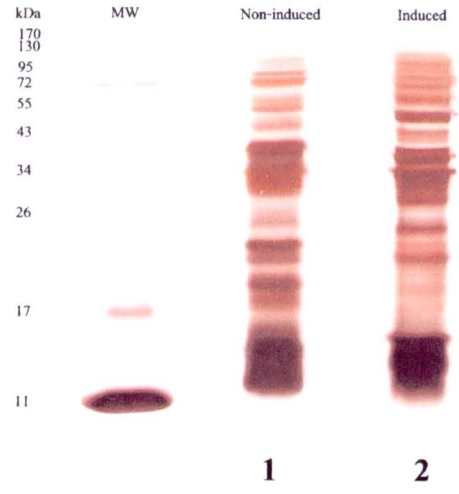


Figure 20 Periplasmic protein of *E. coli* strain u2/4 extracted using PeriPreps™ Periplasting kit; lane MW, molecular weight marker (Page Ruler™; Fermentus INC); lane 1, non induced; lane 2, AM induced at MIC.

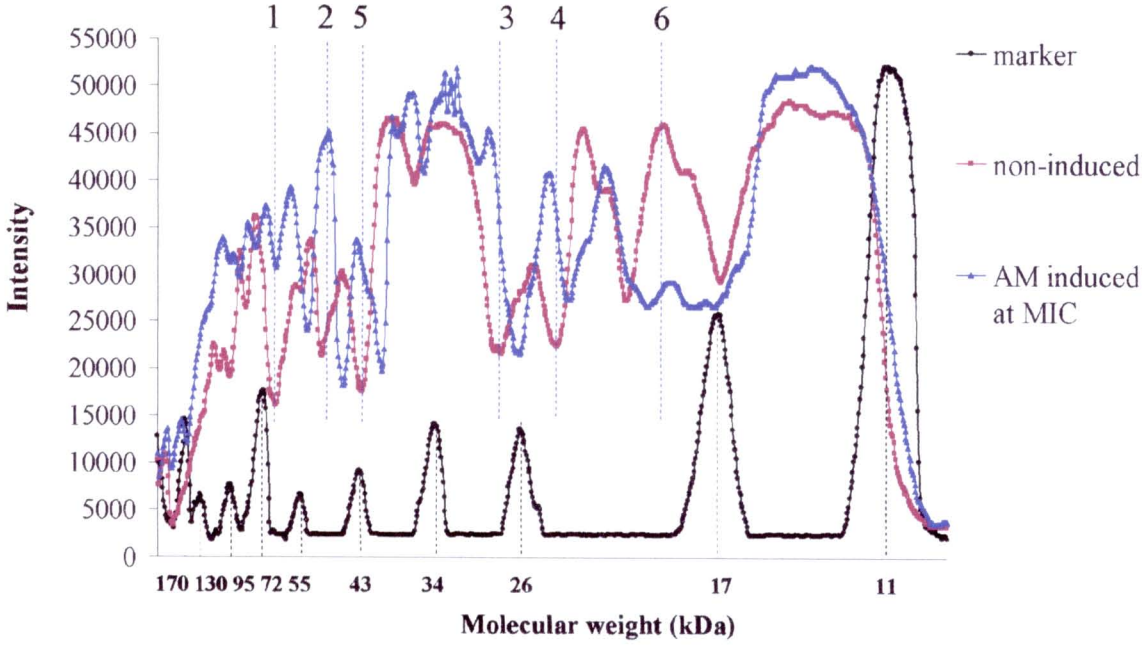


Figure 21 Data analysis of protein bands of *E. coli* strain u2/4 created using OriginPro 7.5 (OriginLab®)

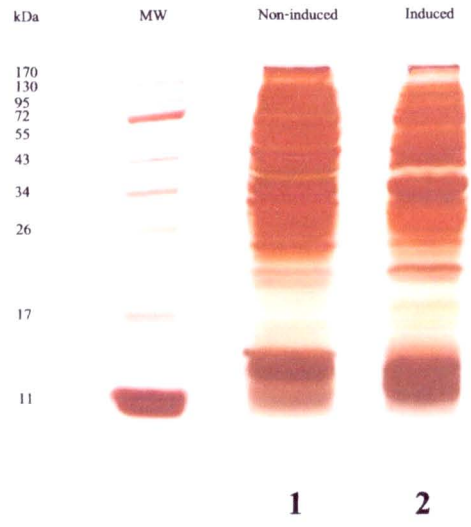


Figure 22 Periplasmic protein of *K. pneumoniae* strain u3/14 extracted using PeriPreps™ Periplasting kit; Lane MW, molecular weight marker (Page Ruler™; Fermentus INC); lane 1, non induction with AM; lane 2, induced with MIC of AM.

