Original Article

Presence of enterobacteria producing extended-spectrum β-lactamases and/or carbapenemases in animals, humans and environment in India

Fateh Singh[1*] S.D. Hirpurkar[1] Sanjay Shakya[2] Nidhi Rawat[1] Prashant Devangan[2]

Foziya Farzeen Khan[1] Sunil Kumar Bhandekar[3]

Abstract

The objective of this study was to isolate antimicrobial resistant bacteria from animals, humans and environment, and to characterize them based on phenotypic attributes of resistance. A total of 521 samples were collected from animals (284), humans (69) and environment (168) which resulted in isolation of 615 non-duplicate resistant isolates (to either amoxycillin-clavulanate, ceftazidime, cefixime, meropenem, or imipenem). Antimicrobial susceptibility test (AST) revealed the highest rate of resistance to cefixime (49.3%) followed by ceftazidime (45%), meropenem (38.1%), amoxicillin-clavulanate (35.3%), and imipenem (13%). Identification recorded *Escherichia coli* (54.6%), *Klebsiella* spp. (17%), *Citrobacter* spp. (7.5%), *Enterobacter* spp. (6.7%), *Acinetobacter* spp. (3.0%), *Serratia* spp. (2.8%), *Proteus* spp. (2.5%), *Shigella* spp. (2.5%), and other bacteria (3%). Production of extended-spectrum beta-lactamases (ESBLs), assessed by double disc diffusion test (DDDT) and double disc synergy test (DDST) using amoxicillin and amoxicillin-clavulanate, and ceftazidime and ceftazidime-clavulanate/ ceftazidime-tazobactam, was seen among 60 (28.6%) and 47 (22.3%) isolates, respectively. Moreover, 22.5% of the isolates expressed carbapenemase phenotype identified by imipenem-EDTA metallo-beta-lactamases (MBLs) DDDT/DDST. Out of 30 carbapenem-resistant isolates tested, five isolates (16.6%) exhibited positive MBL E-test. Moreover, positive modified Hodge test was shown by five bacterial isolates (16.6%). The present study proved the wide occurrence of multidrug-resistant (MDR) bacteria associated with animals, humans and environment. The phenotypic detection of ESBLs and carbapenemases establishes the preliminary background to more reliable genotypic identification of antimicrobial resistance, and thus aids in rapid clinical diagnosis of MDR bacterial pathogens.

Keywords: antimicrobial resistance, carbapenemases, *Enterobacteriaceae*, extended-spectrum beta-lactamases

[1]*Department of Veterinary Microbiology, College of Veterinary Science and Animal Husbandry, Anjora, Durg, Chhattisgarh, India*

[2]*Department of Veterinary Public Health and Epidemiology, College of Veterinary Science and Animal Husbandry, Anjora, Durg, Chhattisgarh, India*

[3]*Department of Instructional Livestock Farm Complex, College of Veterinary Science and Animal Husbandry, Anjora, Durg, Chhattisgarh, India*

***Correspondence**: fateh.ars07@gmail.com*

Introduction

The increasing trend of antimicrobial resistance has become a global problem. Members of *Enterobacteriaceae* are commensal in the intestine of animals and are usually under selective pressure by the use of antibiotics for treatment and prevention of infectious diseases (Schwarz et al., 2001). Indiscriminate use of antibiotics, which leads to emergence of resistant strains, is mainly after an irrational course of antibiotics by medical community (Li et al., 2007). Antimicrobial drugs are used in animals as feed additives and growth promoters to increase livestock production. Hence, these drugs render the meat, milk, and eggs unsafe and becoming the potential source of multidrug-resistant (MDR) bacteria (Schneider and Garrett, 2009). Beta-lactams are the most commonly used antibiotics to combat bacterial infections (Carattoli, 2009). However, the production of bacterial beta-lactamases hydrolyses the beta-lactam ring of antibiotics and renders them inactive. Extended-spectrum beta-lactamases (ESBLs) are capable of targeting a broader spectrum of antibiotics, including penicillins and their complexes (amoxycillin-clavulanate), monobactams (aztreonam), and up to third generation cephalosporins (Paterson and Bonomo, 2005). Earlier, carbapenems have been used successfully to treat infections due to resistant *Enterobacteriaceae bacteria (*Morrill et al., 2015). However, during the past decades, the rate of MDR bacteria and carbapenem-resistant *Enterobacteriaceae* clinical isolates has increased, considerably restricting effective antimicrobial therapy (Pournaras et al., 2016). Surveillance of antibiotic resistance could be a valuable tool to screen the resistance trends on population level (Kwak et al., 2015). The emergence of bacterial antibiotic resistance is usually assessed either by population analysis or susceptibility testing (Firsov et al., 2015). Reports are scanty on beta-lactam-resistant enterobacteria related to livestock animals particularly from Chhattisgarh and Rajasthan states of India. Since the first extensive investigation highlighted the emergence and spread of NDM and KPC carbapenemases from New Delhi and its surrounding areas in India (Kumarasamy et al., 2010), the present study targeted Delhi and Uttar Pradesh along with Chhattisgarh and Rajasthan, which are geographically located in different Agroclimatic zones. Recovery of MDR bacterial isolates from animal, human and environment sources indicates the acquisition of antimicrobial resistance by bacteria.

