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The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2021; SP-10(10): 618-625

www.thepharmajournal.com
Received: 09-08-2021
Accepted: 10-09-2021

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Detection of *β-lactamase* genes among *enterobacteriaceae* members from local vended ice creams sold at different parts of Andhra Pradesh

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Abstract
Microorganisms gain access into milk and milk products either through contaminated water supply or during unhygienic production, processing and handling. *Enterobacteriaceae* are frequently encountered in dairy products including milk, cheese and ice cream. The members of *Enterobacteriaceae* are generally considered as harmless commensals and opportunistic pathogens. However, several recent reports have documented that acquiring the antimicrobial resistance markers made them major concern today. Therefore, the study was conducted to evaluate the microbiological quality of street-vended ice creams sold in different areas of Andhra Pradesh. Ninety-five street vended/ local made ice cream samples were randomly collected and analysed for bacterial contamination. The results revealed *Escherichia coli* (40), *Klebsiella pneumonia* (34), *Klebsiella oxytoca* (7) and *Proteus mirabilis* (14) by PCR method. None of the samples tested positive for *Salmonella* spp. All these isolates were tested for production of different *β*-lactamase genes responsible for antimicrobial resistance by both phenotypic and genotypic methods. Out of 40 isolates of *E. coli*, five showed *bla* $_{TEM}$, one *bla* $_{SHV,}$ one *bla* $_{CTXM}$ group 9, 2 *bla* $_{ACC}$ and 1 isolate showed *bla* $_{KPC}$ gene. Out of 41 isolates of *Klebsiella* (34 *K. pneumonia* and 7 *K. oxytoca*), 23 isolates showed *bla* $_{shv}$ genes. Out of 14 isolates of *Proteus mirabilis*, three isolates showed *bla* $_{TEM}$, one isolate showed *bla*$_{OXA}$, one *bla* $_{CTX-M}$ group-1, one showed *bla* $_{CTX-M}$ group-9, one *bla* $_{FOX}$ and one *bla* $_{ACC}$ gene. It is clear from the current studies that there is a necessity for improving the hygienic status of locally produced ice cream.

Keywords: *Enterobacteriaceae*, antimicrobial resistance, *β*-lactamase genes

Introduction
Ice cream is a scrumptious dairy product commonly consumed by all age groups during all seasons with more access during summer. Due to its nutrient richness that favours the microbial invasion, it can harbour many potent pathogens. Most of the ice creams get contaminated with microorganisms during production, transportation and preservation. Children, elders and immunocompromised patients are prone to the foodborne infections and the contaminated food products can be majorly responsible for infections (HS, 2017) [16].
Enterobacteriaceae are Gram-negative, rod-shaped facultative anaerobes. They able to cause a wide range of illness starting from mild illness to fatal meningitis. Though some are true pathogens, most of them are regarded as opportunistic pathogens (Davin-Regli *et al.*, 2019) [10]. Due to their ubiquitous and widespread distribution, they can easily access the food chain. The family includes a number of important foodborne pathogens such as *Salmonella*; pathogenic *Escherichia coli*, including *Escherichia coli* O157:H7; *Klebsiella* and *Proteus* spp. In the food industry, *Enterobacteriaceae* are commonly used as indicator organisms for poor hygienic practices or improper/poor manufacturing process (Bintsis, 2017) [3].
Antibiotics are widely used to protect the health of humans and animals or as food additive to increase the growth rate of animals. Apart from chemical pollution caused by antibiotics themselves, the improper usage of antibiotics may also accelerate the development of antibiotic resistance genes (ARGs) in bacteria, which cause health risks to humans and animals. These bacteria can be transmitted from environment to human via direct or indirect contact. Of late, Extended spectrum *β*-lactamases among the *Enterobacteriaceae* members are the major concern as they help the bacteria to survive against the *β*-lactam drugs. *β*-lactamases pose a major problem in clinical therapeutics, as infections caused by the organisms are

associated with a higher morbidity and mortality (Rupp and Fey, 2003) [21].

In developed countries, food safety and quality control measures are followed very sensibly to improve the shelf life of milk products with minimal bacterial contamination from raw ingredients to final product to curb public health issues. In the countries like India, adoption of quality control measures may be flawed due to lager economical differences, poverty, lack of public awareness and education. In the recent years, there has been an increased incidence and prevalence of *β-lactamases* in animal derived food products all over the world and in various parts of India. Therefore, the study was planned to assess the microbiological quality (especially *β-lactamases* in foodborne pathogens) of street-vended ice creams sold in different places of Andhra Pradesh.