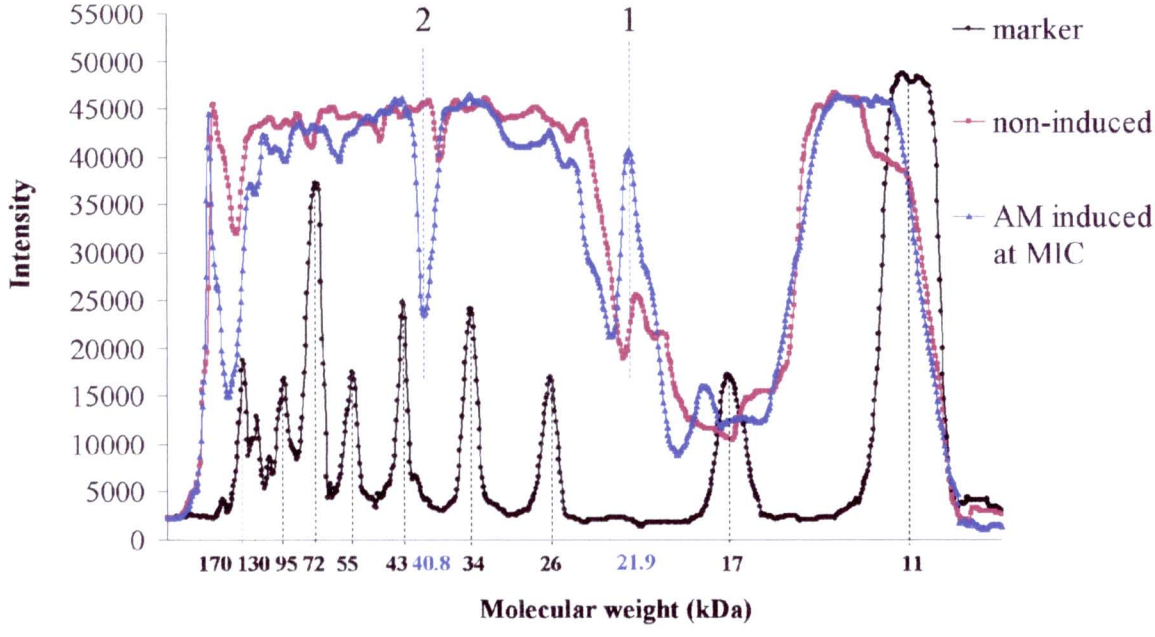


Figure 23 Data analysis of protein bands of *K. pneumoniae* strain u3/14 created using OriginPro 7.5 (OriginLab®)

2.4.4 Determination of β - lactamase activity by spectrophotometric nitrocefin assay

β - lactamase activity (International Unit, IU) of each sample was calculated from its optical density after analyzed with the β - lactamase standard curve. β - lactamase activities were compared between the non - induced and induced conditions with drug. The results of these activities were exhibited in Table 23. Other details were shown in Appendix C. For all isolates, the activities of the induced conditions were higher than non - induced condition. The activities of AM and CAZ induced conditions were not significant different, except for *E. coli* strain u2/4 and *K. pneumoniae* strain u3/14. β - lactamase activities of CAZ - induced condition of 2 isolates were higher by 2.36 and 3.55 times than AM - induced condition.

2.4.5 The minimal concentration of β - lactamases that inhibited antimicrobial activity

The significant differences of the average zones were used to evaluate the antimicrobial activities of each sample. The MICs of their β - lactamases that indicated the 50 % inhibition toward the antibiotic activity of CAZ were shown in Figure 24 - 26. β - lactamase activities of periplasmic protein which extracted from *E. coli* ATCC 25922 in all culture conditions could not be observed as same as PBS control. For non - induced conditions (Figure 24), MIC of all extracted proteins were at 1:8 dilution of the total protein. Except the MIC of the extracted *K. pneumoniae* strain u1/23, it was at undiluted protein. For CAZ induced conditions (Figure 25), MIC of *K. pneumoniae* strain u3/14 and u1/23 were at the non - dilution and 1:2 dilution, respectively. MIC of *E. coli* strain u2/4 and u6/8 were at the 1:4 dilution. For AM induced conditions (Figure 26), MIC of u1/23 and u6/8 were at the non -

dilution. MIC of u3/14 and u2/4 were at the 1:2 and 1:8 dilutions of the total protein concentrations, respectively.

Table 23 Comparison of β - lactamase activities between non - induced, CAZ - induced and AM - induced conditions of the selected isolates

Isolates	**Conditions**	**β - lactamase activity (mIU/mg protein)**	**Ratio**
E. coli	Non - induced	0.07	1.00
ATCC 25922	CAZ - induced	0.12	1.76
	AM - induced	0.12	1.79
E. coli	Non - induced	0.12	1.00
strain u2/4	CAZ - induced	1.13	9.17
	AM - induced	0.48	3.88
E. coli	Non - induced	0.09	1.00
strain u6/8	CAZ - induced	0.19	2.03
	AM - induced	0.09	0.95
K. pneumoniae	Non - induced	0.29	1.00
strain u1/23	CAZ - induced	1.47	4.98
	AM - induced	1.38	4.68
K. pneumoniae	Non - induced	0.23	1.00
strain u3/14	CAZ - induced	2.07	8.96
	AM - induced	0.58	2.52

Ratio; the ratio of β - lactamase activity between each induced condition compared with non - induced condition of each tested isolate.