Materials and Methods

The present study included part of a doctoral thesis research completed at Chhattisgarh Kamdhenu Vishwavidyalaya, Durg, Chhattisgarh, as an in-service assignment from ICAR-Central Sheep and Wool Research Institute, Avikanagar, Rajasthan. To meet the objectives of this study, animal samples were collected from Chhattisgarh and Rajasthan states. Due to high prevalence reports on NDM and KPC carbapenemases in New Delhi areas, environmental samples from the national capital, Delhi, and its adjacent areas (Uttar Pradesh) were collected for the study. Efforts were made to highlight the emergence and clinical importance of antimicrobial resistance, and the phenotypic detection techniques suitable for rapid clinical diagnosis of MDR pathogens in primary health practices and field conditions; however, deficiency in uniformity of sample size, sample type, and study design created weaknesses in this study.

***Collection and processing of samples*:** A total of 521 samples were collected from animals (goat, sheep, cattle and poultry) (284), humans (69) and environment (168) from Chhattisgarh (394) and other states viz. Rajasthan (81), Delhi (23) and Uttar Pradesh (23) (Table 1). The animal samples included meat, milk, faecal and clinical and/or tissue samples. Human samples such as urine and faecal samples were collected from disease diagnostic laboratories. The environmental samples included water samples from small water reservoirs in city confluents, waste water drainages, sewage water, ponds, canals and rivers. Simultaneously, drinking and tap water samples were also collected. Moreover, the samples from a poultry flock were screened for bacterial contamination following the all-in all-out strategy, and comprised drag samples, boot samples, floor water samples, and tap water. The faecal, urine, milk, and meat samples were collected aseptically in sterile vials. The clinical and tissue samples were received in sterile plastic vials brought by animal owners to the laboratory for clinical diagnosis. The water samples were collected in sterile syringes brought immediately to the laboratory. The water, milk, urine, and faecal samples were stored at 4^0C until primary inoculation. The tissue and meat samples (~1.0 g) were triturated in sterilized pestle mortar with sterile normal saline solution and stored at 4^0C before inoculation in nutrient broth.

***Bacterial isolation*:** Bacterial isolation was carried out as per Sleigh and Duguid (1989). One gram each of the faecal and triturated tissue samples was inoculated in 10 ml of sterilized nutrient broth and incubated at 37^0C for 24 h, and then streaked on Mac-Conkey agar plate. Similarly, one ml of each water/ urine/ milk samples was inoculated into 10 ml of sterile nutrient broth, then after incubation at 37^0C for 24 h plated on Mac-Conkey agar. Each of the drag and boot samples from previously disinfected poultry flocks were incubated into 50 ml of tetrathionate broth as pre-enrichment for *Salmonella* spp. and then inoculated on Mac-Conkey agar. Then, the Mac-Conkey agar grown isolates were used for antimicrobial susceptibility test. Moreover, the samples of meat, poultry faecal, and rest of the milk, water, cattle faecal, and clinical and/or tissue samples were incubated in nutrient broth at 37^0C for 18-24 h, and then inoculated on HiCrome ESBL agar for preliminary screening and isolation of ESBL producing bacteria. Bacteria grown on the HiCrome ESBL agar were obtained in pure culture and categorised as resistant isolates.

