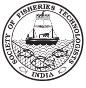

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Research Note

Antibiotic Resistance and Pathogenic Potential of *Escherichia coli* Isolated from Food Contact Surfaces of a Commercial Kitchen

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Food safety in the restaurant is very important to safeguard the health of consumers which in turn will contribute to economic development of countries by fostering tourism. The objective of the study was to understand the prevalence of antibiotic resistance, genes coding for antibiotic resistance and virulence genes among *E. coli* isolated from various food contact surfaces of a commercial kitchen. A total of 15 *E. coli* were isolated from a commercial kitchen. The *E. coli* isolates were resistant to cefpodoxime (75 %) and nitrofurantoin (50 %) followed by ampicillin (43 %). Multiple antibiotic resistance indexes of the isolates ranged from 0.6 to 0.93. Out of the total isolates, 46.6 % carried bla_{TEM} genes and 20% carried $bla_{CTX\text{-}M}$ genes. The virulence gene *hly-A eae* and *stx*1 were detected in 40 % of the isolates, while *stx2* genes was not detected in these isolates. In conclusion, the presence of antibiotic resistant pathogenic *E. coli* isolates in food contact surfaces of the commercial kitchen is a cause of concern since there is a possibility of cross contamination to the food items, thereby posing health risk to the consumer.

Keywords: *Escherichia coli,* commercial kitchen, cross contamination, antibiotic resistance, virulence genes

Ensuring safe food is the fundamental duty of catering and hospitality industry. The primary concern for public health is food safety. Unsafe food poses a threat to consumer health and sometimes poses life threatening consequences *Escherichia coli* has long been regarded as indicator organism for hygiene in itself and as being a member of the 'coliform' group (Eslava et al., 2003). While most *E. coli* strains are commensals in the intestines of warm-blooded animals, the pathogenic strains are responsible for a wide spectrum of diseases because of their virulence traits. (Malik & Memona, 2010). Food-borne pathogenic bacteria have shown the ability to remain viable on various surfaces for extended periods and this has been shown to be important during various cross contamination situations (Kramer et al., 2006; Kusumaningrum et al., 2002).

Worldwide observations concerning antimicrobial resistance of *E. coli* have been reported. Due to the rapid evolution of drug resistant mutants to the majority of first-line antimicrobial drugs, managing the spread of *E. coli* infections has become more challenging (Sabate et al., 2008). There are several ways that food can become contaminated with antimicrobial resistant organisms. One of them is the presence of bacteria that are resistant to antibiotics in food that has been subjected to antibiotic treatment during production, such as the fishes raised in aquaculture systems. Another way is through cross-contamination with antimicrobial resistant bacteria during food processing (Carattoli, 2008; Zirakzadeh & Patel, 2005). The objective of this study was to investigate the prevalence of antibiotic resistance genes and virulence genes of *E. coli* isolated from food contact surfaces in a commercial kitchen.

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Samples were collected aseptically from 10 different sites in the kitchen. Swab samples from the kitchen sink, stove knob, cutting board, refrigerator handle, phone handle; cold room stand, potato peeler, kitchen towel, worker hands and aluminium foil used for covering food items were taken in this study. The area to be swabbed were selected and the swab samples were collected using a sterile swab in an aseptic manner. The swabs after sampling were placed in sterile conical flasks containing 100 ml distilled water and labelled separately as described Mohammed et al. (2018). All swab samples were placed in an ice-cooled box and transported immediately to the laboratory for microbial analysis.

Three tube, most probable number (MPN) methodology was carried out to determine the *E. coli* load in the samples (Moore & Griffth, 2022). Presumptive positive tubes were streaked onto Eosin Methylene Blue (EMB) agar and incubated at 37 °C for 24-48 h (Oyeleke & Manga, 2008). For the confirmation of the *E. coli,* the positive samples (with a green metallic sheen) were streaked on Hi-Crome ECC Agar (Hi-media, Mumbai, India). Dark pink coloured colonies were selected and biochemical characterization of the colonies were carried out using IMViC test (Barrow et al., 1993). *E. coli* isolates (n= 15) were subjected to disc diffusion testing to find susceptibility towards 16 antimicrobials (Bauer et al., 1966). The antibiotics and concentration used were; ampicillin (AMP, 10 μg), chloramphenicol (C, 30 μg), ceftazidime (CAZ, 30 μg), colistin (CL, 10 μg), ciprofloxacin (CIP, 5 μg), cefpodoxime (CPD, 10 μg), ceftriaxone (CTR, 30 μg), cefuroxime (CXM, 30 μg), cefotaxime (CTX, 30 μg), doxycycline hydrochloride (DO, 30 μg), gentamicin (GEN, 10 μg), nalidixic acid (NA, 30 μg), nitrofurantoin (NIT, 300 μg), streptomycin (S, 10 μg), tetracycline (TE, 30 μg) and trimethoprim (TR, 5 μg). The National Committee for Clinical Laboratory Standards (NCCLS, 1997) advised classifying strains as susceptible, having decreased susceptibility, or being resistant. The micro organisms that encode for resistance to three classes of antibiotics were classified as Multidrug resistant (Clinical and Laboratory Standards Institute, 2022)