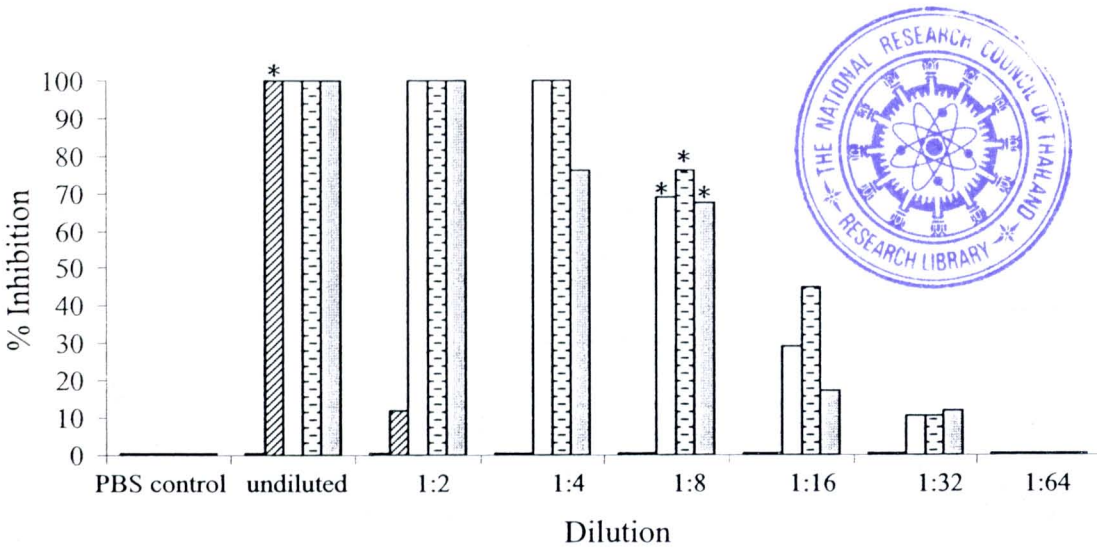


Figure 24 The percentages of inhibition of β - lactamase activity using non - induced condition, toward the activity of CAZ. The data was shown as % inhibition of β - lactamase activity of *E. coli* ATCC 25922 ( ), u1/23 ( ), u2/4 ( ), u3/14 ( ) and u6/8 ( ). *, ≥ 50% inhibition.

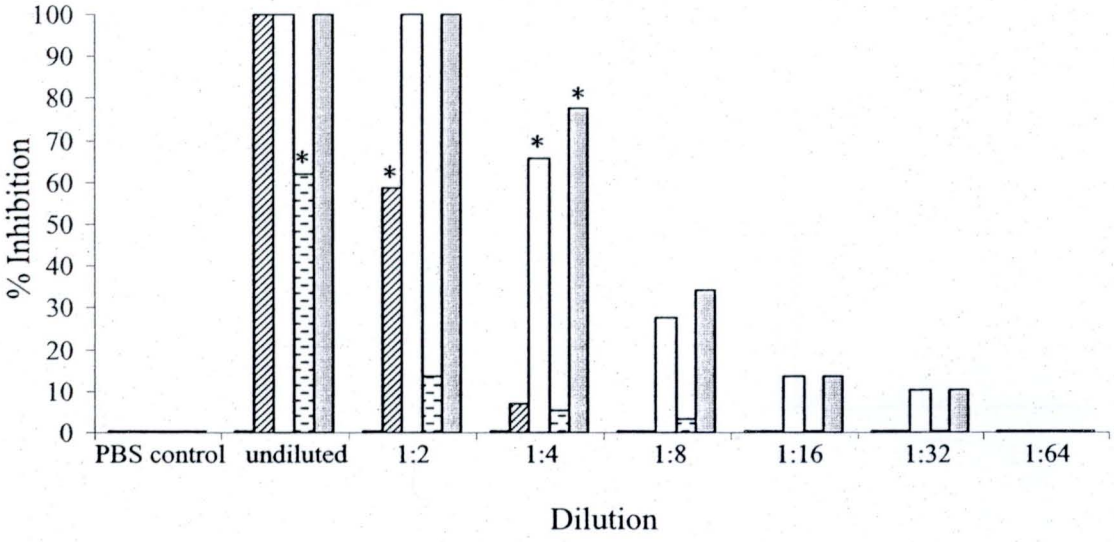


Figure 25 The percentage of inhibition of β - lactamase activity using CAZ induced condition, toward the activity of CAZ. The data was shown as % inhibition of β - lactamase activity of *E. coli* ATCC 25922 (⊡), u1/23 (▨), u2/4 (□), u3/14 (⊟) and u6/8 (▣). *, ≥ 50% of inhibition.

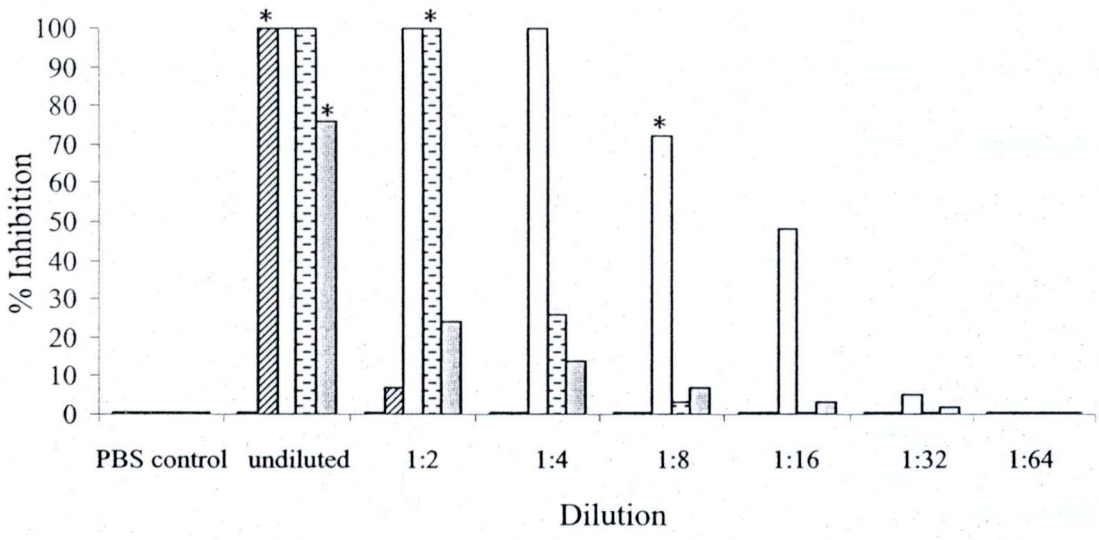


Figure 26 The percentage of inhibition of β - lactamase activity using AM induced condition, toward the activity of CAZ. The data was shown as % inhibition of β - lactamase activity of *E. coli* ATCC 25922 (⊡), u1/23 (▨), u2/4 (□), u3/14 (⊟) and u6/8 (▣). *, ≥ 50% of inhibition.