***Antimicrobial susceptibility testing (AST) and bacterial identification*:** The *in vitro* AST with disc diffusion method was performed using amoxycillin-clavulanate (30 µg), ceftazidime (30 µg), cefixime (5 µg), imipenem (10 µg), meropenem (10 µg), and ciprofloxacin (5 µg) (HiMedia Pvt. Ltd., Mumbai) as

per CLSI (2011). The procedure was optimised using the above antimicrobials against *E. coli* ATCC 25922 reference strain.

All the resistant isolates after AST and HiCrome ESBL screening were stored at 4^0C in pure culture slants. Bacterial isolates were preliminary identified based on cultural characteristics. Further identification of bacterial isolates was carried out on the basis of standard biochemical characteristics using indole, methyl red, Voges-Praukauer, citrate, urease, and sugar fermentation tests and growth on triple sugar iron agar (Sleigh and Duguid, 1989).

Table 1 Samples collected from different sources

Type of samples	Number of samples collected from different areas						Total
	Chhattisgarh					Other states	
	DURG	RJN	BSP	RPR	DMT		
Chevon	20	08	12	10	11	-	61
Milk	32	09	18	20	06	-	85
Cattle faeces	37	-	-	-	-	-	37
Sheep faeces	-	-	-	-	-	81	81
Poultry faeces	02	-	-	06	-	-	08
Clinical/Necropsy	12	-	-	-	-	-	12
Human urine	50	-	15	-	-	-	65
Human faeces	04	-	-	-	-	-	04
Water samples	58	11	13	-	-	46	128
Poultry flock	40	-	-	-	-	-	40
Total	**255**	**28**	**58**	**36**	**17**	**127**	**521**

RJN (Rajnandgaon), BSP (Bilaspur), RPR (Raipur), DMT (Dhamtari) are districts of Chhattisgarh.

***Detection of extended-spectrum beta-lactamases and carbapenemases*:** The double disc diffusion test (DDDT) and double disc synergy test (DDST) were carried out for phenotypic detection of ESBLs using amoxicillin (30 µg) and amoxicillin-clavulanate (30 µg), and ceftazidime (30 µg) and ceftazidime-clavulanate (30 µg) (CAC)/ ceftazidime-tazobactam (30 µg) (CAT) discs as per CLSI (2012). Similarly, carbapenemase (metallo-beta-lactamases; MBLs) production was assessed by imipenem (10 µg) + EDTA (20 µg) disc with imipenem (10 µg) disc alone as per Yong et al. (2009). Minimum inhibitory concentration (MIC) was determined using E-test MBL strips as per Patwardhan et al. (2013) with slight modification in which meropenem was used in place of imipenem. Moreover, Modified Hodge test (MHT) was performed to assess the production of carbapenemases by enterobacteria (Mathers et al., 2013). The indicator organism *Escherichia coli* ATCC 25922 and meropenem and imipenem discs were used to perform MHT.

Results

Morphology based distinct colonies were identified and each distinct colony was counted as a single isolate. One-to-five distinct colonies per sample were observed on Mac-Conkey agar plates and tested for antimicrobial resistance using AST. Similarly, the colonial morphology was also used to differentiate the bacterial isolates grown on HiCrome ESBL agar with appearance of one-to-four distinct colonies per sample on each plate. On HiCrome ESBL agar, each distinct colony was directly counted as resistant isolate.

Out of the 521 samples, 366 samples were screened on Mac-Conkey agar which yielded 614 Gram-negative bacterial isolates. Out of the 614 isolates, 404 isolates were resistant to at least one of the following antimicrobials: amoxycillin-clavulanate, ceftazidime, cefixime, meropenem or imipenem, using AST. Moreover, 155 samples were directly screened on HiCrome ESBL agar which yielded 211 resistant isolates after selective isolation. Overall, 825 (614+211) Gram-negative bacterial isolates were recovered from the 521 samples, out of which 615 were categorised as resistant isolates based on AST (404) and preliminary screening on HiCrome ESBL agar (211). Apart from this, *Pseudomonas* isolates (62) were also identified but not included for further study.

