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Research article

The genotypic identification of colicins produced by clinical isolates of *Escherichia coli* in Iraq

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ABSTRACT

Escherichia coli is natural and essential part of the bacterial flora in the gut of humans and animals. *E. coli* produces colicin, proteinaceous toxin that could inhibit the growth of similar or closely related bacterial strains. The production incidence of four colicin types in *E. coli* were tested in 31 bacterial isolates isolated from different sources (urine, stool, blood, ear swab and sewage water) by using polymerase chain reaction of specific primer for each colicin type. Production of colicin E1 was detected in four bacterial isolates (13.33%), three of them isolated from urine samples, while the last isolated from sewage water. Colicin M was detected in 15 colicinogenic isolates (50%), 12 isolates isolated from urine, 2 from sewage, while last one from stool. However, colicin E3 and E9 were not detected in all colicinogenic isolates. *E. coli* isolated from human urinary tract infections showed high incidence of colicin M and E1 production, respectively

Keywords: Colicin, *E.coli*, Colicin M, Colicin E1.

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INTRODUCTION

Escherichia coli is a common inhabitant of the gastrointestinal tract of humans and animals. Some of *E. coli* strains are harmless commensals of the intestinal tract and others are major pathogens of humans and animals. The pathogenic *E. coli* is divided into strains causing disease inside the intestinal tract and others capable of infection at extra 342 intestinal sites [1]. Bacteriocin production is an important characteristic of *E. coli* and several related species in the enterobacteriaceae family, within the genus *Escherichia,* bacteriocin production is almost exclusively associated with strains of *E. coli* [2]. Bacteriocins may serve as anticompetitors, enabling the invasion of a strain into an established microbial community [3,4]. They may also prevent the invasion of other strains or species into an occupied niche or limit the advance of neighboring cells [5].

The colicins are protein compounds produced by, and active against, *E. coli* and others members of enterobacteriaceae family. At least 34 different colicins have been described and found to share an interesting

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number of features [6]. Colicins are class of antimicrobial compounds produced by bacteria, are thought to be important mediators of intra- and interspecific interactions, and are a significant factor in maintaining microbial diversity [7]. Colicins are plasmid-encoded 3-domain antibacterial proteins that are produced during times of stress by and active against *E. coli* and closely related bacteria [8]. Their toxic activities are of various types; some colicins form ion channels in the cytoplasmic membrane of sensitive cells, while others act as nucleases that degrade DNA or 16s RNA in the cytoplasm, and one, colicin M, inhibits the biosynthesis of murein [9]. Colicins are not active against more distantly related strains because these strains lack the receptor and/or translocation system for uptake of the colicins [10]**.** The aim of this study was to determine the colicin production by *E coli* strain isolated from different sources and detect the genes of it by PCR.

MATERIALS AND METHODS

Specimen's collections

One hundred and five specimens included: urine, stool, ear swab and sewage, were collected in sterilized containers from five hospitals in Baghdad, Al-Imam Ali hospital, Ibn Al-baladi hospital, Fatema Al-Zahraa hospital and Al-sader hospital. The study was conducted following approval from the scientific and academic committee in Department of Biology/ College of Science /University of Baghdad.

Isolation and identification

In the laboratory within aseptic conditions, the collected specimens were streaked directly on MacConkey agar and Eosin methylene blue EMB agar (Himedia/India) and incubated for 24 h at 37ºC. Pink colonies were picked and re-cultured on another MacConkey and EMB gar. Further identification tests included the morphol-ogical characteristics and biochemical tests were carried out depending on Forbes *et al*. [11]. Finally API E20 system was done.

Screening of colicinogenic E. coli by Cup assay methods

Method of Al-Qassab ana Al-Qafagi [12] was followed for screening colicinogenic isolates. Loopful from an overnight LB broth culture of each producer isolates is heavely streaked on brain heart infusion agar (BHI) and incubated at 37C for 18 hs. Wells were made by using cork porer 8 mm diameter. Suspension of indicator isolate were spread on the surface of muller hinton agar then left to dry at 37 ^{o}C for 10 min and double plates were made for each isolate. Discs removed from Brain heart infusion (B.H.I) agar medium were sticked gently on the surface of Muller hinton medium spread by the sensitive isolate then incubated at 37 ^{o}C for overnight. Sensitivity was detected by measured the zone of inhibition. The sensitive strain was procured from College of Science, Al-Mustansiriya University.