3. The inhibitory effects of Lactobacillus culture and its agents on β - lactamase activity and growth of the uropathogenic bacteria

3.1 The inhibitory effects of lactobacillus culture on the growth of the uropathogenic bacteria

The colony forming units (CFU/ml) of the uropathogenic bacteria were count using spread plate method. The results were demonstrated in Figure 27 - 31. Four uropathogenic bacteria and *E. coli* ATCC 25922 could grow in BHI supplemented with 50% MRS broth and the number of colony increased from 0, 6, 12 hrs of the cultured time. The potent lactobacillus culture, L541 and LSS, could totally inhibit

the growth of the uropathogenic bacteria at 6 and 12 hrs of cultured time. However, the uropathogenic bacteria could weakly grow in the B120 culture. The results indicated that these lactobacillus cultures could inhibit the growth of uropathogenic bacteria.

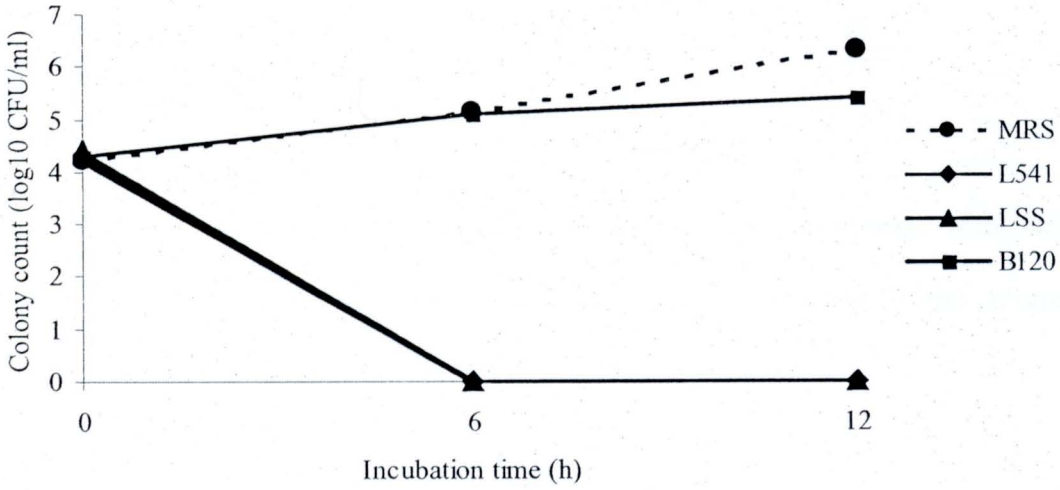


Figure 27 Colony forming units of *E. coli* ATCC 25922 co - cultured with each lactobacillus. The data was shown as colony count at 0, 6 and 12 hrs of the cultured time. MRS broth was used as the control experiment.

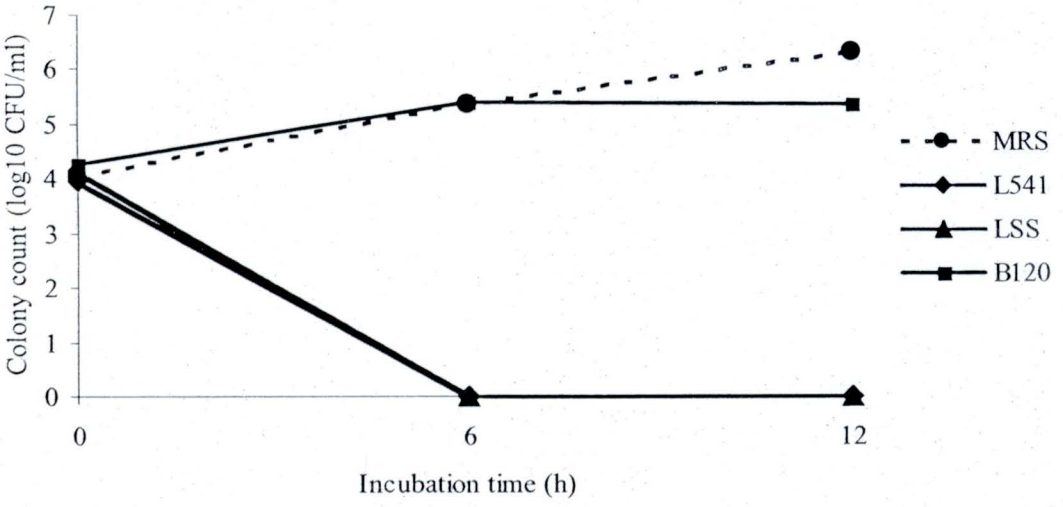


Figure 28 Colony forming units of *E. coli*, u2/4, co - cultured with each lactobacillus. The data was shown as colony count at 0, 6 and 12 hrs of the cultured time. MRS broth was used as the control experiment.