Among all isolates tested by AST, the resistance rate was highest to cefixime (49.3%) and least to imipenem (13%) (Table 2). Results showed that food samples yielded 95% resistant isolates compared to only 59% from other samples of animal origin (Table 3). However, the degree of resistance to all tested antimicrobials except imipenem was highest among the isolates recovered from human urine (Table 2).

Out of the total of 615 bacterial isolates (resistant to either of the targeted antimicrobial agents), 357 isolates could be revived on subculture and used for bacterial identification. The identification revealed the highest number for *E. coli* (54.6%) isolates followed by *Klebsiella* spp. (17%), *Citrobacter* spp. (7.5%), *Enterobacter* spp. (6.7%), *Acinetobacter* spp. (3%), *Serratia* spp. (2.8%), *Proteus* spp. (2.5%), *Shigella* spp. (2.5%), and other bacteria (3%) (Table 4).

Out of 210 isolates screened for phenotypic expression of ESBL using DDDT/DDST, 104 isolates (49.5%) exhibited resistance to either amoxicillin or amoxicillin-clavulanate or both, whereas 91 isolates (43.3%) were resistant to either ceftazidime or ceftazidime-clavulanate/ ceftazidime tazobactam or both. Sixty bacterial isolates (28.6%) developed zone of inhibition of ≥ 5 mm higher for amoxicillin-clavulanate than amoxicillin alone with (Fig. 1D) and without (Fig. 1B) synergy. Forty-seven (22.3%) bacterial isolates exhibited zone of inhibition of ≥ 5 mm higher for ceftazidime-tazobactam/ ceftazidime-clavulanate than ceftazidime alone with (Figs. 1B, 1C) and without (Fig. 1A) synergy (Table 5). Furthermore, few bacterial isolates (n=58) displayed zone of inhibition of < 5 mm higher or equal to or lower for amoxicillin-clavulanate than amoxicillin alone (Fig. 1C). Similarly, forty-six bacterial isolates developed zone of inhibition of < 5 mm higher or equal to or lower for ceftazidime-

tazobactam when compared with ceftazidime alone (Fig. 1D).

DDDT/DDST for MBL was applied on 160 isolates which manifested imipenem resistance among 31 isolates (19.3%). Out of the 31 imipenem resistant isolates, seven isolates (23.3%) showed zone of inhibition of ≥ 5 mm higher for imipenem-EDTA compared to imipenem alone.

Out of 30 randomly selected isolates (resistant to third generation cephalosporins and meropenem) screened by MBL double disc synergy E-test strips, five isolates demonstrated MIC of ≥ 8 times less for meropenem-EDTA compared to meropenem alone. Moreover, 15 (50%), 6 (20%), 3 (10%) and 6 (20%) isolates evidenced the MIC of 24 µg, 32 µg, 64 µg and > 256 µg, respectively, to meropenem.

Positive MHT (Fig. 2) was observed among five bacterial isolates when compared with carbapenemase producing reference stain (*Klebsiella pneumoniae* ATCC BAA-1705). All five isolates produced positive MHT with imipenem, but not with meropenem. All MHT producing isolates co-expressed ESBL confirmed by DDDT using amoxicillin with and without clavulanate.

Table 2 Antimicrobial resistance pattern of bacterial isolates

Sample type	Isolates tested (n)	Number of isolates resistant (% resistance) to different antimicrobials					
		AMC	CAZ	CFM	IPM	MRP	CIP
Milk	29	02 (6.9)	18 (62)	13 (44.8)	01 (3.4)	10 (34.4)	06 (20.6)
Sheep faecal	140	35 (25)	38 (27.1)	52 (37.1)	24 (17.1)	45 (32.1)	18 (12.8)
Cattle faecal	60	35 (58.3)	38 (63.3)	36 (60)	04 (6.6)	15 (25)	20 (33.3)
Poultry faecal	04	00	02 (50)	01 (25)	00	02 (50)	02 (50)
Clinical/ tissue (animals)	10	02 (20)	07 (70)	04 (40)	03 (30)	06 (60)	03 (30)
Human urine	112	66 (58.9)	82 (73.2)	90 (80.3)	14 (12.5)	76 (67.8)	62 (55.3)
Human faecal	09	02 (22.2)	04 (44.4)	02 (22.2)	01 (11.1)	02 (22.2)	01 (11.1)
Water	214	65 (30.4)	68 (31.8)	88 (41.1)	27 (12.6)	63 (29.4)	26 (12.1)
Poultry flock	36	10 (27.8)	19 (52.8)	17 (47.2)	06 (16.6)	15 (41.6)	07 (19.4)
Total	**614**	**217 (35.3)**	**276 (45)**	**303 (49.3)**	**80 (13)**	**234 (38.1)**	**145 (23.6)**