The presence of bla_{TEM} (Marynard et al., 2003) and bla_{CTX-M} genes (Batchelor et al., 2005) were detected using polymerase chain reaction (PCR). Genomic DNA of the isolates were extracted using the boiling method as described by Devi et al. (2009). The PCR was carried out with a PCR mix of 25 μl containing 2.5 mM $MgCl_2$, 1X Taq buffer (Tris (pH 9.0) at 25 °C, KCl and Triton X-100), 200 μ Md NTP, 1 pmol/μl of each of the primers, 1 U of Taq polymerase ($GeNei_{TM}$, India) and 1 μl of the DNA template. PCR conditions were, initial denaturation at 94 °C for 5 min, 30 cycles of denaturation at 94 °C for 30 sec, annealing at 60 °C for 30 sec, extension at 72 °C for 90 sec, and final extension at 72 °C for 7 min. The sequence of the PCR primers are given in Table 1.

Each isolate was subsequently screened using PCR for the entero pathogenic *E. coli* virulence genes. (*hlyA, eae, stx1 and stx2*) as described by Reza & Sakineh (2013) as given in Table 1. Each reaction mixture contained 1 μl of 25 pmol of each primer, 4 mM $MgCl_2$, 2 μl of 200 μM dNTPs, 2 μl of 1X Taq buffer, 1 U of Taq DNA polymerase ($GeNei_{TM}$ India) and 1 μl DNA template in 20 μl reaction volume. The PCR was carried out by initial denaturation at 94 °C for 2 min, 35 cycles of denaturation at 95 °C for 1 min, annealing at 65 °C for 2 min and extension at 72 °C for 1 min. Final extension was carried out at 72 °C for 7 min. PCR products were electrophoresed on a 1.5 % agarose gel containing ethidium bromide.

Table 1. Primers used for the detection of antibiotic resistant genes and virulence genes

Genes investigated	Primer sequence (5′ - 3′)		Reference
	Forward	Reverse	
bla_{TEM} (857 bp)	gagtattcaacattttcgt	accaatgcttaatcagtga	Marynard et al., 2003
bla_{CTXM} (585 bp)	cgatgtgcagtaccagta	ttagtgaccagaatcagcg	Batchelor et al., 2005
hlyA (534 bp)	gcatcatcaagcgtacgttcc	aatgagccaagctggttaagct	Schmidt et al., 1995
eae (384 bp)	gacccggcacaagcataagc	ccacctgcagcaacaagagg	Yu & Kaper, 1992
stx1 (180 bp)	ataaatcgccattcgttgactac	agaacgcccactgagatcatc	Jackson et al., 1987
stx2 (288 bp)	ggcactgtctgaaactgctcc	tcgccagttatctgacattctg	Jackson et al., 1987

Statistical analyses of the results were carried out using IBM SPSS version 22 (IBM Corporation, Armonk, New York, USA). A Pearson's Chi-squared test was applied to test differences in the prevalence of *E. coli* among different sites of commercial kitchen Statistical significance was set at a P value of < 0.05.

Unhygienic preparation of food provides plenty of opportunity for contamination. The present study investigated the prevalence of antibiotic resistance, antibiotic resistance genes and virulence genes among *E. coli* isolated from food contact surfaces in a commercial kitchen. A total of 15 *E. coli* were isolated from food contact surfaces such as kitchen sink (2 nos.) stove knob (3 nos.) freezer handle (3 nos.) phone handle (2 nos.), cold room stand (2 nos.) and kitchen towel (3 nos.). In our study, there was significant difference in the prevalence of *E. coli* between freezer handle and the kitchen sink ($p < 0.05$). All the isolates were subjected to antibiotic

Table 2. Detection of virulence genes in the *E. coli* isolates

Strain No.	Source	*hlyA* gene	*eae* gene	*stx1* gene	*stx2* gene
ESK1	Stove knob	+	-	+	-
EKS1	Kitchen sink	-	+	-	-
EKS2	Kitchen sink	+	+	+	-
EFH1	Freezer handle	+	-	+	-
EFH2	Freezer handle	+	-	+	-
EFH3	Freezer handle	-	+	+	-
EPH1	Phone handle	+	+	-	-
ECRS1	Cold room stand	-	+	-	-
EKT1	Kitchen towel	+	+	+	-

Table 3. MAR indexing and antibiotic resistance patterns of *E. coli* isolated from food contact surfaces in a commercial kitchen