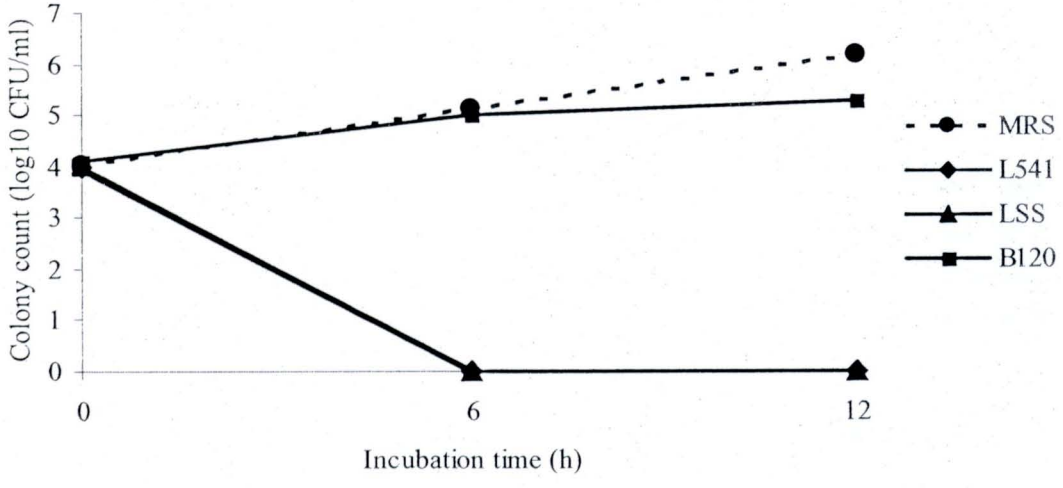


Figure 29 Colony forming units of *E. coli*, u6/8, co - cultured with each lactobacillus. The data was shown as colony count at 0, 6 and 12 hrs of the cultured time. MRS broth was used as the control experiment.

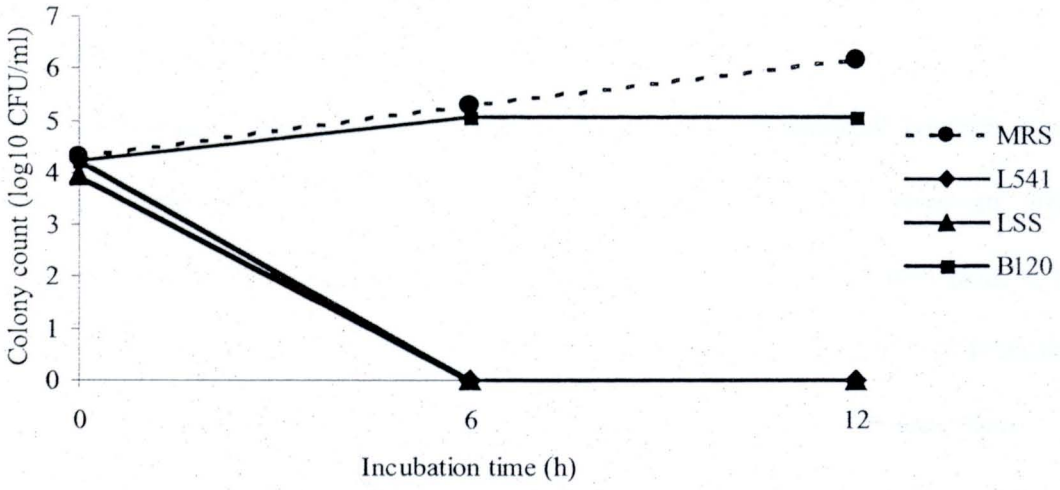


Figure 30 Colony forming units of *K. pneumoniae*, u1/23, co - cultured with each lactobacillus. The data was shown as colony count at 0, 6 and 12 hrs of the cultured time. MRS broth was used as the control experiment.

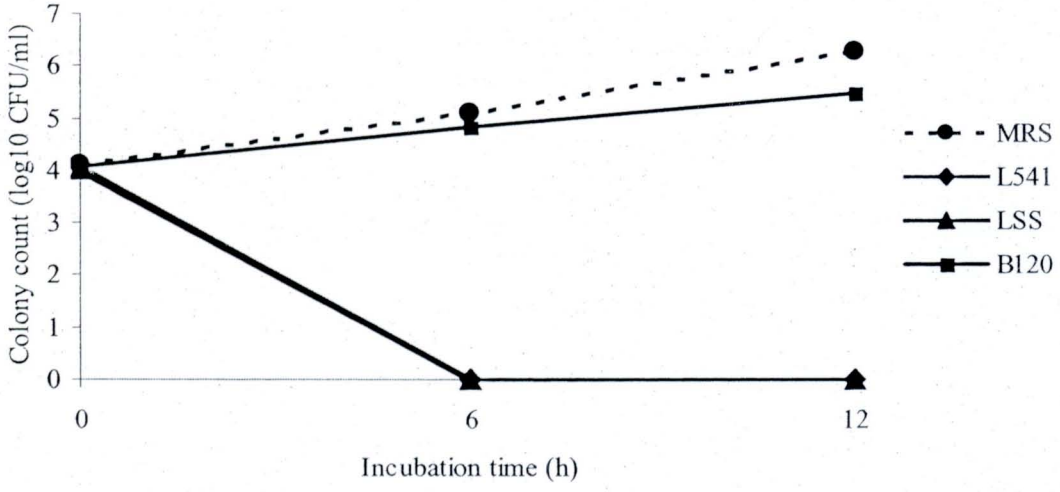


Figure 31 Colony forming units of *K. pneumoniae*, u3/14, co - cultured with each lactobacillus. The data was shown as colony count at 0, 6 and 12 hrs of the cultured time. MRS broth was used as the control experiment.

