



Evaluation of a new selective agar medium for detection of carbapenem-resistant *Enterobacteriaceae*



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ABSTRACT

CRE-JU is a novel selective agar for carbapenem-resistant *Enterobacteriaceae* that contains ceftazidime, cloxacillin, meropenem, and vancomycin. This study evaluated the ability of 63 carbapenem-resistant isolates and 53 non-carbapenem-resistant *Enterobacteriaceae* strains clinically isolated in Japan, Myanmar, Nepal, and Vietnam to grow on CRE-JU. CRE-JU showed 92.1% sensitivity and 100% specificity for detecting carbapenem-resistant *Enterobacteriaceae* compared with drug susceptibility profiles.

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The emergence and spread of carbapenem-resistant *Enterobacteriaceae* (CRE) and carbapenemase-producing *Enterobacteriaceae* (CPE) have become serious problems in medical settings worldwide (Logan and Weinstein 2017). CRE and CPE cause nosocomial outbreaks, and infections with these bacteria are associated with high rates of morbidity and mortality in patients with serious underlying disorders and those in intensive care units (Potter et al. 2016). Many CPE isolates harbor transmissible plasmids carrying genes encoding carbapenemases, including class A β -lactamases (KPC and GES-5), class B metallo- β -lactamases (IMP, KHM, NDM and VIM), and class D β -lactamases (OXA-48-like) in general (Potter et al. 2016). CPE isolates harboring these carbapenemase-encoding genes are globally disseminated, and it is important for minimizing the dissemination of such plasmids (Baran and Aksu 2016).

Timely detection of CRE and CPE by clinical bacteriology laboratories is important in patient diagnosis and treatment. Three screening media for detection of these bacteria are currently available commercially: SUPERCARBA medium (Girlich et al. 2013), Oxoid Brilliance™ CRE agar (Cohen Stuart et al. 2013), and CHROMager (Nordmann et al., 2012) mSuperCARBA medium (Garcia-Quintanilla et al. 2018). In addition, several kits and tests for detection of CPE are used by bacterial laboratories, including the modified Hodge test (Clinical and Laboratory Standards Institute 2010); the modified carbapenem inactivation method (mCIM)

(Pierce et al. 2017); the improved carbapenem inactivation method (CIMTrisII) (Uechi et al. 2019); and the RAPIDEC® CARBA NP test (bioMérieux, France), a commercially available modified version of the Carba NP test (Aktaş et al. 2017). CRE-JU medium is a new cystine lactose electrolyte deficient (CLED) agar containing ceftazidime (8 mg/L), cloxacillin (126 mg/L), meropenem (2 mg/L), and vancomycin (2 mg/L). This study describes the ability of this new selective agar medium to detect CRE.

CRE was defined as strains showing resistance to imipenem and/or meropenem (MIC ≥ 4 μ g/mL) (Clinical and Laboratory Standards Institute 2018). A total of 116 clinical isolates of *Enterobacteriaceae* were tested. Of them, 63 were CRE and the remaining 53 were non-CRE. Of the 63 CRE isolates, 60 were carbapenemase producers, including isolates of *Citrobacter freundii* producing NDM-type ($n = 2$) and KHM-type ($n = 1$); *Enterobacter cloacae* KPC-type ($n = 2$) and NDM-type ($n = 7$); *Escherichia coli* NDM-type ($n = 19$); *Klebsiella pneumoniae* GES-5-type ($n = 1$), KPC-type ($n = 2$), NDM-type ($n = 15$), and OXA-type ($n = 3$); *Klebsiella varicola* NDM-type ($n = 2$); *Morganella morganii* NDM-type ($n = 1$); *Providencia rettgeri* NDM-type ($n = 2$); and *Serratia marcescens* GES-5-type ($n = 3$) (Table 1). Three isolates of *Proteus mirabilis* were carbapenem-resistant but carbapenemase nonproducers. The remaining 53 isolates were carbapenem-susceptible carbapenemase nonproducers, including *E. cloacae* ($n = 4$), *E. coli* ($n = 33$), *K. pneumoniae* ($n = 13$), *Proteus mirabilis* ($n = 3$), *P. rettgeri* ($n = 1$), and *S. marcescens* ($n = 1$) (Table 1). Of these 53 carbapenemase nonproducers, 26 were extended spectrum β -

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Table 1
Evaluation of CRE-Jun medium for growth of carbapenemase producers and nonproducers.