The present work carry out to-

- To isolate and identify *Enterobacteriaceae* species in street vended ice cream samples.
- To detect the presence of *β*-lactamases among the identified *Enterobacteriaceae* isolates by both phenotypic and genotypic methods.
- Extended spectrum *β-lactamases* detection, including detection of (Extended spectrum *β-lactamases*), *Amp C*-type *β-lactamases* and *carbapenamases* in *Enterobacteriaceae* isolates.

2. Material and methods

2.1 Standard control and primers

ATCC (American Type Culture Collection) culture of *Proteus mirabilis* (ATCC 12453), *Salmonella typhimurium* standard culture (ATCC 14028) and *Klebsiella pneumonia* (ATCC 700603) were used as standard controls. Oligonucleotide primers were custom synthesized from M/s. Bioserve Biotechnologies Pvt. Ltd. (Hyderabad).

2.2 Sample collection and processing

The samples comprised of street-vended ice creams sold in different areas of Andhra Pradesh. Ninety-five street vended/ local made ice cream samples were randomly collected and analysed for bacterial contamination. The samples were processed at the Department of Veterinary Public Health and Epidemiology, NTR College of Veterinary Science, Gannavaram. All *Enterobacteriaceae* isolates were confirmed to species level as per standard protocol.

The samples were homogenized (10 g) with 90 ml of Tryptic Soya Broth and incubated for 24 h at 37ºC. The enriched cultures were streaked on to MacConkey Agar (MCA) and the plates were incubated for 24 h at 37ºC. For isolation of *Salmonella* spp., first step enrichment achieved by inoculating 1ml of the sample from the transport media into 9 ml of buffered peptone water (BPW) and incubate at 37°C for 18 h. Later for selective enrichment, 0.1 ml of the pre-enriched inoculum was transferred to 10 ml of Rappaport-Vassiliadis broth (RV broth) and incubated at 42°C for 24 h. After enrichment, a loop full (10 µl) of inoculum was streaked on to Xylose Lysine Desoxycholate (XLD) agar (Hi Media) and incubated at 37°C for 24 h. Presumptive *Salmonella* colonies (4-5 colonies/plate) appearing slightly transparent red halo with a black centre surrounded by a pink-red zone on XLD agar were screened further for its biochemical characterization.

2.3 DNA extraction from enriched broth samples

DNA was extracted from all the *Enterobacteriaceae* isolates by using boiling and snap chilling (Bobbadi *et al.,* 2020) [4] method. 1.5 ml of enriched broth was taken into micro centrifuge tubes and centrifuged at 8000 rpm for 10 min. Supernatant from microcentrifuge was discarded and 50 µl of nuclease free water. Bacterial pellet with nuclease free water was placed in boiling water bath for 10 min 100 ^{0}C. Immediately subjected to dry ice temperature (snap chilled) for 10 min and centrifuged at 10,000 rpm for 5 min. The supernatant was taken as template and was subjected to different m-PCR assays for genus and species level confirmation.

2.4 Molecular Confirmation of *Enterobacteriaceae* members by m-PCR

Molecular confirmation of presumptive colonies was done by targeting the genus and species-specific genes of *E. coli* (Sun *et al.,* 2011) [23], *Klebsiella* spp. (Brisse & Verhoef, 2001; Chander *et al.,* 2011 and Kovtunovych *et al.,* 2003) [5, 6, 17], *Salmonella* spp. (Soumet *et al.,* 1999) [22], *Proteus mirabilis* (Zhang *et al.,* 2013) [26]. PCR assays were optimized in 25 µL reaction mixture containing 2 µL of DNA, 12.5 µL of 2X master mix (GoTaq Promega Green Master Mix), 0.5 µL quantity of both forward and reverse primers (10 pmol/µL) and the rest of the volume is make up with nuclease free water. The cycling conditions and primer specifications are mentioned in the Table-I. PCR products were subjected to gel electrophoresis using 1% agarose with ethidium bromide as fluorescent dye (Sambrook & Russell, 2001) and visualized using Gel Documentation unit (BIORAD).

2.5 Phenotypic detection of *β*-lactamase production

The screening and confirmation for *β-lactamases* production was done as per Clinical and Laboratory Standards Institute (CLSI) guidelines 2014. Resistance to at least one of the four antibiotics used was considered as positive screening test for possible ESBL production as per CLSI (2014) guidelines. The screen positive isolates were confirmed by the double disc synergy test (DDST) and phenotypic confirmatory disc diffusion test (PCDDT), Amp C detection Ezy MICTM Strip and imipenem (IPM)/imipenem-EDTA Etest strips. E-test was performed according to the recommendations of the manufacturer (Hi media). A reduction in the MIC of imipenem of three or more 2-fold dilutions in the presence of EDTA was interpreted as a positive test indicative of MBL production (Walsh *et al.,* 2002) [25].

