Clonal Spread of Carbapenem Resistant *Acinetobacter baumannii* in the Patients and their Environment at BMA Medical College and Vajira Hospital

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Objective: *To determine the clonal spread of carbapenem-resistant Acinetobacter baumannii (CRAB) in the patients and their environment at BMA Medical College and Vajira Hospital.*
Material and Method: *A prospective study on CRAB isolated from the clinical specimens of 30 patients and 300 from their environmental samples were carried out from September 1-15, 2008. The CRAB isolates were genotyped using PCR-based typing method.*
Results: *Twenty-six (86.7%) and 20 (66.7%) cases of 30 patients had their environment contaminated with A. baumannii and CRAB, respectively Environmental contamination rates of A. baumannii and CRAB were 18.0%(54/300) and 13.0%(39/300), respectively. The most contaminated sites with CRAB were bedside cupboards (26.7%), followed by bedrails and bed sheets (20%), BP cuffs (16.7%), over bed tables and nurse station counters (13.3% each) and push carts (10%). Four molecular types were classified among 65 CRAB isolates. Molecular type 1 was the most prevalent (90.7%) and found in all kinds of environmental samples except patient record folder and computer keyboard/mouse. About 37% of the patients had at least one of their environmental samples contaminated with CRAB clonally related with their own types.*
Conclusion: *Clonal spread of CRAB was demonstrated to emphasize the important of hand hygiene, contact precaution and patient's environmental decontamination in controlling the spread of CRAB in the hospital.*

Keywords: *Carbapenem resistant A. baumannii, Clonal spread, PCR-based typing method, REP, M13*

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Outbreaks of carbapenem resistance *A. baumannii* (CRAB) have been reported, and becoming a threat in critical care settings[1,2]. At Bangkok Metropolitan Administration Medical College and Vajira Hospital (BMA-MCVH), *A. baumannii* was the most common nosocomial pathogen with increasing isolation rates of CRAB[3]. Survival of these bacteria and persistence in the hospital environment are believed to be important factors in the development and continuation of the outbreaks[4-6]. A significant correlation between *A. baumannii* infection rates and their environmental contamination suggested that the contamination is the cause, rather than the effect, of the acquisition of *A. baumannii* infection or colonization[4,6,7]. Therefore, the environmental surrounding patients and common areas in the wards of patients infected or colonized with CRAB may act as a reservoir for this microorganism and transfer to other patients. The aim of this study was to determine clonal spread of CRAB in the patients and their environment at BMA-MCVH by using PCR based typing method.

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Material and Method

A prospective study on CRAB isolated from the patients and their environment were carried out during 1-15 September, 2008 at BMA MCVH. Upon identification of CRAB from the clinical specimens,

the patients were located and 10 environmental samples were collected from each case. A total of 300 environmental samples were collected from patient's environment including bed rail, bed sheet, over bed table, bedside cupboard, bed curtain, blood pressure cuff; and from non patient's environment *i.e.* push cart, patient's record folder, computer keyboard/mouse and nurse station counter. The patient's information was obtained from the infection control surveillance record.

A. baumannii was identified using Gram staining and biochemical tests, *i.e.* Gram negative, oxidase negative, non-motile, citrate positive, grow at 44°C and utilize glucose by oxidation but not by fermentation[8] . Antimicrobial susceptibility testing was determined by the disk diffusion method according to the Clinical and Laboratory Standards Institute guidelines[9]. The following antimicrobial discs were included; amikacin, ampicillin/sulbactam, cefoperazone/sulbactam, cefepime, cefpirome, ceftazidime, ciprofloxacin, colistin, imipenem, meropenem, piperacillin/tazobactam and tigecycline. CRAB was defined as an isolate that was resistant to imipenem and meropenem.

The genomic DNAs were extracted by using modified spin filter-silica based method (NucleoSpin® Tissue kit). Molecular typing of *A. baumannii* was done by using PCR-based typing method with REP-1, REP-2[10], and M-13 primers[11]. Amplification reactions were carried out as previously described[12] in a thermal cycler (Thermo Hybaid Scientific, USA), with an initial denaturation at 94°C, 10 min followed by 30 cycles of denaturation (1 min at 94°C), annealing (1 min at 45°C) and extension (2 min at 72°C), with a single final extension of 16 min at 72°C. Amplified products were analyzed by electrophoresis in 1.2% agarose gel containing ethidium bromide (0.2 μg/ml) and detected by an UV transilluminator (BIS 303 PC, Jerusalem, Israel). The PCR fingerprint patterns visualized on gel were saved and analyzed on the basis of similarity in numbers and matching positions of all major bands. Similarity analysis of the data was carried out by the UPGMA clustering method (Geneious 2.5.2; Biomatters, New Zealand). The study has been reviewed and approved by Ethical Committee of Bangkok Metropolitan Administration (No.96.51).