Species	Strain	Carbapenemase	MIC ($\mu\text{g/mL}$)		Growth on CRE-JU	Lowest detection limit	Country of isolation	Remarks (ESBL)	Reference
			IPM	MEM					
<i>Citrobacter freundii</i>	IOMTU157	NDM-1	4	8	+	10^1	Nepal	This study	
<i>Citrobacter freundii</i>	MyNCGM447-1	NDM-4	2	8	+	10^1	Myanmar	This study	
<i>Citrobacter freundii</i>	KHM243	KHM-1	2	4	+	10^2	Japan	Sekiguchi et al. 2008	
<i>Enterobacter cloacae</i>	VNCEc2	KPC-2	16	16	+	10^1	Vietnam	This study	
<i>Enterobacter cloacae</i>	VNCEc13	KPC-2	8	16	+	10^1	Vietnam	This study	
<i>Enterobacter cloacae</i>	VNCEc1	NDM-1	16	16	+	10^1	Vietnam	This study	
<i>Enterobacter cloacae</i>	VNCEc15	NDM-4	16	32	+	10^1	Vietnam	This study	
<i>Enterobacter cloacae</i>	VNCEc16	NDM-7	16	32	+	10^1	Vietnam	This study	
<i>Enterobacter cloacae</i> ssp. <i>cloacae</i>	MyNCGM10	NDM-1	32	32	+	10^1	Myanmar	This study	
<i>Enterobacter cloacae</i> ssp. <i>cloacae</i>	MyNCGM55	NDM-1	4	4	+	10^3	Myanmar	This study	
<i>Enterobacter cloacae</i> ssp. <i>cloacae</i>	MyNCGM30	NDM-7	32	64	+	10^1	Myanmar	This study	
<i>Enterobacter cloacae</i> subsp. <i>dissolvens</i>	MyNCGM72	NDM-1	4	4	+	10^3	Myanmar	This study	
<i>Escherichia coli</i>	IOMTU324	NDM-1	16	8	+	10^1	Nepal	This study	
<i>Escherichia coli</i>	IOMTU340	NDM-1	16	8	+	10^1	Nepal	This study	
<i>Escherichia coli</i>	MyNCGM12-1	NDM-1	4	8	+	10^1	Myanmar	This study	
<i>Escherichia coli</i>	MyNCGM13	NDM-1	32	32	+	10^1	Myanmar	This study	
<i>Escherichia coli</i>	IOMTU388_1	NDM-12	16	64	+	10^1	Nepal	Tada et al. 2014b	
<i>Escherichia coli</i>	IOMTU558	NDM-13	32	128	+	10^1	Nepal	Shrestha et al. 2015	
<i>Escherichia coli</i>	IOMTU355	NDM-3	32	32	+	10^1	Nepal	Shrestha et al. 2017	
<i>Escherichia coli</i>	NCGM77	NDM-3	16	32	+	10^1	Japan	Shrestha et al. 2017	
<i>Escherichia coli</i>	IOMTU328	NDM-4	8	32	+	10^1	Nepal	Shrestha et al. 2017	
<i>Escherichia coli</i>	MyNCGM73	NDM-4	16	128	+	10^1	Myanmar	This study	
<i>Escherichia coli</i>	IOMTU351	NDM-5	64	32	+	10^1	Nepal	Shrestha et al. 2017	
<i>Escherichia coli</i>	IOMTU365	NDM-5	16	64	+	10^1	Nepal	Shrestha et al. 2017	
<i>Escherichia coli</i>	IOMTU387	NDM-5	8	16	+	10^1	Nepal	Shrestha et al. 2017	
<i>Escherichia coli</i>	MyNCGM32	NDM-5	8	32	+	10^1	Myanmar	This study	
<i>Escherichia coli</i>	MyNCGM48	NDM-5	8	32	+	10^1	Myanmar	This study	
<i>Escherichia coli</i>	MyNCGM66	NDM-5	8	64	+	10^1	Myanmar	This study	
<i>Escherichia coli</i>	IOMTU372	NDM-7	8	32	+	10^1	Nepal	Shrestha et al. 2017	
<i>Escherichia coli</i>	MyNCGM53	NDM-7	16	64	+	10^1	Myanmar	This study	
<i>Escherichia coli</i>	IOMTU11	NDM-8	256	256	+	10^1	Nepal	Tada et al. 2013	
<i>Klebsiella pneumoniae</i>	RYU-U7005-3	GES-5	128	64	+	10^1	Japan	This study	
<i>Klebsiella pneumoniae</i>	VBKP105	KPC-2	64	64	+	10^1	Vietnam	Tada et al. 2017	
<i>Klebsiella pneumoniae</i>	VNCKp29	KPC-2	4	4	+	10^1	Vietnam	Tada et al. 2017	
<i>Klebsiella pneumoniae</i>	IOMTU154	NDM-1	16	32	+	10^1	Nepal	Tada et al. 2013	