2.6 Identification of different types of *β*-lactamase genes by m-PCR

Molecular characterization of the *β-lactamases* producing strains were done by polymerase chain reaction (PCR) specific for Extended spectrum *β*-lactamase genes, *Amp C* - type *β-lactamases* and *carbapenamases* (Dallenne *et al.,* 2010) [9]. DNA obtained from all the phenotypic confirmatory positive isolates were subjected to four m-PCR assays optimized in 25 µl reaction mixture, containing 2 µl of DNA template, 12.5 µl of 2x master mix (Go Taq Green Master Mix, Promega), 0.5µl each of forward and reverse primers (10 pmol/µl) and the rest of the volume is made by adding nuclease free water. The *β*-lactamase Group-specific primers used for the assays and specifications mentioned in Table-II. The m-PCR I, II & III were subjected under standardized cycling conditions: initial denaturation at 94 ^{0}C for 10 min; 30 cycles of 94 ^{0}C for 40 s, 60 ^{0}C for 40 s and 72 ^{0}C for 1 min and a final elongation step at 72 ^{0}C for 7 min.

For the detection of carbapenemase genes, multiplex PCR assay subjected under standardized cycling conditions: initial denaturation at 94 ^{0}C for 10 min; 30 cycles of 94 ^{0}C for 40 s, 55 ^{0}C for 40 s and 72 ^{0}C for 1 min and a final elongation step at 72 ^{0}C for 7 min.

3. Results and Discussion

In the present study, a total of 95 street vended Ice cream samples collected from different parts of Andhra Pradesh were analysed for isolation and identification of Enterobacteriaceae members. Out of 95 Ice cream samples, 95 isolates were positive for *Enterobacteriaceae* members (40 *Escherichia coli* (42.1%), 34 *Klebsiella pneumonia* (35.78%), 7 *Klebsiella oxytoca* (7.36%) and 14 *Proteus mirabilis* (14.73%)) both by cultural and confirmatory PCR assays (showed in Fig-1to 4).

E. coli is generally considered as indicator for faecal contamination and potential presence of other enteric pathogens. *E. coli* was found in 40 (42.1%) ice cream samples, which is in accordance with the HS (2017) [16] 48.96% incidence. On the other hand, Abou-El Khair *et al*. (2014) [1] reported a higher incidence of *E. coli* (96%) recovered from packaged Ice-Cream samples in Gaza City. It was noticed that the prevalence of *Proteus* and *Klebsiella* species of the present study was almost similar to the results of Gaza (2013) showing 37% of *Klebsiella* spp. whereas Badr (2018) reported a lower prevalence of *Proteus* (12%) and *Klebsiella* (28%) recovered from the ice cream samples in Hyderabad.

In current study, *Salmonella* could not be detected in the Ice cream samples. This is in agreement with Fadel and Ismail (2009) [13]. On the contrary, Omar *et al*, (2018) [20], detected *Salmonella* spp. in 56% of ice cream samples (14/25).

These results indicated that the local vended ice creams in present study are of poor microbiological quality. As ice cream is a rich source of nutrients, it can act as medium for luxurious multiplication of microorganisms. Though the bacteria isolated in the present study are mesophilic, they can also undergo dormant stage in refrigerated temperatures for ice cream storage. *E. coli*, *Klebsiella* spp. and *Proteus mirabilis* are susceptible to pasteurization but poor heat treatment and post pasteurization contamination made them alive. The difference between results from current study and the previous studies may be attributed to sampling techniques, sources of samples (home or factory-made), handling of samples and types of media used.

All these isolates were tested for production of different *β*-lactamase genes responsible for antimicrobial resistance by both phenotypic and genotypic methods (Table-III and IV). In the ESBL phenotypic screening and confirmation test, involving detection of resistance against four cephalosporin antibiotics (Cefotaxime, Ceftazidime, ceftriaxone and aztreonam), zone size expanded by a minimum of 5 mm in presence of *β*-lactamase inhibitors as defined by CLSI (2014) [8] guidelines. The present study findings were in accordance with earlier studies of *β*-lactamase antimicrobial resistance in *Enterobacteriaceae* family, where ESBL production was confirmed in *Enterobacteriaceae* isolates using combination discs of *β*-lactam antibiotics and *β*-lactamase inhibitors (Garrec *et al.*, 2011) [14].