AMC = amoxicillin-clavulanate, CAZ = ceftazidime, CFM = cefixime, IPM = imipenem, MRP = meropenem, CIP = ciprofloxacin

Table 3 Antimicrobial resistant bacterial isolates recovered from different sources

Type of samples	Nature of samples	Basis of antimicrobial resistance	No. of samples tested	No. of Gram-negative isolates	No. of resistant isolates (%)
Food samples (animal origin)	Milk	AST	33	29	19
	Milk	ESBL agar	52	53	53
	Chevon	ESBL agar	61	115	115
	Total		**146**	**197**	**187 (95)**
Faecal samples (animal origin)	Cattle	AST	35	60	45
	Cattle	ESBL agar	02	03	03
	Sheep	AST	81	140	70
	Poultry	AST	02	04	02
	Poultry	ESBL agar	06	05	05
	Total		**126**	**212**	**125 (59)**
Clinical/ tissue samples (animals)	Tissue	AST	10	10	08
	Tissue	ESBL agar	02	01	01
	Total		**12**	**11**	**09 (82)**
Human samples	Urine	AST	65	112	94
	Faecal	AST	04	09	04
	Total		**69**	**121**	**98 (81)**
Environmental samples	Water	AST	96	214	132
	Water	ESBL agar	32	34	34
	Poultry flock	AST	40	36	30
	Total		**168**	**284**	**196 (69)**
	Net Total		**521**	**825**	**615 (75)**

Discussion

Among the majority of isolates, the antimicrobial resistance to beta-lactams (third generation cephalosporins) compared to beta-lactams with enzyme inhibitors (amoxicillin-clavulanate/ ceftazidime-clavulanate/ ceftazidime-tazobactam) indicated possible expression of ESBLs (Table 2) (CLSI, 2011; Patwardhan et al., 2013). Amoxicillin-clavulanate resistance among isolates might be due to their ability to express the inhibition resistant beta-lactamases. Ciprofloxacin (fluoroquinolone) resistance was an additional confounding factor to MDR and common among ESBL-producing bacteria (Kanayama et al., 2015).

Among all, food source carried maximum MDR bacterial threats with their possible spread to human community through food chain. Higher yield of resistant isolates from food sources might be due to animal faecal contamination of food animal products. Moreover, unhygienic handling and processing of food sources might have contributed to the bacterial load. Previous studies reported isolation of resistant enterobacteria from animals (Mandakini et al., 2015,

Samanta et al., 2015), milk and milk products (Sheikh et al., 2013), human urine (Khajuria et al., 2014; Singh et al., 2015), and environment (Srinivasan et al., 2015; Upadhyay and Joshi, 2015) in India. Indiscriminate use of antibiotics to maintain human and animal health and to increase livestock production might be associated with the emergence of MDR bacteria. The confluence of important factors such as poor public health infrastructure, rising incomes, high burden of disease, and unregulated sales of antibiotics has created suitable environment for rapidly emerging multidrug-resistance in India (Laxminnarayan and Chaudhury, 2016). Isolation of resistant enterobacteria (69%) from the environmental samples indicated possible release of bacteria from animal and human sources to the environment. These bacteria may be the further sources that infect human as well as animal population and are maintained due to any kind of interaction between them.

Table 4 Identification of resistant bacteria recovered from different sources
Numbers in parenthesis indicate % cultural recovery of resistant bacteria.