<i>Klebsiella pneumoniae</i>	IOMTU208	NDM-1	16	32	+	10^1	Nepal	This study	
<i>Klebsiella pneumoniae</i>	MyNCGM111	NDM-1	16	64	+	10^1	Myanmar	This study	
<i>Klebsiella pneumoniae</i>	MyNCGM191	NDM-1	8	64	+	10^1	Myanmar	This study	
<i>Klebsiella pneumoniae</i>	MyNCGM201	NDM-1	16	64	+	10^1	Myanmar	This study	
<i>Klebsiella pneumoniae</i>	VNCKp39	NDM-1	8	8	+	10^1	Vietnam	Tada et al. 2017	
<i>Klebsiella pneumoniae</i>	VNCKp42	NDM-1	32	64	+	10^1	Vietnam	Tada et al. 2017	
<i>Klebsiella pneumoniae</i>	VNCKp77-1	NDM-1	16	64	+	10^1	Vietnam	Tada et al. 2017	
<i>Klebsiella pneumoniae</i>	VNCKp77-2	NDM-1	16	64	+	10^1	Vietnam	Tada et al. 2017	
<i>Klebsiella pneumoniae</i>	MyNCGM36	NDM-4	32	128	+	10^1	Myanmar	This study	
<i>Klebsiella pneumoniae</i>	VNBM37	NDM-4	32	128	+	10^1	Vietnam	Tada et al. 2017	
<i>Klebsiella pneumoniae</i>	VNCKp73	NDM-4	128	256	+	10^1	Vietnam	Tada et al. 2017	
<i>Klebsiella pneumoniae</i>	MyNCGM444	NDM-5	4	32	+	10^1	Myanmar	This study	
<i>Klebsiella pneumoniae</i>	MyNCGM235	NDM-7	32	128	+	10^1	Myanmar	This study	
<i>Klebsiella pneumoniae</i>	MyNCGM362	NDM-7	16	64	+	10^1	Myanmar	This study	
<i>Klebsiella pneumoniae</i>	VNCKp05	OXA-48	8	8	+	10^1	Vietnam	Tada et al. 2017	
<i>Klebsiella pneumoniae</i>	VBKP112	OXA-48	4	8	+	10^1	Vietnam	Tada et al. 2017	
<i>Klebsiella pneumoniae</i>	VNCKp30	OXA-48	4	4	+	10^1	Vietnam	Tada et al. 2017	
<i>Klebsiella variicola</i>	IOMTU169	NDM-1	16	64	+	10^1	Nepal	This study	
<i>Klebsiella variicola</i>	IOMTU197	NDM-1	64	32	+	10^1	Nepal	This study	
<i>Morganella morganii</i>	MyNCGM218	NDM-1	4	4	+	10^3	Myanmar	This study	
<i>Proteus mirabilis</i>	BML16	—	4	<0.5	—	> 10^7	Japan	CTX-M-2 This study	
<i>Proteus mirabilis</i>	BML17	—	4	<0.5	—	> 10^7	Japan	CTX-M-2 This study	
<i>Proteus mirabilis</i>	BML18	—	4	<0.5	—	> 10^7	Japan	CTX-M-2 This study	
<i>Providencia rettgeri</i>	IOMTU001	NDM-1	32	64	+	10^1	Nepal	Tada et al. 2014a	
<i>Providencia rettgeri</i>	IOMTU091	NDM-1	64	64	+	10^1	Nepal	Tada et al. 2014a	
<i>Serratia marcescens</i>	RYU-636-3	GES-5	16	4	+	10^2	Japan	This study	
<i>Serratia marcescens</i>	RYU-7002-1	GES-5	8	1	—	> 10^7	Japan	This study	
<i>Serratia marcescens</i>	RYU-U437	GES-5	8	1	—	> 10^7	Japan	This study	
<i>Enterobacter cloacae</i>	VNCEc14	—	2	2	—	> 10^7	Vietnam	This study	
<i>Enterobacter cloacae</i>	VNCEc17	—	<0.5	<0.5	—	> 10^7	Vietnam	This study	
<i>Enterobacter cloacae</i>	VNCEc18	—	<0.5	<0.5	—	> 10^7	Vietnam	This study	
<i>Enterobacter cloacae</i>	VNCEc19	—	<0.5	<0.5	—	> 10^7	Vietnam	This study	
<i>Escherichia coli</i>	MyNCGM312_2	—	<0.5	<0.5	—	> 10^7	Myanmar	This study	
<i>Escherichia coli</i>	MyNCGM355	—	<0.5	<0.5	—	> 10^7	Myanmar	This study	
<i>Escherichia coli</i>	MyNCGM479_1	—	<0.5	<0.5	—	> 10^7	Myanmar	This study	
<i>Escherichia coli</i>	MyNCGM479_2	—	<0.5	<0.5	—	> 10^7	Myanmar	This study	
<i>Escherichia coli</i>	TI36	—	1	<0.5	—	> 10^7	Japan	Ieda et al. 2015	