Among the 95 *Enterobacteriaceae* isolates, 54 isolates (16 *E. coli*, 26 *K. pneumonia*, 4 *K. oxytoca* and 8 *P. mirabilis*) were phenotypically confirmed positive for beta lactamase production (Fig-5). Out of 54 ESBL producing *Enterobacteriaceae* isolates, 36 isolates (7 *E. coli* (19.44%), *19 K. pneumonia* (52.77%), 4 *K. oxytoca* (11.11%) and 6 *P. mirabilis* (16.66%) were genotypically positive as shown in the Fig-7 and 8. The findings of the present study are contradictory to the findings of Odenthal *et al*. (2016) [19] who reported major ESBL-producing species was *Escherichia coli* (75.6%), followed by *Citrobacter* spp. (9.6%), *Enterobacter cloacae* (6.1%), and *Klebsiella oxytoca* (3.7%).

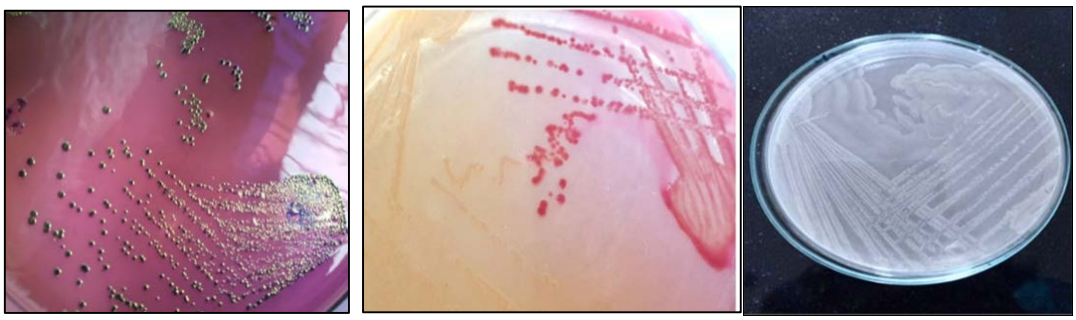
The present study findings are in accordance with Gundogan and Avci (2013) [15], who reported 20% prevalence for *E. coli* whereas, a lower prevalence for *K. pneumonia* with 6.7% and high prevalence for *K. oxytoca* with 86.7% from ice cream samples collected in turkey. In contrary to the present study, Tekiner and Özpınar (2016) [24] reported not to have recovered ESBL positive *Enterobacteriacae* members from milk and meat products.

Out of 40 isolates of *E. coli*, 5 showed *bla* TEM (12.5%), one *bla* SHV (2.5%) and one *bla* CTXM group 9 (2.5%). Out of the 34 isolates of *Klebsiella* obtained (27 *K. pneumonia* and 7 *K. oxytoca*), 23 isolates showed *bla* SHV (55.88% *K. pneumonia* and 57.14% of *K. oxytoca*). Out of 14 isolates of *Proteus mirabilis*, 3 isolates showed *bla* TEM (42.86%), *bla* OXA, *bla* CTX-M group1 and *bla* CTX-M group 9 gene was observed in one isolate each (14.29%).

The phenotypic AmpC betalactamase and Metallo betalacatamases detection was performed by using AmpC detection Ezy MICTM Strip and imipenem (IPM)/imipenem-EDTA Etest strips, respectively (Fig-6). Out of 95 isolates obtained, 17 of the isolates (5 *E.coli* (12.5%), 8 *K. pneumonia* (23.53%), 3 *P. mirabilis* (21.43%) and one *K. oxytoca* (14.29%)) were phenotypically positive for AmpC betalactamase and 4 isolates (2 *E. coli* and 2 *P. mirabilis*) further confirmed by PCR (Fig-8). However, one *E. coli* isolate (1.05%) showed phenotypically positive test result and was further confirmed by PCR (Fig-9).

Of 14 isolates of *Proteus* mirabilis, *bla* FOX and one *bla* ACC gene showed by one isolate each (14.29%). In contrary to the present study, chinnam *et al*., (2021) [7] reported none of the isolates were carrying *bla* ACC, *bla* MOX and carbapenamase genes (*bla* VIM , *bla* IMP, *bla* KPC and *bla* NDM-1) from the 1093 different samples obtained from foods of animal origin and intestinal samples.

Our results showed that one thirds of *Enterobacteriaceae* isolates recovered from ice cream samples were ESBL positive however, 4.2% and 1.05% of isolates were positive for Amp C betalactamses and carbapenamases, respectively. To the best of our knowledge, this is the first study conducted to determine the prevalence on different types of *β*-lactamases from the street vended ice creams. The detection of *β*-lactamase and carbapenem resistant isolates harbouring the resistant genes raises serious public health concerns since carbapenems are considered the first resort drugs for the treatment of serious infections due to ESBL possessing bacteria (Livermore and Hawkey, 2005) [18].