Plasmid extraction of *E. coli*

In this study, 30 isolates of *E. coli* were selected for detection colicin (E1, E3, E9 and M) genes, 30 isolates were colicin producers and 1 isolate was not producer that used as a control. Plasmid DNA was extracted from these isolates by using commercial Accuprep® PCR plasmid mini extraction kit (Bioneer,Korea) and then DNA concentration and purity were determined by using Nano-drop system.

PCR amplification

The sequence of four specific pairs primers were used according to previous studies [13,14]. PCR reaction was used to detect bacteria those posses colicin genes (**Table 1**). The PCR reactions were done in 25 µl volume and comprised 12.5 µl of green master mix (Promega, USA), 1 µl of each primer (10 Pmol) and 2 µl DNA template. Deionized distilled water was used to bring the final reaction volume to 25 µl. The thermocycler cycling conditions were 1 cycle of denaturation at 94 ^{o}C for 2 min, annealing for 1 min, elongation at 72 ^{o}C for 1 min then 30 cycles of denaturation at 94 ^{o}C for 1 min. The PCR reaction products were stored at -20 C or immediately separated on 2% agarose gels.

RESULT AND DISCUSSION

Detection the colicinogenic isolate

Locally isolates *E. coli* were screened in order to select the efficient isolates in colicin production. The ability of these isolates in colicin production was assayed after culturing at 37 ^{o}C in BHI agar, then wells were made on this agar and put on Muller hinton agar that contained the sensitive strain. The antagonistic effect against the sensitive strain was detected by measuring the inhibition zone according to cup assay method (**Fig. 1**).

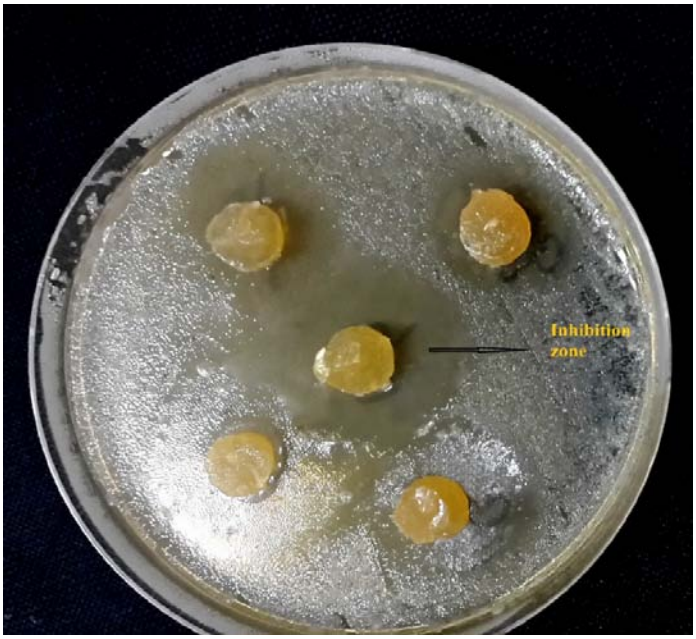

Fig 1. Cup assay method for detection coliciongenic *E. coli*.

Table 1. Primers used for colicin detection.