Table 1 (continued)

Species	Strain	Carbapenemase	MIC ($\mu\text{g/mL}$)		Growth on CRE-JU	Lowest detection limit	Country of isolation	Remarks (ESBL)	Reference
			IPM	MEM					
<i>Escherichia coli</i>	TI64	—	<0.5	<0.5	—	>10 ⁷	Japan		leda et al. 2015
<i>Escherichia coli</i>	BML1	—	<0.5	<0.5	—	>10 ⁷	Japan	CTX-M-101	This study
<i>Escherichia coli</i>	BML10	—	<0.5	<0.5	—	>10 ⁷	Japan	CTX-M-2	This study
<i>Escherichia coli</i>	BML2	—	<0.5	<0.5	—	>10 ⁷	Japan	CTX-M-27	This study
<i>Escherichia coli</i>	BML3	—	<0.5	<0.5	—	>10 ⁷	Japan	CTX-M-15	This study
<i>Escherichia coli</i>	BML4	—	<0.5	<0.5	—	>10 ⁷	Japan	CTX-M-27	This study
<i>Escherichia coli</i>	BML5	—	<0.5	<0.5	—	>10 ⁷	Japan	CTX-M-27	This study
<i>Escherichia coli</i>	BML6	—	<0.5	<0.5	—	>10 ⁷	Japan	CTX-M-14	This study
<i>Escherichia coli</i>	BML7	—	<0.5	<0.5	—	>10 ⁷	Japan	CTX-M-15	This study
<i>Escherichia coli</i>	BML8	—	<0.5	<0.5	—	>10 ⁷	Japan	CTX-M-15	This study
<i>Escherichia coli</i>	BML9	—	<0.5	<0.5	—	>10 ⁷	Japan	CTX-M-15	This study
<i>Escherichia coli</i>	TI15	—	<0.5	<0.5	—	>10 ⁷	Japan	CTX-M-14	leda et al. 2015
<i>Escherichia coli</i>	TI67	—	0.5	<0.5	—	>10 ⁷	Japan	CTX-M	leda et al. 2015
<i>Escherichia coli</i>	TI70	—	<0.5	<0.5	—	>10 ⁷	Japan	CTX-M-14	leda et al. 2015
<i>Escherichia coli</i>	NCGMEC88	—	<0.5	<0.5	—	>10 ⁷	Japan		This study
<i>Escherichia coli</i>	RYU 2912 C-11	—	<0.5	<0.5	—	>10 ⁷	Japan		This study
<i>Escherichia coli</i>	TI19	—	<0.5	<0.5	—	>10 ⁷	Japan		leda et al. 2015
<i>Escherichia coli</i>	TI38	—	<0.5	<0.5	—	>10 ⁷	Japan		leda et al. 2015
<i>Escherichia coli</i>	TI39	—	<0.5	<0.5	—	>10 ⁷	Japan		leda et al. 2015
<i>Escherichia coli</i>	TI65	—	<0.5	<0.5	—	>10 ⁷	Japan		leda et al. 2015
<i>Escherichia coli</i>	ATCC25922	—	<0.5	<0.5	—	>10 ⁷	-		A type strain
<i>Escherichia coli</i>	IOMTU318	—	<0.5	<0.5	—	>10 ⁷	Nepal		This study