Results

The patients' characteristics were shown in Table 1. They were admitted in 13 wards during the 2 weeks of sample collection. 40% were admitted in the ICUs. The most common specimen, from which CRAB were isolated, was sputum/tracheal secretion (60%). Fifty per cent of CRAB isolates were present in mixed cultures with other nosocomial microorganisms such as MDR *Pseudomonas aeruginosa* (resistant to beta-lactams, fluoroquinolones and aminoglycosides)[13], methicillin-resistant *Staphylococcus aureus* (MRSA), *Escherichia coli* (ESBL)[9] and *Candida albicans*. The CRAB nosocomial infections and colonization were 66.7% (20/30) and 33.3% (10/30), respectively.

A. baumannii and CRAB in the environmental samples

Fifty-four *A. baumannii* (18%) and 39 CRAB (13.0%) isolates were identified among 300 of the environmental samples (Fig. 1). *A. baumannii* was isolated from all types of environmental samples investigated. The highest rate of *A. baumannii* contamination was found on bedside cupboards (30.0%), followed by bedrails, push carts and blood pressure cuffs (26.7% each), bed sheets (23.3%), nurse station counters (16.7%), over bed tables (13.3%) and bed curtains (10.0%). Patient's record folders and computer keyboards/mouse at nurse station showed

Table 1. Characteristics of the patients infected or colonized with CRAB (n = 30)

Characteristics	Number	%
Sex		
Male	17	56.7
Female	13	43.3
Age (years)		
0-20	1	3.3
21-40	4	13.3
41-60	6	20.0
> 60	19	63.4
Range = 9-90 years		
Median = 69 years		
Major clinical problems		
Respiratory diseases	16	53.3
Cerebrovascular diseases	8	26.7
Trauma	4	13.3
Cardiovascular diseases	2	6.7
Clinical samples of CRAB		
Sputum	18	60.0
Urine	5	16.7
Wound swab	4	13.3
Blood	3	10.0
CRAB infection/colonization		
Nosocomial infection	20	66.7
Colonization	10	33.3

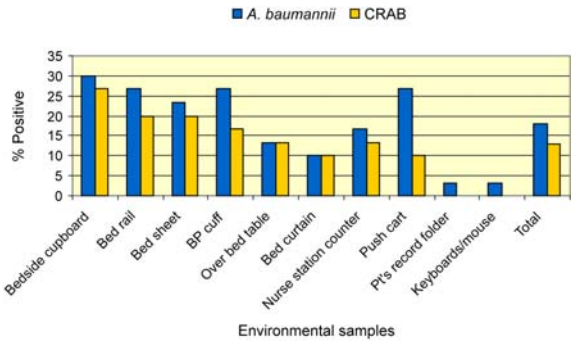

Fig. 1 Percentage of environmental cultures positive for *A. baumannii* and CRAB by items among 300 environmental samples

the lowest contamination rate of *A. baumannii* (3.3%).

Distributions of CRAB were slightly different from those of *A. baumannii*. Bedside cupboards was the predominant source with 26.7%, followed by bedrails and bed sheets (20.0% each), blood pressure cuffs (16.7%), over bed tables and nurse station counters (13.3% each), bed curtains and push carts (10.0% each). Over all *A. baumannii* and CRAB contamination rates of the patient's environment were 13% and 10.7% and non patient's environment were 5% and 2.3%, respectively.