Bacteriocin type	Primer na	Sequence 5' 3'	Length	References
E1	Col E1	TGTGGCATCGGGCGAGAATA CTGCTTCCTGAAAAGCCTTTT	650	Gordon D.M. and O Brien C.L.,2006)
E3	Col E3	TAAGCAGGCTGCATTTGATG TCGGATCTGGACCTTTCAAC	413	Smajs,D.*et al.*,2010
E9	Col E9	TAAGCAGGCTGCATTTGATG GACTTTTCTCCCTCCGACCT	418	Smajs,D.*et al.*,2010
M	Col M	GCTTACCACTTCGCAAAACC GAGCGACTCTCCGATAATGC	429	Smajs,D.*et al.*,2010

Results indicated in **table 2** showed that 30 isolates (54.54%) from 55 isolates were colicin producers according to inhibition zone against the sensitive strain. Diameters of inhibition zone ranged from 12 to 40 mm. Local study by Al-sa'edi, [15] reported that 30.55% from isolates were colicin producers, while others not, that is why the study was not agreed with the present study. However, Tishvarian, [16] in other local study showed that 70.83% from *E. coli* isolates were colicinogenic producer and this result was higher than the present study.

Table 2. Frequency of colicinogenic isolates depending on diameter of inhibition zone against sensitive strain of *E. coli*.

Id	*E. coli* isolates	Diameter of inhibition zone	Id	*E.coli* isolates	Diameter of inhibition zone	Id	*E.coli* isolates	Diameter of inhibition zone
1	E 1	25 mm	**20**	E 57	12 mm	**38**	E 83	12 mm
2	E 2	17 mm	**21**	E 58	15 mm	**39**	E 84	_
3	E 3	_	**22**	E 60	_	**40**	E 85	15 mm
4	E 5	_	**23**	E 62	15 mm	**41**	E 87	33 mm
5	E 8	_	**24**	E 63	_	**42**	E 88	35 mm
6	E 9	_	**25**	E 64	40 mm	**43**	E 89	20 mm
7	E 12	20 mm	**26**	E 65	_	**44**	E 90	_
8	E 13	15 mm	**27**	E 66	12 mm	**45**	E 91	_
9	E 20	17 mm	**28**	E 67	45 mm	**46**	E 92	_
10	E 23	_	**29**	E 69	_	**47**	E 93	15 mm
11	E 27	20 mm	**30**	E 70	_	**48**	E 94	_
12	E 35	_	**31**	E 71	15 mm	**49**	E 95	_
13	E 37	_	**32**	E 72	15 mm	**50**	E 96	15 mm
14	E 51	12 mm	**33**	E 74	_	**51**	E 97	15 mm
15	E 52	12 mm	**34**	E 75	42 mm	**52**	E 98	_
16	E 53	_	**35**	E 79	_	**53**	E 99	17 mm
17	E 54	13 mm	**36**	E 80	15 mm	**54**	E102	_
18	E 55	15 mm	**37**	E 82	_	**55**	E103	35 mm
19	E 56	_						

Feldgarden and Rily, [17] demonstrated that most *E. coli* are resistant to most colicins. On average, 93% of the isolates were resistant to all colicin types, and 33% were multiply resistant to all the colicins tested. James *et al.* [18] reported that approximately 30 % of the natural population of *E. coli* produced colicin with high diversity of colicin type among them and Riley and Gordon, (19) indicated that 25 types of colicin were characterized. Hossneara *et al.* [20] reported that 39 % from *E. coli* isolates were colicin producers and this result was disagreeing with the present study.

PCR analysis

Monoplex PCR technique was carried on to detect colicin encoding genes in 31 *E. coli* isolates (30) isolates were colicin producers and 1 isolates were not producer. The colicins was detected in the isolates by PCR were E1, E3, E9 and M. In this assay, specific primers were used, the results showed band of PCR product with 650 bp that represent colicin *E1* gene and band with 429 bp that represent colicin M. Monoplex PCR studies demonstrated that 15 isolates of *E. coli* contain colicin M, 4 isolates contain colicin E1,while E3,E9 were not found in any of these isolates (**Fig 2** and **3**).