<i>Escherichia coli</i>	IOMTU391	—	<0.5	<0.5	—	>10 ⁷	Nepal		This study
<i>Escherichia coli</i>	IOMTU792	—	<0.5	2	—	>10 ⁷	Nepal		This study
<i>Escherichia coli</i>	MyNCGM54	—	<0.5	<0.5	—	>10 ⁷	Myanmar		This study
<i>Escherichia coli</i>	VNBM20	—	<0.5	<0.5	—	>10 ⁷	Vietnam	CTX-M-15	This study
<i>Escherichia coli</i>	VNBM31	—	<0.5	<0.5	—	>10 ⁷	Vietnam	CTX-M-27	This study
<i>Escherichia coli</i>	VNBM32	—	<0.5	<0.5	—	>10 ⁷	Vietnam	CTX-M-14	This study
<i>Klebsiella pneumoniae</i>	BML11	—	<0.5	<0.5	—	>10 ⁷	Japan	CTX-M-14	This study
<i>Klebsiella pneumoniae</i>	BML12	—	<0.5	<0.5	—	>10 ⁷	Japan	CTX-M-14	This study
<i>Klebsiella pneumoniae</i>	BML13	—	<0.5	<0.5	—	>10 ⁷	Japan	CTX-M-14	This study
<i>Klebsiella pneumoniae</i>	BML15	—	<0.5	<0.5	—	>10 ⁷	Japan	CTX-M-14	This study
<i>Klebsiella pneumoniae</i>	MyNCGM174	—	<0.5	<0.5	—	>10 ⁷	Myanmar		This study
<i>Klebsiella pneumoniae</i>	MyNCGM328_1	—	<0.5	<0.5	—	>10 ⁷	Myanmar		This study
<i>Klebsiella pneumoniae</i>	MyNCGM328_2	—	<0.5	<0.5	—	>10 ⁷	Myanmar		This study
<i>Klebsiella pneumoniae</i>	VNBM14	—	<0.5	<0.5	—	>10 ⁷	Vietnam	SHV-129, CTX-M-27, VEB-1	This study
<i>Klebsiella pneumoniae</i>	VNBM3	—	<0.5	<0.5	—	>10 ⁷	Vietnam	SHV-28, CTX-M-15	This study
<i>Klebsiella pneumoniae</i>	VNBM8	—	<0.5	<0.5	—	>10 ⁷	Vietnam	CTX-M-27	This study
<i>Klebsiella pneumoniae</i>	VNBM9	—	<0.5	<0.5	—	>10 ⁷	Vietnam	SHV-12, CTX-M-15	This study
<i>Proteus mirabilis</i>	BML19	—	2	<0.5	—	>10 ⁷	Japan	CTX-M-2	This study
<i>Proteus mirabilis</i>	BML20	—	2	<0.5	—	>10 ⁷	Japan	CTX-M-2	This study
<i>Proteus mirabilis</i>	TI73	—	2	<0.5	—	>10 ⁷	Japan	CTX-M-2	leda et al. 2015
<i>Providencia rettgeri</i>	IOMTU094	—	2	1	—	>10 ⁷	Nepal		Tada et al. 2014b
<i>Serratia marcescens</i>	MyNCGM68	—	<0.5	<0.5	—	>10 ⁷	Myanmar		This study