3.2 The inhibitory effects of the bacteriocins on the growth of uropathogenic bacteria

The results of bacteriocins toward the growth of uropathogenic bacteria were shown in Figure 32 - 36. The uropathogenic bacteria could grow in BHI supplemented with 50% MRS broth and the numbers of colony increased from 0, 6, 12 hrs of the cultured time. At 6 and 12 hrs, the crude bacteriocin extracted from the potent lactobacillus, L541 and LSS, could inhibit the growth of the uropathogenic bacteria as well as plantaricin and imipenem, the control agents. However all uropathogenic bacteria could weakly inhibit in the experiment of the crude extract of *L. crispatus* strain B120. The results indicated that crude bacteriocins extracted from all lactobacilli had the inhibition activity toward the growth of uropathogenic bacteria.

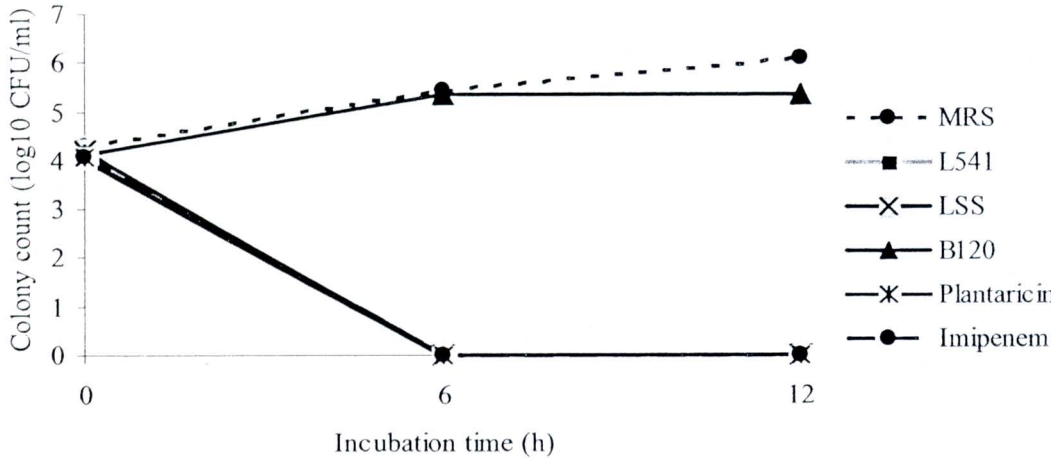


Figure 32 Colony forming units of *E. coli* ATCC 25922 co - cultured with each lactobacillus. The data was shown as colony count at 0, 6 and 12 hrs of the cultured time. MRS broth was used as the control experiment.

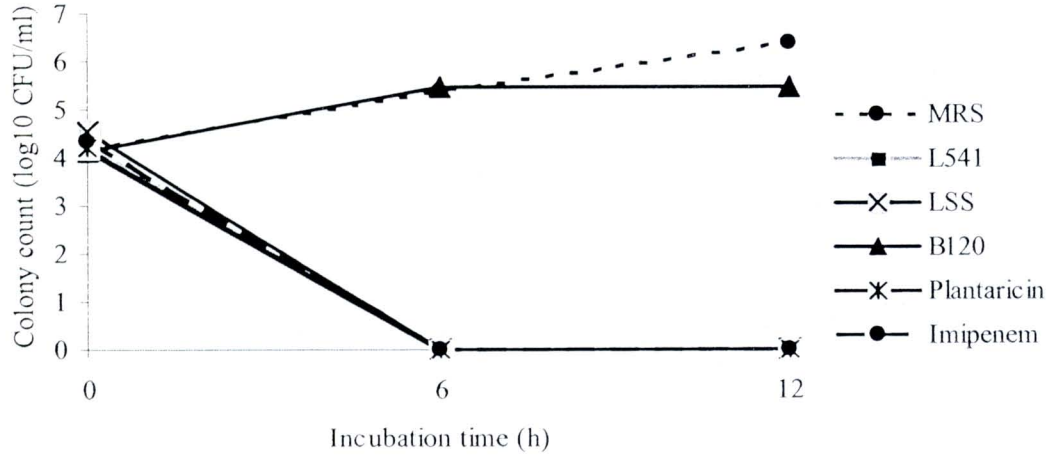


Figure 33 Colony forming units of *E. coli*, u2/4, co - cultured with each lactobacillus. The data was shown as colony count at 0, 6 and 12 hrs of the cultured time. MRS broth was used as the control experiment.

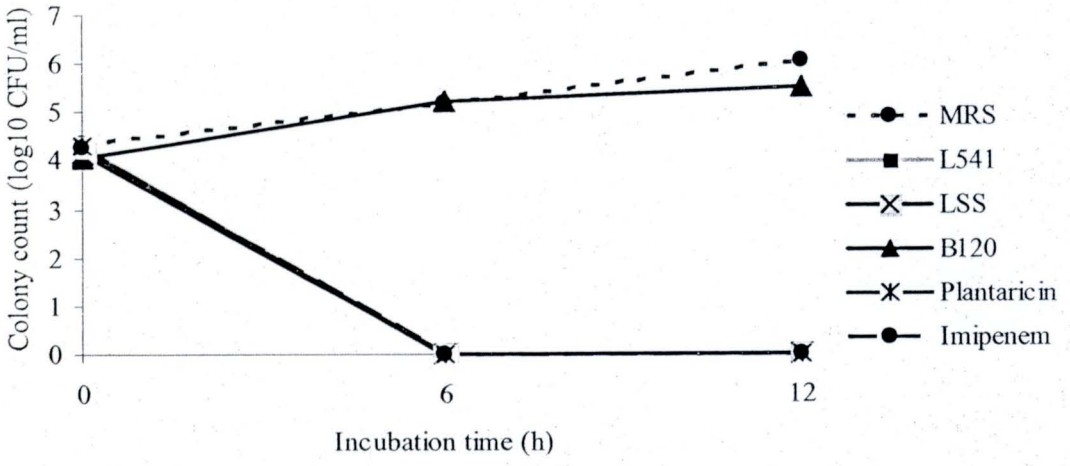


Figure 34 Colony forming units of *E. coli*, u6/8, co - cultured with each lactobacillus. The data was shown as colony count at 0, 6 and 12 hrs of the cultured time. MRS broth was used as the control experiment.