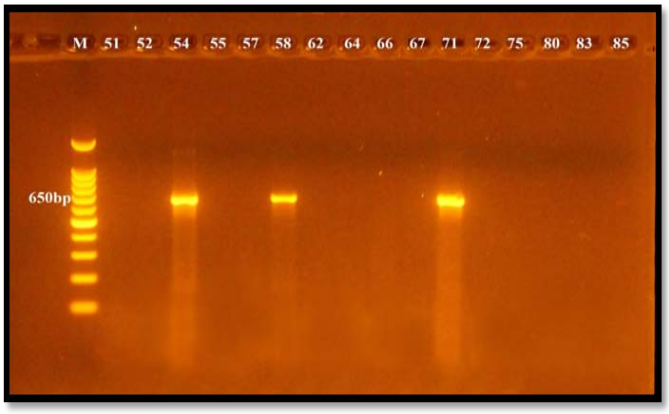

Fig 2. Gel electrophoresis of amplified PCR product of *Col E1*gene (650 bp) of colcinogenic isolates in monoplex pattern agarose (2%), 3 isolates were positive, TBE buffer (1x), 65 volt for 1 h. stained with ethidium bromide. M: DNA ladder (100 bp); Lanes 4,7,11 isolates were positive while other lanes represent isolates were negative to *col E1* gene, all these isolates represent colicinogenic isolates

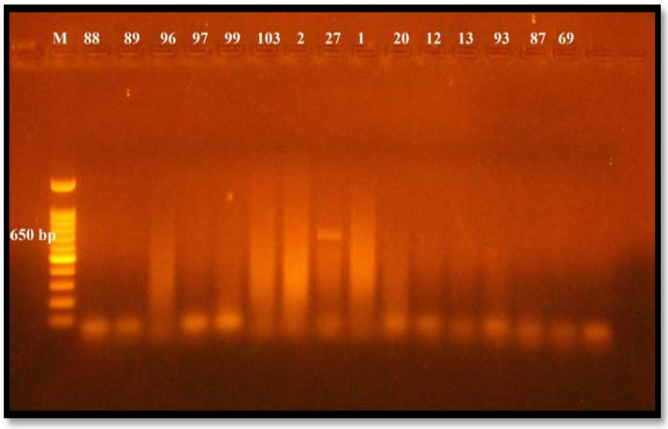

Fig 3. Gel electrophoresis of amplified PCR product of Col E1gene (650 bp) in monoplex pattern,agarose (2%), one isolate was positive, TBE buffer (1x), 75 volt for 1 h. stained with ethidium bromide. M, DNA ladder (100 bp). Lane E1 positive isolate; lane, 16 was non colcinogenic isolte that used as control.

Colicin E1 was found in 4 isolates (13.33%), 3 isolates obtained from urine samples, while last one from sewage, Smajs *et al.* [14] found a statistical increase in UTI strains producing colicin E1 compared to control. Colicin E1 is also known to have toxic effects on eukaryotic cells and is considered to be a virulence factor in UPEC strains [21,22]. Enzymatic E group colicins are 60 kDa toxins that translocate their C-terminal cytotoxic domains across the inner membrane in order to elicit cell death through the hydrolysis of nucleic acid. Four H-N-H endonuclease colicins have been identified, E2, E7, E8 and E9, all of which target the bacterial chromosome**.** The gene encoding colicin E1, is located in the ColE1 plasmid. *cea* forms an operon with the downstream kil gene, encoding the Kil protein, whose function is to make the cell membrane leaky to release colicin E1 into the surrounding environment, killing the producing cells in the process [23,24]**.** The result showed that 15 colicinogenic *E. coli* had colicin M gene with 429 bp, while other isolates were not had this gene. Band of PCR product, which was detected and confirmed by gel electrophoresis by using 100 bp DNA ladder (**Fig. 4** and **5**). In this study, most frequent detected gene was colicin M that found in 50% of colicinogenic *E. coli***.** Colicin M was earlier demonstrated to provoke *E. coli* cell lysis via inhibition of cell wall peptidoglycan (murein) biosynthesis. As the formation of the*O*-antigen moiety of lipopolysaccharides was concomitantly blocked, it was hypothesized that the metabolism of undecaprenyl phosphate, an essential carrier lipid shared by these two pathways, should be the target of this colicin [25]. In the absence of colicin M, the lipid-linked precursor of murein biosynthesis, undecaprenyl-N-acetyl-muramyl pentapeptide-N-acetylglucosamine (lipid II), is transferred across the cytoplasmic membrane and incorporated into murein. Undecaprenyl pyrophosphate is released and converted to the monophosphate, which reenters the reaction cycle. Colicin M inhibits this undecaprenylphosphate (lipid) carrier regeneration step of murein synthesis [26]. Colicins B and M have been found to co-occur at a significantly greater frequency than expected by chance [27]. The colicin B and M gene operons are almost always found in close proximity on the same large conjugative plasmid [28,29]. The present results showed that colicin E3 and E9 were not detected in any one of isolates, this results were in an agreement with Smajs *et al.* [14], who reported that these genes were not found in UTI strains.