IPM = imipenem, MEM = meropenem.

lactamase (ESBL) producers (Table 1). The entire genome of carbapenem-resistant isolates and ESBL producers was sequenced by MiSeq (Illumina, San Diego, CA). Sequences of drug-resistance genes were determined by CLC genomics workbench version 9.0.1.

The minimal inhibitory concentrations (MICs) of imipenem (IMP) and meropenem (MEM) were determined by a microdilution method according to Clinical Standards Laboratory Institute guidelines (Clinical and Laboratory Standards Institute 2018). The break point of each isolate was cultured overnight on tryptic soy agar (TSA) plates, suspended in sterile saline and adjusted to 0.5 McFarland turbidity. Ten microliters of each suspension was inoculated onto CRE-JU selective ager medium, followed by incubation at 37 °C for 18 h; if colonies were not detected, the medium was incubated for an additional 48 h. To determine the lowest detection limit of inoculated isolates on CRE-JU, isolate concentrations were adjusted at 1×10^8 CFU/mL based on their absorbances at 600 nm, and 100 μL of serial 10-fold dilutions were plated onto CRE-JU and TSA plates, which were cultured at 37 °C for 18 h. The numbers of bacterial colonies were counted after incubation.

Of the 63 carbapenem-resistant isolates tested, 58 (92.1%) grew on the CRE-JU agar (Table 1). Of them, 60 were carbapenem producers. Of these

producers, 58 grew on CRE-JU. These 58 isolates were producers of GES-5, KHM-1, KPC-2, NDM, and OXA-48 type carbapenemases. The MICs of IPM and MEM for these 58 isolates grown on CRE-JU ranged from 2 to 256 mg/L (median: 16 mg/L) and 4–256 mg/L (median: 32 mg/L), respectively. Of the 58 isolates, 51 had MICs ≥ 8 mg/L on MEM, with lowest limits of detection of 10^1 CFU/plate. The remaining 7 isolates had MICs of 4 mg/L on MEM, with lowest limits of detection ranging from 10^1 to 10^3 CFU/plate. Five isolates (7.9%) did not grow on CRE-JU (Table 1). Three were carbapenem-nonproducing *Proteus mirabilis* with MIC on IMP and MEM of 4 mg/L and 0.5 mg/L, respectively. The remaining 2 isolates were GES-5 producing *S. marcescens*, with MICs on IPM and MEM of 8 mg/L and 1 mg/L, respectively. A third GES-5 producing *S. marcescens* isolate grew on CRE-JU, indicating that CRE-JU is a poor detector GES-5 producing *S. marcescens* and that CRE-JU does not detect MEM-sensitive, but IMP-resistant, CPE isolates (Table 1).

None of the 53 carbapenem-susceptible isolates tested, including 32 ESBL producers, grew on CRE-JU (Table 1).

Because CRE-JU is a CLED-based medium, it was able to distinguish lactose-fermenting (yellow colonies) from lactose-nonfermenting colonies (data not shown). Carbapenemase-producing *C. freundii*, *E. cloacae*.

E. coli, and *K. pneumoniae* isolates grew on CRE-JU, yielding yellow-colored colonies. Although carbapenemase-producing and lactose-nonfermenting *Enterobacteriaceae*, including *M. organii*, *P. rettgeri*, and *S. marcescens*, also grew on CRE-JU, their colonies were not yellow. These findings suggest that CRE-JU could easily distinguish carbapenemase-producing and lactose-fermenting *Enterobacteriaceae* from nonfermenting Gram-negative bacteria.

When stored at 4 °C, CRE-JU had an expiration date of 75 days, as determined when tested using carbapenemase-producing *C. freundii* and carbapenemase-nonproducing *K. pneumoniae* isolates.

Collectively, CRE-JU screening medium had a sensitivity of 92.1% (58/63) and a specificity of 100% ($P < 0.001$), indicating that CRE-JU can effectively detect CRE, including CPE. CRE-JU is useful for detecting KPC-, NDM-, and OXA-producing *Enterobacteriaceae*, but not GES-5-producing *S. marcescens*.

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Declaration of competing interest

J. S. and Y. I. are employees of Kojin Bio Co., Ltd., and other authors have no conflict interest.

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Ethical statement

This study was approved by Ministry of Health and Sports in the Republic of the Union of Myanmar (Letter No. Ethical Committee 2016) and the Biosafety Committee, Juntendo University School of Medicine (approval number: BSL2/30-1).

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