Sample sources	Isolates tested	Identified bacterial isolates (%)								
		E. coli	*Klebsiella* spp.	*Citrobacter* spp.	*Enterobacter* spp.	*Acinetobacter* spp.	*Serratia* spp.	*Proteus* spp.	*Shigella* spp.	Other bacteria
Cattle faeces	48	35 (72.9)	10 (20.8)	01 (2.1)	01 (2.1)	00	00	01 (2.1)	00	00
Sheep faeces	47	25 (53.1)	07 (14.9)	01 (2.1)	07 (14.9)	01 (2.1)	01 (2.1)	01 (2.1)	03 (6.4)	01 (2.1)
Poultry faeces	05	05 (100)	00	00	00	00	00	00	00	00
Chevon	57	29 (50.8)	14 (24.5)	08 (14.0)	00	02 (3.5)	00	01 (1.8)	00	03 (5.2)
Milk	41	26 (63.4)	05 (12.2)	04 (9.8)	01 (2.4)	02 (4.8)	01 (2.4)	01 (2.4)	00	01 (2.4)
Clinical /necropsy	06	03 (50.0)	01(16.6)	00	00	00	00	01(16.6)	00	01(16.6)
Human urine	63	34 (54)	10 (15.8)	07 (11.1)	06 (9.5)	00	00	03 (4.7)	03 (4.7)	00
Human faeces	03	01 (33.3)	00	00	01 (33.3)	00	00	00	00	01 (33.3)
Water	71	35 (49.3)	12 (16.9)	06 (8.5)	06 (8.5)	05 (7)	01 (1.4)	01 (1.4)	02 (2.8)	03 (4.2)
Poultry flock samples	16	02 (12.5)	02 (12.5)	00	02 (12.5)	01 (6.25)	07 (43.8)	00	01 (6.25)	01 (6.25)
Total	**357**	**195 (54.6)**	**61 (17.0)**	**27 (7.5)**	**24 (6.7)**	**11 (3.0)**	**10 (2.8)**	**09 (2.5)**	**09 (2.5)**	**11 (3.0)**

Table 5 Phenotypic detection of beta-lactamases

Type of beta-lactamases	Method/ assay	Antimicrobial used	No. of isolates tested	No. of positive isolates (%)
ESBLs	Double disc diffusion	AMX, AMX-AMC	210	60 (28.6)
		CAZ, CAZ-CAT/CAC	210	47 (22.3)
Carbapenemases	MBL-double disc diffusion	IPM, IPM-EDTA	31/160	07 (22.5)
	MBL E-test	MRP, MRP-EDTA	30	05 (16.6)
	MHT	IMP	30	05 (16.6)

DDDT/DDST indicated phenotypic expression of ESBL by bacterial isolates (CLSI, 2011; CLSI, 2012). Several studies applied DDDT/DDST for phenotypic confirmation of ESBL among enterobacteria (Patwardhan et al., 2013; Sharma et al., 2013).

For carbapenemases, the present findings are in accord with those of Datta et al. (2012), who recorded carbapenemase production in *K. pneumoniae* and *E. coli* isolates using imepenem-EDTA combined disc test. Other researchers have the opinion that phenotypic detection of combined mechanisms of resistance such as MBL or KPC expression in ESBL-expressing isolates is important for epidemiological purposes and for implementing rapid and specific infection control measures (Birgy et al., 2012; Patwardhan et al., 2013). Higher MIC of meropenem compared to meropenem-EDTA indicated expression of MBL (carbapenemase) by the bacterial isolates (Patwardhan et al., 2013).

The findings of MHT accord with those of Galani et al. (2008) and Mathers et al. (2013), however, the results of MHT using imipenem in place of meropenem was contrary to those of CLSI (2011). The co-expression of ESBL among MHT producing isolates agreed with that of Du et al. (2014). The negative MHT by carbapenem-resistant isolates might be due to low expression of carbapenemases or due to expression of MBL by bacterial isolates (Castanheira et al., 2011; Birgy et al., 2012). However, MHT based on *in vivo* production of carbapenemase has been suggested to

detect carbapenemase producers (CLSI, 2011; Patwardhan et al., 2013; Du et al., 2014).

The present study highlights the occurrence of beta-lactamase harbouring *Enterobacteriaceae* bacteria from animals, humans and environment. *Enterobacteriaceae* bacteria restrict therapeutic alternatives not only due to ESBLs and carbapenemases, but also because of the linkage with non-beta-lactam resistance. Therefore, it is important to check constantly the emergence of resistance among Gram-negative organisms. Hence, early detection of MDR using phenotypic methods is essential to limit the spread of infection due to these organisms. Although the sample size and design of the present study lack uniformity due to the inability to establish appropriate correlation between the animals, humans and environment, this study will still be valuable to clinicians dealing with rapid diagnosis and treatment of resistant pathogens. Moreover, it visualizes the importance for the need for planning and execution of effective control programs to emerging antimicrobial resistant pathogens. This study may provide substantial information for more reliable molecular investigations which is usually used only by reference laboratories.