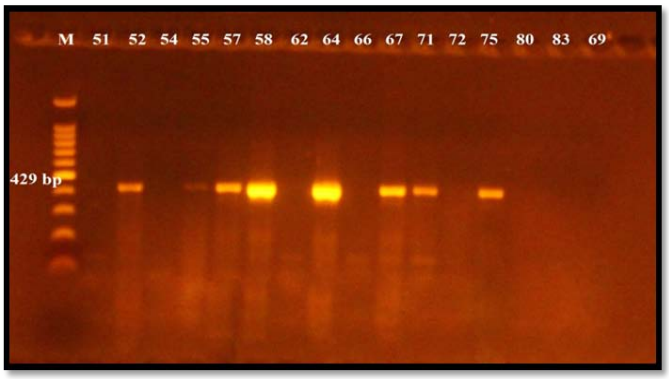

Fig 4. Gel electrophoresis of amplified PCR product of *col M* gene (429bp) of *S.epidermidis isolates* in monoplex pattern,agarose (2%),8 isolate were positive, TBE buffer (1x), 75 volt for 1 hrs. stained with ethidium bromide. M, DNA ladder (100 bp). Lanes 3, 5, 6, 7, 9, 11,12,14 were *Col M* positive isolates.

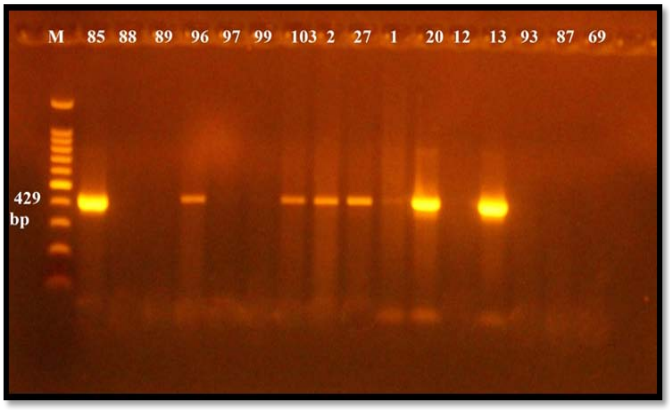

Fig 5. Gel electrophoresis of amplified PCR product of colicin M gene in monoplex pattern,7 isolate were positive to it. Agarose (2%), TBE buffer (1x), 75 volt for 1 h. stained with ethidium bromide. M, DNA ladder (100 bp) .Lanes 2,5,8,9,10,12,14 were ColM positive isolates; lane 17 was non-colicinogenic isolate (control).

The frequency of bacteriocin-encoding genes was found to be positively correlated with the frequency of *E. coli* virulence determinants [30]. Smajs *et al.* [14] reported that Colicin E1 was co-associated with colicin M, but only if the strain did not also encode colicin B. The later was in an agreement with present study because colicin M was detected in colicinogenic isolates that had colicin E1 also. Imren *et al*. [31] reported that out of 129 uropathogenic *E. coli* strains, 33% (25.5) produced colicin, Among these colicin types the group E colicin was more than 50%. None of the strains produced colicin E6, Out of 129 *E. coli* strains 22 (17%) produced colicin V. Tahamtan *et al.,*[32] in Iran reported that approximately 100% of isolates contained at least one colicin gene and data indicated 39.19 of isolates harbored at least one colicin, while 21,73,17.42,13.04 and 3.34 present of isolates posses 2,3,4 and five genes of colicin respectively. these isolates were taken from animals.

Conflict of interest

The author declares that she has no conflict of interests.

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