EMB agar (*E. coli*) *Proteus mirabilis* nutrient EMB agar (*E. coli*)

Fig 1: Phenotypic detection of different *Enterobacteriaceae* members

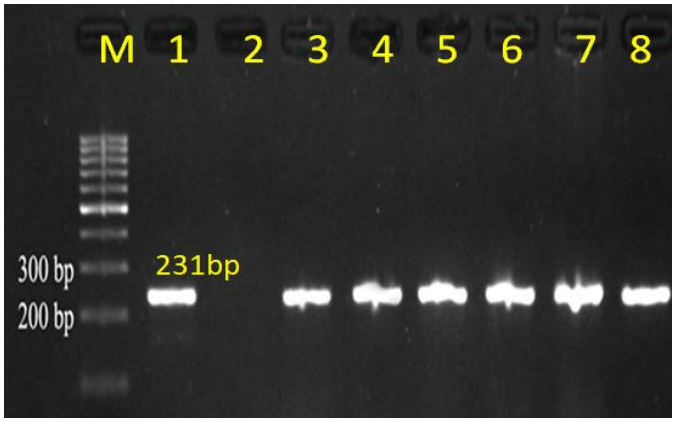


Fig 2: Gel photograph of *E.coli* species-specific PCR Lane M DNA ladder (100bp) Lane 1 Amplified DNA (231bp) Lane 2 Negative control Lane 3-8 Amplified DNA of *E.coli*

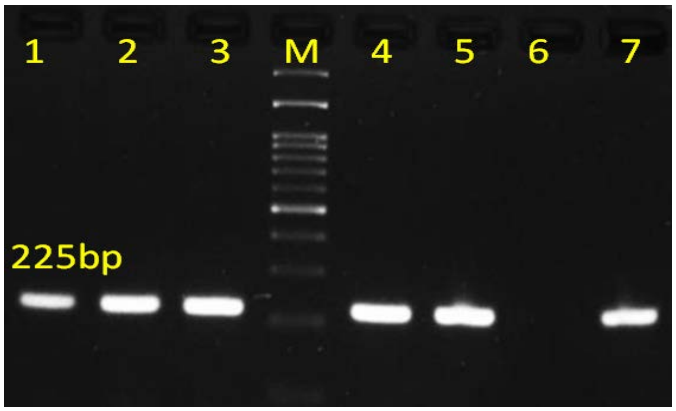


Fig 3: Gel photograph of *ureR* specific PCR for *P. mirabilis* Lane 1-5 Amplified DNA of P. mirabilis M DNA ladder (100bp) Lane 6 Negative control Lane 7 Positive control of *P. mirabilis*

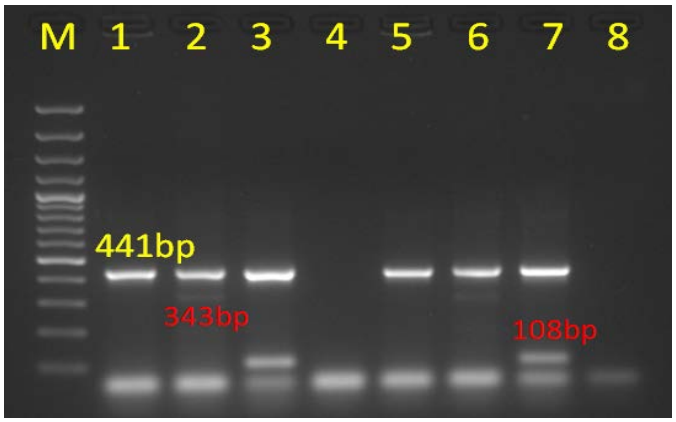


Fig 4: Gel photograph of *Klebsiella* genus and species species-specific PCR Lane M DNA ladder (100bp) Lane 1 Amplified DNA *Klebsiella* genus standard (441bp) Lane 2 Amplified DNA *Klebsiella. oxytoca* standard (343bp) Lane 3 Amplified DNA of *K. pneumoniae* (108) Lane 4 Negative control Lane 5, 6&7 Amplified DNA positive for genus, *K. oxytoca* and *K. pneumonia*

Phenotypic screening test for ESBL Phenotypic confirmatory tests (PCTs) for ESBL production

Fig-5: Phenotypic detection of different Extended spectrum *β*-lactamses in *Enterobacteriaceae* members