Antimicrobial susceptibility of A. baumannii isolates

All of 30 CRAB isolated from the patients and 72.2% (39/54) of the environmental isolates were CRAB and MDR-*A. baumannii*, which defined as those resistance to 3 or more different classes of antibiotics including aminoglycosides, fluoroquinolones, beta-lactams, and 3rd generation of cephalosporin[14]. All CRAB isolated from the patients were resistant to ampicillin/sulbactam, cefpirome, ciprofloxacin, piperacillin/tazobactam. While 96.7% and 93% were resistant to ceftazidime and cefepime, respectively. The isolates were resistant to amikacin 83% and cefoperazone/sulbactam 16.7%. *A. baumannii* recovered from the environment were resistant to piperacillin/tazobactam (74.1%), imipenem, meropenem, cefpirome (72.2% each), and cefepime, ciprofloxacin (70.4% each). Colistin and tigecycline were the most active agents against all of the CRAB isolates (susceptibility rates of 100%).

CRAB molecular typing

Molecular typing was determined in 65 CRAB isolates (30 from the patients and 35 from the environment). On the basis of 90% similarity, four different types were identified. Molecular type 1 was the most common (90.7%), 59 out of 65 isolates (Fig. 2, Table 2) and found in all kinds of the environmental samples except patient record folder and computer keyboard/mouse. Bedside cupboards was the most common contaminated with CRAB (26.7% of 30 cases), which six isolates belonged to type 1. CRAB that contaminated at nurse station counters and push carts from four wards, all were molecular type 1. Moreover, there were 11 out of 30 cases (36.7%) that had at least one of their environmental samples contaminated with CRAB clonally related with their own types. Five of these 11 cases who were admitted in 4 different wards, infected with clonally related CRAB type 1(1). Of the five cases, 2 cases (P16 and P19) were admitted in female medical 1 ward and infected with type 1(1) at the same time. Another 5 cases who were admitted in 4 different wards infected/colonized with type 1(2) CRAB.

Discussion

The carbapenem-resistant *A. baumannii* (CRAB) causing nosocomial infection was an increasing problem in BMA-MCVH and tended to spread rapidly. Ratio of CRAB nosocomial infection (66.7%) to CRAB colonization (33.3%) in the present study was 2:1. The CRAB colonization pressure was quite high and could lead to CRAB infection later on. The risk of CRAB acquisition particularly in ICU patients are associated with CRAB colonization pressure and ICU antibiotic uses over the preceding three months[15]. Half of the clinical specimens, from which CRAB were isolated, were also found other major microorganisms such as *Pseudomonas aeruginosa* (MDR), methicillin-resistant *Staphylococcus aureus* (MRSA), *Escherichia coli* (ESBL) and *Candida albicans*. The co-infection with these drug resistant organisms may provide the opportunity for them to exchange genetic materials and mobile genetic elements such as plasmids[11], transposons[16], and integrons[17] which allow the acquisition of a multidrug-resistance among them.

About 72% of *A. baumannii* isolated from the environment were CRAB. Contamination of *A. baumannii* and CRAB could frequently be isolated from patient's environment that are often touched by patients, visitors and hospital personnel such as bedside cupboards, bedrails, and bed sheets forming a possible source of exogenous transmission[12,18,19]. However, *A. baumannii* isolates were also found on common area that are not surrounding the patient but

Table 2. Distribution of CRAB molecular types of the patients and environmental isolates