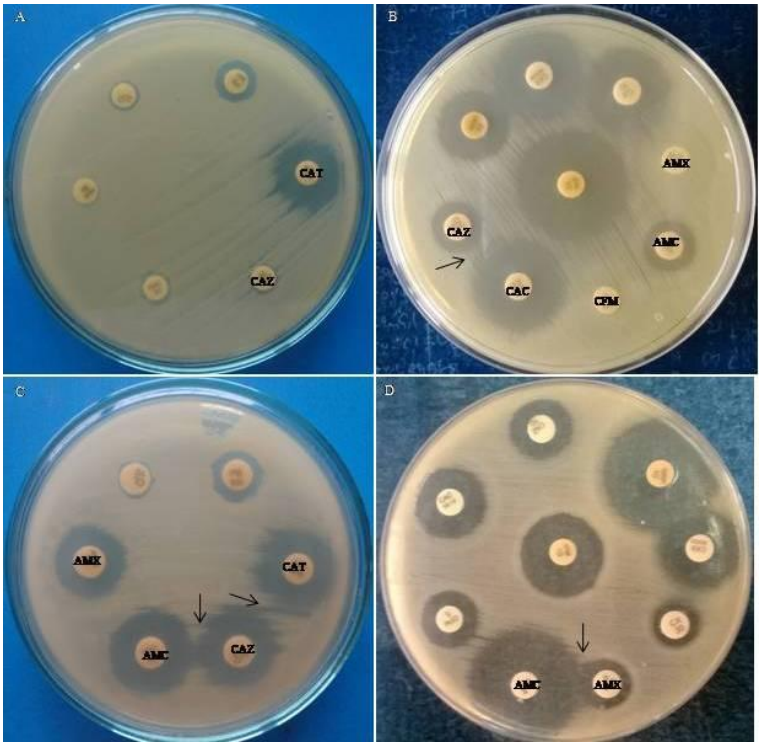

Figure 1 **(A-D):** Bacterial isolates showing DDDT with CAZ and CAT (A), AMX and AMC, CFM and CAC (B). Arrow directions showing DDST with CAZ and CAC (B), CAZ and AMC, CAZ and CAT (C), AMX and AMC (D).

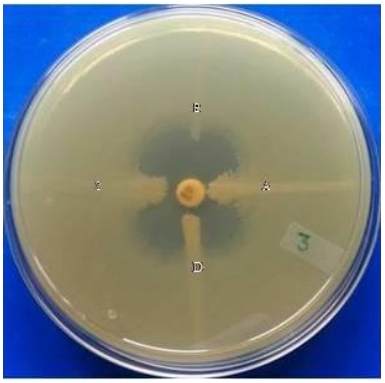

Figure 2 MHT showing carbapenemase production by bacterial isolate. A, KPC positive control; C, Test positive (KPC producer); D, Weak MHT (MBL producer); B, Test negative.

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บทคัดย่อ

การแยกเชื้อแบคทีเรียที่สร้าง extended-spectrum β-lactamases และ/หรือ carbapenemases ในสัตว์ มนุษย์ และสิ่งแวดล้อม ในประเทศอินเดีย

ฟาเตห์ ซิงห์[1*] หิปรู์คาร์[1] สัญชัย ศักขา[2] นิธิ ราวัต[1] ประชาติ เทวาการ[2]
โฟสิยา ฟาซิน ข่าน[1] สุนี กุมาร ปานเลขา[3]