Fig 6: Phenotypic detection of ESBL production (AmpC β-lactamases and Metallo β-lactamases) A. Ezy MIC strip- Cefotaxime with and without clavulanic acid B. Ezy MIC impenem with and without EDTA strip for MBL detection

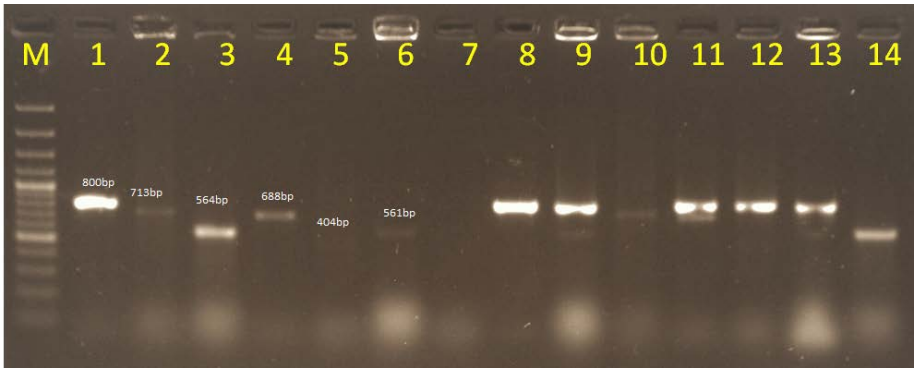


Fig 7: Gel photograph of m- PCR targeting *blaTEM*, *blaOXA*, *blaSHV*, *blaCTX-M* group 1, group 2 and group 9 genes in ESBL-producing *Enterobactereacae members* Lane M Molecular weight marker (100bp) Lane 1 Known DNA standard for *blaTEM* (800 bp) Lane 2 Known DNA standard for *blaSHV* (713 bp) Lane 3 Known DNA standard for *blaOXA* (564 bp) Lane 4 Known DNA standard for *blaCTX-M* group 1gene (688bp) Lane 5 Known DNA standard for *blaCTX-M* group 2 gene (404bp) Lane 6 Known DNA standard for *blaCTX-M* group 9 gene (561bp) Lane 8 *E. coli* isolate carrying *blaTEM* Lane 9 *E. coli* isolate carrying *blaTEM* and *blaCTX-M* group 9 gene Lane 10 *K. oxytoca* isolate carrying *blaSHV* gene Lane 11 *P. mirabilis* isolate carrying *blaTEM* & *blaSHV* gene Lane 12 *P. mirabilis* isolate carrying *bla TEM* gene *blaCTX-M* group 2 gene Lane 13 *K. pneumoniae* isolate carrying *blaTEM* & *bla CTX-M* group 9 gene Lane 14 *P. mirabilis* isolate carrying *blaOXA* gene

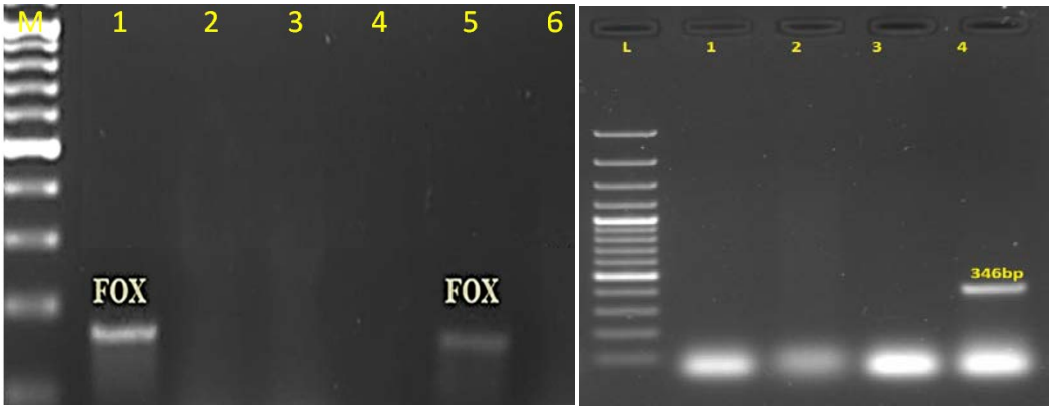


Fig 8: Photographs of PCR targeting *bla*AmpC genes Lane M Molecular weight marker (100bp) Lane 1&5 (gel-1) DNA standard for *bla*AmpC gene (FOX) (190) Lane 5 (gel-2) P. mirabilis isolate carrying *blaAmp*C gene (ACC) (346 bp)