Patient No.	CRAB molecular type											
	Patient	Bedrail	Bed sheet	Over bed table	Bedside cup board	Bed curtain	Record folder	BP cuff	Nurse station counter	Key board/ mouse	Push cart	Ward
P1	①	-	-	1	①	-	-	1	-	-	1	EM-ICU
P2	1	-	-	-	-	-	-	-	-	-	-	MF1
P3	①	-	-	-	①	-	-	-	-	-	-	MF2
P4	1	-	-	-	4	-	-	-	-	-	-	SF
P5	1	4	-	-	-	-	-	-	-	-	-	OF
P6	1	-	-	-	-	-	-	-	-	-	-	MS2
P7	1	-	1	-	-	-	-	-	1	-	-	MS4
P8	❶	-	❶	-	-	-	-	-	-	-	-	MM2
P9	❶	-	-	-	1	❶	-	-	-	-	-	EM-ICU
P10	1	-	-	-	-	-	-	-	-	-	-	M-ICU
P11	1	-	-	3	3	-	-	-	-	-	-	MM1
P12	1	-	-	-	-	-	-	-	-	-	-	SF
P13	1	-	-	-	-	-	-	-	-	-	-	S-ICU
P14	1	-	-	-	-	-	-	-	-	-	-	MF1
P15	1	-	-	-	1	-	-	-	1	-	-	NS-ICU
P16	①	①	1	-	-	-	-	-	-	-	-	MF1
P17	**1**	1	-	-	1	-	-	**1**	-	-	-	EM-ICU
P18	2	-	1	-	-	-	-	1	-	-	-	EM-ICU
P19	①	-	-	-	-	-	-	-	①	-	1	MF1
P20	1	-	-	-	-	-	-	-	-	-	-	S-ICU
P21	❶	❶	-	-	-	❶	-	-	-	-	-	MM2
P22	3	1	-	-	-	-	-	1	-	-	-	M-ICU
P23	1	-	-	-	-	-	-	-	-	-	-	M-ICU
P24	1	-	1	-	-	-	-	-	-	-	-	M-ICU
P25	1	-	-	-	-	-	-	-	-	-	-	SM
P26	❶	-	-	-	❶	-	-	-	-	-	-	MM1
P27	1	-	1	-	-	-	-	-	-	-	-	MM2
P28	1	-	-	-	-	-	-	-	-	-	-	M-ICU
P29	❶	❶	-	❶	-	-	-	-	-	-	-	OF
P30	①	-	-	①	-	-	-	-	-	-	-	MS2
Total isolates	30	6	6	4	8	2	-	4	3	-	2	65

P, patient; P1, number indicates patient number; EM-ICU, emergency medical-ICU; MF1/2, female medical 1/2; SF, female surgical ward; OF, female orthopedic ward; MS2/4, medical special ward 2/4; MM1/2, male medical 1/2; M-ICU, medical ICU; S-ICU, surgical ICU; NS- ICU, neurosurgical-ICU; SM, male surgical ward. ① Identical type 1(1) ❶ identical type 1(2)

frequently touched by healthcare staff such as push carts, nurse station counters, patient's record folders, computer keyboards/mouse, medical equipments[4,20,21]. In this study, *A. baumannii* and CRAB contamination rates of the patients' environment (13% and 10.7%) were higher than those of non patients' environment (5% and 2.3%). All of them were used and touched by healthcare staff and cleaned infrequently. *A. baumannii* contamination rate was found 26.7% on push cart and 37.5% of them were CRAB. Push cart was normally used by many staff in the ward but its cleaning might be infrequent or done by housekeeping staff rather than healthcare staff. Therefore, push cart usage and cleaning as well as other items in the ward with the common uses should be paid attention and be specific in routine and protocol of cleaning.

The highest rates of *A. baumannii* and CRAB environmental contamination were found on bedside cupboard (30.0% and 26.7%). Bedside cupboard is reported to be contaminated in many studies[4,22,23]. Six