Isolate No.	MAR Index	Resistance pattern	Resistance genes tested	
			bla_{TEM}	bla_{CTXM}
ESK1	0.37	CL, CPD, CXM, DO, NIT, TE	+	-
EKS1	0.12	CPD, NA	+	+
EKS2	0.37	AMP, CIP, CL, CPD, DO, NIT	+	+
EFH1	0.18	CPD, CXM, NA	+	+
EFH2	0.5	AMP, CAZ, CPD, CTX, CXM, NA, NIT, TR	+	+
EFH3	0.37	AMP, CL, CPD, DO, NIT, TE	+	+
EFH4	0.43	AMP, CAZ,CL, CPD, NA, NIT,TR	+	+
EPH1	0.31	CL, DO, NIT, TE, TR	-	-
EPH2	0.06	CPD	-	-
ECS1	0.12	CPD, CTX	+	-
ECS2	0.31	CL, CPD, DO, NA, TE	+	-
EKT1	0.5	AMP, CPD, GEN, S, TE, NA, NIT, TR	+	-
EKT2	0.93	AMP, C, CAZ, CIP, CPD, CTR, CTX, CXM, DO, GEN, NA, NIT, S, TE, TR	+	-

ESK- Stove knob, EKS- Kitchen sink, EFH-freezer handle, EPH-Phone handle, ECS-Cold room stand, EKT-Kitchen towel

resistance profiling. The higher percentage of resistance was exhibited against cefpodoxime (75 %) and nitrofurantoin (50 %) followed by ampicillin (43 %). Moderate level of resistance was found against nalidixic acid (37.50 %), doxycycline (37.50 %), colistin (37.50 %), tetracycline (31.25 %), trimethoprim (25 %), cefuroxime (25 %) and ceftazidime (18 %) (Fig. 1). Several studies have suggested that food might be a source of antibiotic-resistant *E. coli*. ESBL-producing *E. coli* was commonly identified from gloves and cutting board used for raw poultry reprocessing (Ilse et al., 2011; Abgottspon et al., 2014). MAR indices and resistance pattern of *E. coli* isolates from food contact surface are given in Table 3. The MAR indexes of the isolates ranged from 0.6 to 0.93. One of the isolates form kitchen towel (EKT2) were resistant to 15 antibiotics tested. It is frequently believed that multiple antibiotic resistance (MAR) indices higher than 0.2 came from high-risk sources of contamination (Krumperman, 1983). The bla_{TEM} resistance genes were present in *E. coli* isolated from stove knob, kitchen sink, freezer handle, cold room stand and kitchen towel. The $bla_{CTX\text{-}M}$ gene was detected in *E. coli* isolated from the kitchen sink and freezer handle. The results also revealed that forty percent of *E. coli* isolates carried the *hlyA*, *eae*, and *stx1* genes. The prevalence of bla_{TEM} and $bla_{CTX\text{-}M}$ was 73.3 % and 40 % respectively. In a previous study, it was reported that kitchen food contact surface can easily become contaminated with β-lactamase producing *E. coli* (Tschudin-Sutter et al., 2017). Food preparation and food-adjacent surfaces such as cutting boards, microwave oven controls, faucet handles on sinks, various handles and ingredient lids that were found to be contaminated with microorganisms (Sharp & Walker, 2003). β-lactamase producing *E. coli* was commonly recovered from the kitchen sink, stove knob, freezer handles, cold room stand, phone handle and kitchen towel.

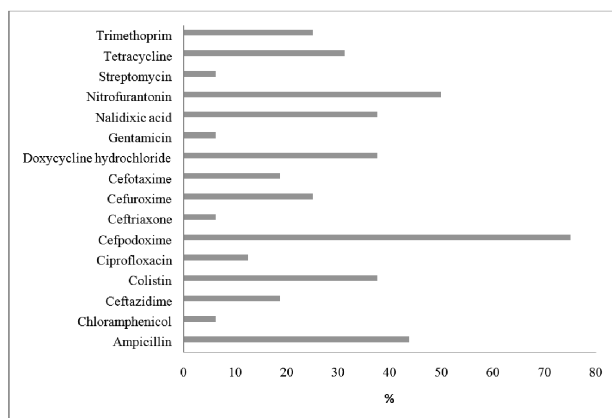

Fig. 1. Prevalence of antibiotic resistance among *E. coli* isolates from food contact surfaces

PCR results showed that *hlyA* and *eae* were detected from *E. coli* isolated from the kitchen sink, phone handle and kitchen towel. The *stx1* gene was detected from stove knob, kitchen sink, freezer handle and kitchen towel isolates (Table 2). Rasheed et al. (2014) reported similar results on the distribution of the *hly*A, *stx2, stx1* genes and antibiotic profiling in Shiga-toxigenic *E. coli* strains isolated from food sources. The *stx2* virulence genes was not detected in any of the isolates studied. Our results indicated that pathogenic *E. coli* was present in kitchen sink, stove knob, freezer handle, phone handle, cold room stand and kitchen towel. There exists a possibility of the *E. coli* from the above surfaces to contaminate the food items and food harbouring these isolates might pose a potential health risk to the consumer. Preventing *E. coli* contamination from food contact surface can be achieved by following standard sanitation operation procedure (SSOP), good manufacturing practice (GMP) and good hygiene practice (GHP) (Habeeb et al., 2018). Utmost care shall be taken at the restaurants to strictly adhere to these procedures.

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