วัตถุประสงค์ของการศึกษานี้ เป็นการแยกเชื้อแบคทีเรียที่ดื้อต่อยาต้านจุลชีพในสัตว์ มนุษย์ และสิ่งแวดล้อม และอธิบายลักษณะของเชื้อแบคทีเรียดังกล่าวตามลักษณะฟีโนไทป์ โดยศึกษาในตัวอย่างจำนวน 521 ตัวอย่าง แบ่งเป็น ตัวอย่างจากสัตว์จำนวน 284 ตัวอย่าง มนุษย์จำนวน 69 ตัวอย่าง และสิ่งแวดล้อมจำนวน 168 ตัวอย่าง พบว่าสามารถแยกเชื้อแบคทีเรียได้จำนวน 615 ตัวอย่างที่ดื้อต่อยาต้านจุลชีพที่ไม่ซ้ำกัน (amoxicillin-clavulanate, ceftazidime, cefixime, meropenem หรือ imipenem อย่างใดอย่างหนึ่ง) ผลการทดสอบความไวต่อยาต้านจุลชีพ (AST) พบอัตราการดื้อยาสูงสุดต่อ cefixime (49.3%) ตามด้วย ceftazidime (45%), meropenem (38.1%), amoxicillin-clavulanate (35.3%) และ imipenem (13%) และเมื่อจำแนกตามชนิดเชื้อแบคทีเรีย ตรวจพบเชื้อ *Escherichia coli* (54.6%), *Klebsiella* spp. (17%), *Citrobacter* spp. (7.5%), *Enterobacter* spp. (6.7%), *Acinetobacter* spp. (3.0%), *Serratia* spp. (2.8%), *Proteus* spp. (2.5%), *Shigella* spp. (2.5%) และแบคทีเรียอื่น ๆ (3%) และพบเชื้อแบคทีเรียที่มี extended-spectrum B-lactamases (ESBLs) เมื่อตรวจด้วยวิธี double disc diffusion test (DDDT) และ double disc synergy test (DDST) โดยใช้ amoxicillin และ amoxicillin-clavulanate จำนวน 60 ตัวอย่าง (28.6%) และ ceftazidime และ ceftazidime-clavulanate / ceftazidime-tazobactam จำนวน 47 ตัวอย่าง (22.3%) นอกจากนี้ พบเชื้อแบคทีเรียที่มี carbapenemase เมื่อตรวจด้วยวิธี imipenem-EDTA metallo-beta-lactamases (MBLs) DDDT / DDST จำนวน 30 ตัวอย่าง (22.5%) โดยพบว่าจากเชื้อ 30 ตัวอย่างที่ดื้อต่อยา carbapenem พบเชื้อจำนวน 5 ตัวอย่าง (16.6%) ให้ผลบวกต่อ MBL E-test และเชื้อแบคทีเรียจำนวน 5 ตัวอย่าง (16.6%) ให้ผลบวกต่อ modified Hodge test โดยสรุปการศึกษาครั้งนี้พิสูจน์ให้เห็นถึงการเกิดเชื้อแบคทีเรียดื้อยาหลายชนิดพร้อมกัน (MDR) อย่างกว้างขวางในสัตว์ มนุษย์ และสิ่งแวดล้อม การตรวจพบฟีโนไทป์ของเชื้อแบคทีเรียที่มี ESBLs และ carbapenemases ทำให้การศึกษาพันธุกรรมของเชื้อดื้อยามีความสำคัญมากขึ้น และส่งผลให้การวินิจฉัยโรคที่เกิดจากเชื้อแบคทีเรียดื้อยาหลายตัวทำได้อย่างรวดเร็ว

คำสำคัญ: ความต้านทานต่อยาต้านจุลชีพ carbapenemases Enterobacteriaceae extended-spectrum B-lactamases

[1]ภาควิชาจุลชีววิทยา วิทยาลัยสัตวแพทยศาสตร์และสัตวบาล แอนโจรา เดิกจ์ รัฐฉัตติสครห์ ประเทศอินเดีย
[2]ภาควิชาสัตวแพทยสาธารณสุขและระบาดวิทยา วิทยาลัยสัตวแพทยศาสตร์และสัตวบาล แอนโจรา เดิกจ์ รัฐฉัตติสครห์ ประเทศอินเดีย
[3]ภาควิชาฟาร์มปศุสัตว์ วิทยาลัยสัตวแพทยศาสตร์และสัตวบาล แอนโจรา เดิกจ์ รัฐฉัตติสครห์ ประเทศอินเดีย
***ผู้รับผิดชอบบทความ** E-mail: fateh.ars07@gmail.com*