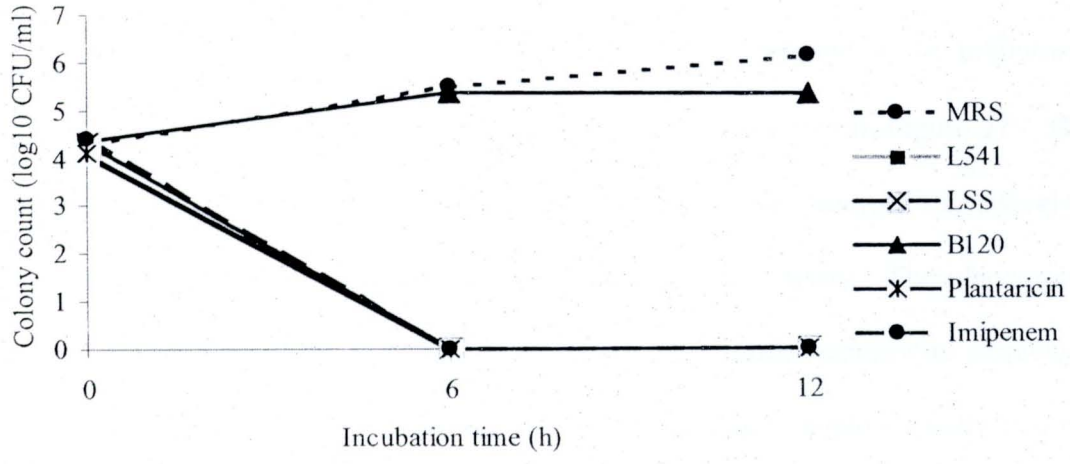


Figure 35 Colony forming units of *K. pneumoniae*, u1/23, co - cultured with each lactobacillus. The data was shown as colony count at 0, 6 and 12 hrs of the cultured time. MRS broth was used as the control experiment.

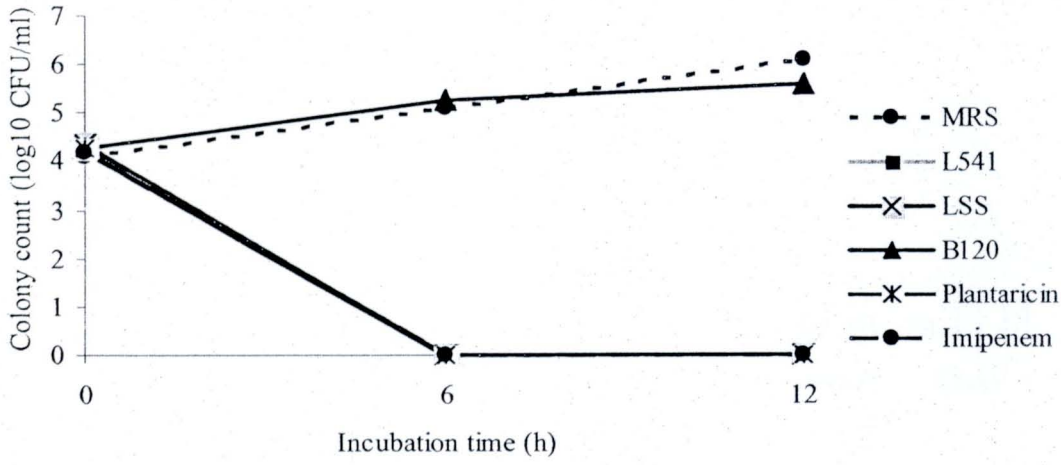


Figure 36 Colony forming units of *K. pneumoniae*, u3/14, co - cultured with each lactobacillus. The data was shown as colony count at 0, 6 and 12 hrs of the cultured time. MRS broth was used as the control experiment.

3.3 The inhibitory effects of the bacteriocins on β - lactamase activity

The reduction of optical density at 486 nm which referred to the inhibitory effects of bacteriocins toward β - lactamase activities were shown in Figure 37 - 39. PBS and clavulanate were used as negative and positive control, respectively. Clavulanate showed 100% inhibition of the β - lactamase activity. The plantaricin was carried out to use as the compared bacteriocins. The crude bacteriocins extracted from the potent *Lactobacillus*, L541 and LSS, and plantaricin could partially inhibit the β - lactamase activity. Their inhibitions were less than 50% inhibition at all conditions. While, the crude bacteriocins extracted from the poor antimicrobial activity strain, B120, showed the low % inhibition in all conditions.