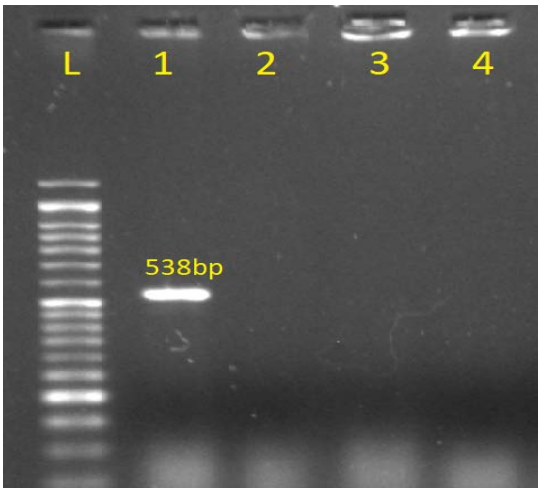


Fig 9: Gel photograph of PCR targeting *bla* KPC gene Lane M Molecular weight marker (50bp) Lane 5 DNA amplified positive for *bla*KPC gene (KPC) (538)

Table I: *Enterobacteriaceae* genus and species-specific PCR primers sequences and PCR conditions

Gene target and Primer Amplicon size	Primer sequence	PCR conditions
Uniplex PCR for *Proteus mirabilis* ((Zhang *et al.*, 2013) [26])		
UreR gene (225bp)	GGTGAGATTTGTATTAATGG	Initial denaturation at 94°C for 4 min; 30 cycles of 94°C for 40 s, 58°C for 1 min and 72°C for 20 sec and a final elongation step at 72°C for 10 min
	ATAATCTGGAAGATGACGAG	
m-PCR for *salmonella* genus and Species ((Soumet *et al.*, 1999) [22])		
Genus *Salmonella* (429bp)	GCCAACCATTGCTAAATTGGCGCA	Initial denaturation at 950 for 5 min; 35 cycles of 94^{0}C for 30 s, 56^{0}C for 90 s and 72^{0}C for 30 sec and a final elongation step at 72^{0}C for 10 min
	GGTAGAAATTCCCAGCGGGTACTGG	
S. Typhimurium fliC (559bp)	CGGTGTTGCCCAGGTTGGTAAT	
	ACTCTTGCTGGCGGTGCGACTT	
S. Enteritidis sefA (312)	AGGTTCAGGCAGCGGTTACT	
	GGGACATTTAGCGTTTCTTG	
m-PCR conditions for *Klebsiella* genus and Species (Brisse and Verhoef, 2001; Chander *et al.*, 2011 and Kovtunovych *et al.*, 2003) [5, 6, 17]		
rpo B (108) *K. pneumoniae*	CAACGGTGTGGTTACTGACG	Initial denaturation of 95^{0}C for 5 min: 35 cycles of final denaturation at 95 ^{0}C for 1 min, annealing at 55^{0}C for 1 min and initial extension at 72^{0}C for 2 min followed by final extension at 72^{0}C for 10 min
	TCTACGAAGTGGCCGTTTTC	
peh X (343) *K. oxytoca*	GATACGGAGTATGCCTTTACGGTG	
	TAGCCTTTATCAAGCGGATACTGG	
gyr A (441) *Klebsiella* genus	CGCGTACTATACGCCATGAACGTA	
	ACCGTTGATCACTTCGGTCAGG	
Uniplex PCR for *E. coli* (Sun *et al.*, 2011) [23]		
E16S (231bp)	ATCAACCGAGATTCCCCCAGT	Initial denaturation of 95^{0}C for 5 min: 35 cycles of final denaturation at 95^{0}C for 1 min, annealing at 50^{0}C for 1 min and initial extension at 72^{0}C for 1 min followed by final extension at 72^{0}C for 10 min
	TCACTATCGGTCAGTCAGGAG	

Table II: *β*-lactamase Group-specific primers used for the assays (Dallenne *et al.*, 2010) [9]