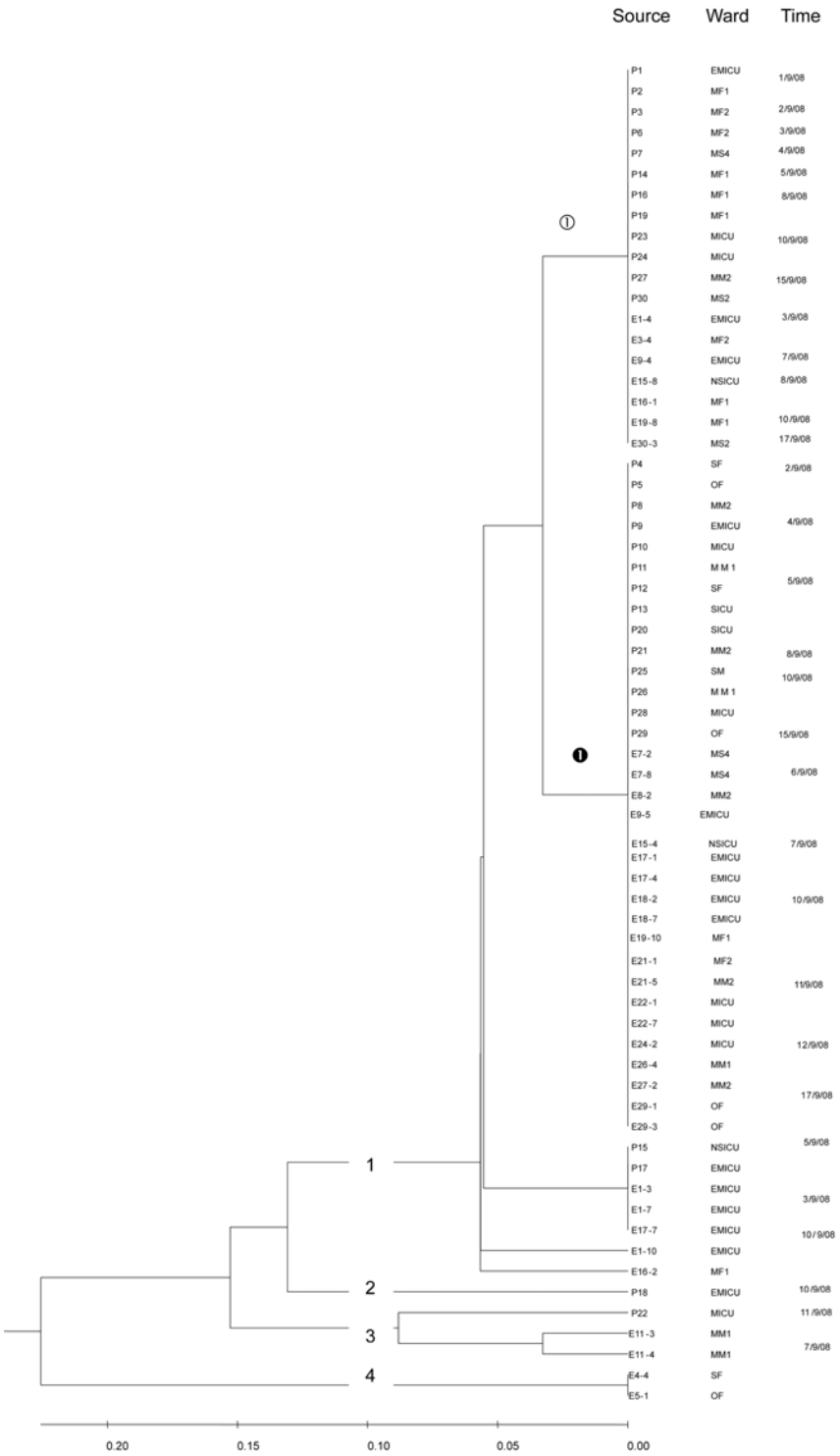

Fig. 2 Dendrogram showing genetic relationship of 4 molecular types CRAB isolates from the patients (P1-P30) and environment (E); E1-1, number after E indicates corresponding to patient's number and type of environmental sample, *i.e.* bedrail-1, bed sheet-2, over bed table-3, bedside cupboard-4, bed curtain-5, patient's record folder-6, blood pressure cuff-7 nurse station counter-8, keyboards/ mouse-9, push cart-10

of 8 CRAB isolates of bedside cupboard samples were molecular type 1 and matched with those of the patients' isolates. Bedside cupboard was most often occupied by many patient belongings as well as medical equipment for personal uses. Therefore, it could be easily contaminated with hand flora of the patient, healthcare staff and visitors. Bed rails and bed sheets were items closely related and found to be contaminated with CRAB with high rate (20%) and second only to bedside cupboard CRAB contamination (26.7%). All 6 CRAB isolates from the patients' bed sheet samples showed the same molecular type with the patients' isolates. While 4 of 6 CRAB isolates from bed rail samples were matched with the patients' isolates and both were molecular type 1. The results demonstrated the cross contamination of CRAB from the patients to their bedside cupboards, bed sheets and bed rails or vice versa. Bed curtain was reported to be the predominate source of *A. baumannii* and found widely spread during the outbreak[24]. In this study, the contamination rates of curtain with both *A. baumannii* and CRAB were lower when compared with previous studies[24,25]. It was possible due to the replacement of cotton curtains with synthetic curtains in most ICU of BMA-MCV hospital. The synthetic curtain was easily clean and did not adsorb dust as the natural fabric curtain.

Most of the environmental isolates (72.2%) were CRAB. By and large, the environmental isolates were more susceptible to the antimicrobial agents than patient's isolates. Nonetheless, the potential for acquisition of transferable carbapenem resistance in environmental isolates are high especially in area where CRAB is endemic[26]. All CRAB isolates were susceptible to colistin and tigecycline, similar to other studies that these 2 drugs had a good activity against MDR- *A. baumannii* and CRAB[27,28]. However the risk for colistin induced-nephrotoxicity is the clinical concern, tigecycline might be a useful alternative to colistin, nevertheless MDR- *A. baumannii* was recently reported to resist tigecycline[29].