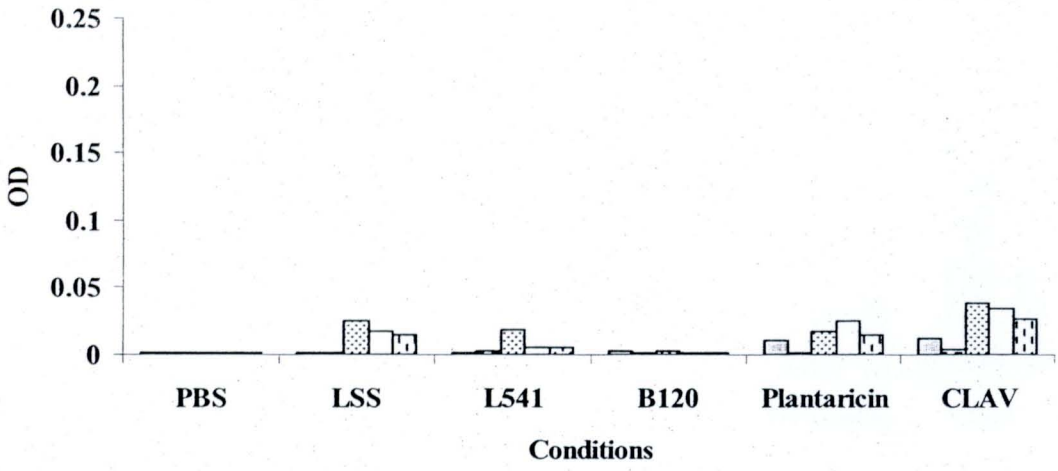


Figure 37 The optical density at 486 nm of bacteriocins toward the β - lactamase activity in the absence of induction. The data was shown as the reduction of OD at 486 nm of the β - lactamase activity of *E. coli* ATCC 25922 ( ), u1/23 ( ), u2/4 ( ), u3/14 ( ) and u6/8 ( ).

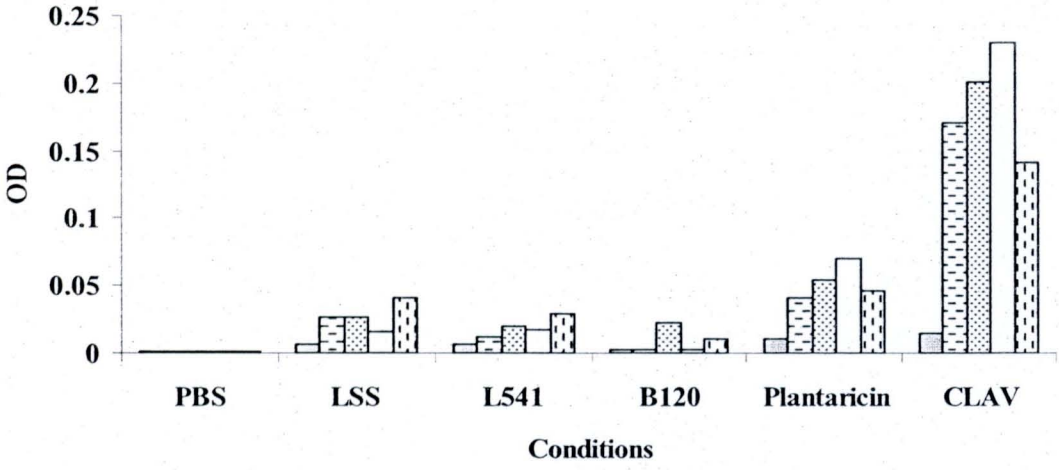


Figure 38 The optical density at 486 nm of bacteriocins toward the β - lactamase activity in the presence of CAZ induction. The data was shown as the reduction of OD at 486 nm of the β - lactamase of *E. coli* ATCC 25922 ( ), u1/23 ( ), u2/4 ( ), u3/14 ( ) and u6/8 ( ).

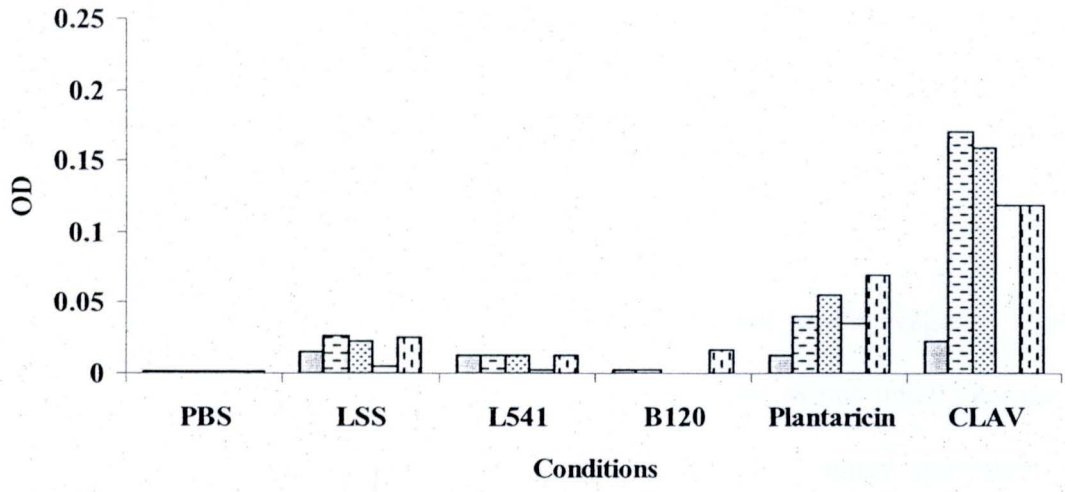


Figure 39 The optical density at 486 nm of bacteriocins toward the β - lactamase activity in the presence of AM induction. The data was shown as the reduction of OD at 486 nm of the β - lactamase of *E. coli* ATCC 25922 (▣), u1/23 (▤), u2/4 (▦), u3/14 (□) and u6/8 (▩).