PCR Name	*β*-Lactamase genes targeted	Primer sequence	Amplicon size
mPCR-I (extended-spectrum *β*-lactamases)			
TEM	TEM-1 & 2	CATTTCCGTGTCGCCCTTATTC CGTTCATCCATAGTTGCCTGAC	800
SHV	SHV-1	AGCCGCTTGAGCAAATTAAAC ATCCCGCAGATAAATCACCAC	713
OXA	OXA-1,4 & 30	GGCACCAGATTCAACTTTCAAG GACCCCAAGTTTCCTGTAAGTG	564
mPCR-II (extended-spectrum *β*-lactamases)			
CTX-M1	CTX-M-1, CTX-M-3, and CTX-M-15	TTAGGAAATGTGCCGCTGTA CGATATCGTTGGTGGTACCAT	404
CTX-M9	CTX-M-9 and CTX-M-14	TCAAGCCTGCCGATCTGGT TGATTCTCGCCGCTGAAG	561
mPCR-III (AmpC *b*eta lactamases)			
ACC	ACC-1 and ACC-2	CACCTCCAGCGACTTGTTAC GTTAGCCAGCATCACGATCC	346
FOX	FOX-1 to FOX-5	CTACAGTGCGGGTGGTTT CTATTTGCGGCCAGGTGA	162
MOX	MOX-1, MOX-2, CMY-1, CMY-8 to CMY-11 and CMY-19	GCAACAACGACAATCCATCCT GGGATAGGCGTAACTCTCCCAA	895
DHA	DHA-1 and DHA-2	TGATGGCACAGCAGGATATTC	997

		GCTTTGACTCTTTCGGTATTCG	
CIT	LAT-1 to LAT-3, BIL-1, CMY-2 to CMY-7, CMY-12 to CMY-18 and CMY-21 to CMY-23	CGAAGAGGCAATGACCAGAC ACGGACAGGGTTAGGATAGY[b]	538
EBC	ACT-1 and MIR-1	CGGTAAAGCCGATGTTGCG GATTTGCTCCGTGGCCGAAA	683
Multiplex IV (metallo- *β* lactamases)			
IMP	IMP variants except IMP-9, IMP-16, IMP-18, IMP-22 and IMP-25	TTGACACTCCATTTACDG[b] GATYGAGAATTAAGCCACYCT[b]	139
VIM	VIM variants including VIM-1 and VIM-2	GATGGTGTTTGGTCGCATA CGAATGCGCAGCACCAG	390
KPC	KPC-1 to KPC-5	CATTCAAGGGCTTTCTTGCTGC ACGACGGCATAGTCATTTGC	538

Table III: Prevalence of different types *β-Enterobacteriaceae* in animal foods and their environment

Number of the isolates	Extended-spectrum *β*-lactamases		AmpC – *β* lactamases		metallo- *β* lactamases	
	Phenotypic	Genotypic	Phenotypic	Genotypic	Phenotypic	Genotypic
E. coli (40)	16 (40%)	7 (17.5%)	5 (12.5%)	2 (5%)	1 (2.5%)	1 (2.5%)
K. pneumonia (34)	26 (76.47%))	19 (55.88%)	8 (23.53%)	-	-	-
K. oxytoca (7)	4 (57.14%)	4 (57.14%))	1 (14.29%)	-	-	-
P. mirabilis (14)	8 (57.14%)	6 (42.86%)	3 (21.43%)	2 (14.29%)	-	-
Total (95)	54 (56.84%)	36 (37.89%)	17 (17.89%)	4 (4.21%)	1 (1.05%)	1 (1.05%)

Table IV: Different *β*-lactamase genes observed in *Enterobacteriaceae* members

β-Lactamase genes targeted	*E. coli* (40)	*K. pneumonia* (34)	*K. oxytoca* (7)	*P. mirabilis* (14)	Total
TEM	5 (12.50%)	-	-	3 (42.86%)	8
SHV	1 (2.5%)	19 (55.88%)	4 (57.14%)	-	24
OXA	-	-	-	1 (14.29%)	1
CTX-M1	-	-	-	1 (14.29%)	1
CTX-M9	1 (2.5%)	-	-	1 (14.29%)	2
ACC	2 (5%)	-	-	1 (14.29%)	3
FOX	-	-	-	1 (14.29%)	1
KPC	1(2.5%)	-	-	-	1

4. Conclusion

The current study results emphasized that there is compulsion to improve the hygienic status of locally vended ice cream. The present study exposed that the microbiological quality of the ice cream is below recommended standard level and are carrying transmissible antimicrobial resistant markers (genes of ESBL's). The children of lower socio-economic class usually consume these local vended ice creams, it is crucial to monitor the quality to ensure well-being of the class of consumers. This contamination may be a result of the improper practices followed in ice cream production, low/poor hygienic practices, faulty packaging and storage.

Implementation of HACCP practices should be enforced. However, providing training regarding the hygiene measures, imparting knowledge about the safe food production among the workers can be an effective way to limiting contamination. Periodical microbiological and quality analysis of dairy products to safeguard the health of consumers must be practiced. Implementation of reward and punishment policy to the local vendors for application of good hygienic standards might be helpful to improve microbiological quality of ice creams.

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