The PCR-based molecular typing results showed that molecular type 1 was the most common CRAB circulated in the BMA-MCV Hospital and responsible for all 20 nosocomial infections (66.7%) in this study. 36.7% of the patients had the clinical isolates clonally related to at least one of the isolates from their environment. In addition, clonally related CRAB isolates were infected or colonized in 10 cases during the study period, suggesting the transmission of CRAB between patients had occurred in the same ward and to other wards as well. These results demonstrated cross contamination and the patients' environment as a reservoir in the spread of CRAB in the hospital. Besides hand hygiene and the routine infection control measures, patient's environment should be target for decontamination and cleaning on the regular basis in order to control the spread of this multidrug resistant microorganism.

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การแพร่กระจายเชื้อ Carbapenem resistant Acinetobacter baumannii ของผู้ป่วยและสิ่งแวดล้อมในวิทยาลัยแพทยศาสตร์กรุงเทพมหานครและวชิรพยาบาล

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วัตถุประสงค์: *ศึกษาการแพร่กระจายของเชื้อ Carbapenem resistant Acinetobacter baumannii (CRAB) สายพันธุ์เดียวกัน ในผู้ป่วยและสิ่งแวดล้อม ณ วิทยาลัยแพทยศาสตร์กรุงเทพมหานครและวชิรพยาบาล*

วัสดุและวิธีการ: *เก็บและวิเคราะห์ 300 ตัวอย่างจากสิ่งแวดล้อมของผู้ป่วยทันทีเมื่อแยกเชื้อ CRAB ได้จากผู้ป่วย 30 ราย ระหว่างวันที่ 1-15 กันยายน พ.ศ. 2551 เชื้อ CRAB ที่แยกได้นำมาจัดกลุ่มระดับโมเลกุลด้วยวิธี PCR-based typing*

ผลการศึกษา: *พบการปนเปื้อนเชื้อ A. baumannii และ CRAB ในสิ่งแวดล้อมของผู้ป่วยร้อยละ 86.7 และร้อยละ 66.7 ตามลำดับ อัตราการปนเปื้อน A. baumannii และ CRAB ในตัวอย่างสิ่งแวดล้อมทั้งหมดคือร้อยละ 18 (54/300) และ 13 (39/300) ตามลำดับ ตัวอย่างสิ่งแวดล้อมที่พบเชื้อ CRAB มากคือตู้ข้างเตียง (ร้อยละ 26.7), เหล็กกั้นเตียงและผ้าปูที่นอน(ร้อยละ 20), ปลอกแขนวัดความดันโลหิต (ร้อยละ 16.7), โต๊ะคร่อมเตียงและเคาน์เตอร์พยาบาล (ร้อยละ 13.3) และรถเข็นสำหรับใส่อุปกรณ์ทำแผล (ร้อยละ 10) การจัดกลุ่มในระดับโมเลกุลจำแนกเชื้อ CRAB 65 ไอโซเลตได้ 4 ไทป์ โดยร้อยละ 90.7 เป็นไทป์ 1 ซึ่งพบในตัวอย่างสิ่งแวดล้อมทุกชนิด ยกเว้นแฟ้มประวัติผู้ป่วย และแป้นพิมพ์ของเครื่องคอมพิวเตอร์/เมาส์ มีผู้ป่วยร้อยละ 37 ที่พบสิ่งแวดล้อมอย่างน้อยหนึ่งชนิดปนเปื้อนเชื้อ CRAB ไทป์เดียวกันกับผู้ป่วย*

สรุป: *การแพร่กระจายของ CRAB สายพันธุ์เดียวกันในผู้ป่วยและสิ่งแวดล้อมแสดงให้เห็นในการศึกษานี้ ย้ำความสำคัญของการล้างมือ มาตรการป้องกันทางการสัมผัส และการทำความสะอาดสิ่งแวดล้อมผู้ป่วยอย่างถูกต้อง เพื่อควบคุมการแพร่กระจายเชื้อ CRAB ในโรงพยาบาล*