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Screening of *E. coli* for its antimicrobial susceptibility pattern in milk and dairy products in Chennai, India

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Abstract
Milk and dairy products harbor bacteria that are resistant to one or more antimicrobial drugs. This study was conducted to determine the antimicrobial susceptibility pattern of *E. coli* isolated from milk and dairy products collected randomly from Chennai. A total of 567 samples comprising raw milk, pasteurized milk, dairy products *viz*., channa based sweets, khoa based sweets, fermented dairy products, concentrated or partially desiccated dairy products, heat and acid coagulated dairy products, frozen dairy products and other dairy products like butter, cheese were processed to isolate *E. coli* and to study their antibiotic susceptibility pattern. Out of 567 samples, 34 *E. coli* were isolated and its phenotypic resistant profile was 94.11 per cent for penicillin-G and cephalothin followed by ampicillin (88.23%), amoxicillin (82.35%) and clindamycin (76.47%). Among these 34 *E. coli* isolates, 28 (82.35%) were multiple drug resistant (MDR). This alarms the need for devising appropriate strategies to prevent the spread of resistance.

Keywords: *E. coli*, milk and dairy products, multiple drug resistant, antimicrobial susceptibility pattern

Introduction
Food acts as an important vehicle for the transfer of antimicrobial resistant (AMR) factor to humans very efficiently. The presence of antimicrobial resistant bacteria in milk and dairy products was considered as an important public health issue due to its potential for the transfer of antimicrobial resistant food-borne pathogens to human population. Food contaminated with antibiotic resistant bacteria act as an ideal vehicle for the transmission of antibiotic resistant strains [1, 2]. *Escherichia coli* (*E. coli*) was considered as a reliable indicator of contamination by manure, soil and contaminated water among many pathogenic microorganisms and can even get access to milk and dairy products [3]. Raw milk, pasteurized milk, yoghurt and other dairy products have been reported to be contaminated with *E. coli* and are usually harmless to the host but, there had been a possibility of acquiring virulence genes by non-pathogenic *E. coli* through horizontal gene transfer which may result in emergence of virulent strains [4]. Hence this study was carried out to determine the antimicrobial susceptibility pattern along with multiple drug resistance (MDR) of various *E. coli* isolates obtained from milk and dairy products.

Materials and methods
A total of 567 samples (75 raw milk, 45 pasteurized milk, 447 dairy products viz., channa based sweets, khoa based sweets, fermented milk products, concentrated or partially desiccated milk products, heat and acid coagulated milk products, frozen milk products and other milk products like butter, cheese and cream) were collected from local milk vendors, cooperative milk dairies, dairy/confectionery shops, supermarkets and sweet shops in different zones of Chennai. *E. coli* was isolated from milk and dairy products by conventional method using Eosin methylene blue agar, MacConkey agar (Himedia). Presumptive *E. coli* isolates were confirmed by species-specific PCR amplification of the *E. coli usp*A gene [5]. Disc diffusion method was performed to identify the antimicrobial susceptibility pattern of *E. coli* isolates using Muller Hinton agar [6] (Himedia).
Test organisms were suspended in normal saline and it was made to 0.5 McFarland standard and then inoculated on Muller Hinton agar plates.

The phenotypic antimicrobial sensitivity response of each isolate was evaluated using 22 antibiotics namely amikacin (30µg), cephoxitin (30µg), cephalothin (30µg), nystatin (100µg), sulphadiazine (300µg), sulphasomidine (300µg), ampicillin (10µg), streptomycin (10µg), enrofloxacin (10µg), cotrimaxazole (25µg), methicillin (5µg), vancomycin (30µg), piperacillin (100µg), imipenam (10µg), gentamicin (10µg), penicillin-G (2units/disc), tetracycline (30µg), clindamicin (2µg), amoxicillin (10µg), ciprofloxacin (5µg), azithromycin (30µg) and cefotaxime (30µg).

Antibiotic discs were gently pressed onto the inoculated Muller Hinton agar and the plates were incubated aerobically at 37°C for 18 to 24 hr [7]. The diameter of the zone of inhibition around the discs was measured to the nearest millimetre using rulers. The isolates were classified as susceptible, intermediate and resistant according to the interpretative standards of CLSI [6]. Isolates showing resistance to three or more antimicrobial subclass were considered as multiple drug resistant (MDR) [8, 9]. A reference strain - *E. coli* MTCC 443 was included in the testing.

Results

A total of 9 and 25 isolates of *E. coli* from 75 raw milk samples and 447 dairy products, respectively were obtained by conventional culture method. The cultural examination of all the presumptive *E. coli* (34) isolates revealed the growth of characteristic green metallic sheen appearance in eosin methylene blue (EMB) agar and flat, dry, pink colonies in MacConkey agar. *E. coli* was seen as Gram negative rod shaped organism by Gram's staining on microscopic examination. On biochemical tests, the isolates were positive for catalase, indole and methyl red and negative for oxidase, Voges- Proskauer and citrate utilization test.

A total of 34 isolates which was recovered by conventional culture was further confirmed by PCR targeting *usp*A gene with a product size of 884 bp. All the 34 isolates were found to be positive by PCR.

All the 34 PCR positive isolates of *E. coli* were subjected to disc diffusion test using commercial discs. Per cent sensitivity of the isolates were 100 per cent, 94.11 per cent, 91.17 per cent, 88.23 per cent and 70.58 per cent for tetracycline, enrofloxacin, imipenam, vancomycin and azithromycin, respectively. Intermediate sensitivity was highest in amikacin (79.4%) followed by piperacillin (73.52%). The phenotypic resistant profile of these isolates were 94.11 per cent for penicillin-G and cephalothin followed by ampicillin (88.23%), amoxicillin (82.35%) and clindamycin (76.47%) (Fig 1) (Table 1).

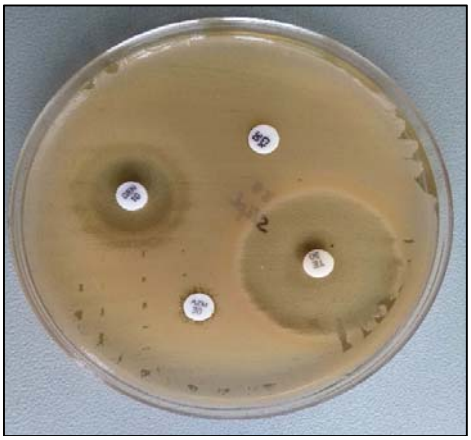

Fig 1: Antimicrobial susceptibility pattern of *E. coli* isolates

Table 1: Overall antimicrobial susceptibility pattern of *E. coli* isolates

Antimicrobial drug tested	Conc. Per disc (µg)	Sensitive isolates		Intermediate sensitive isolates		Resistant isolates	
		No.	Percent	No.	Percent	No.	Percent
Amikacin	30	4	11.76	27	79.4	3	8.82
Cephoxitin	30	2	5.88	21	61.76	11	32.35
Cephalothin	30	0	0	2	5.8	32	94.11
Nystatin	100	5	14.70	22	64.7	7	20.58
Sulphadiazine	300	0	0	23	67.64	11	32.35
Sulphasomidine	300	8	2.35	13	38.23	3	8.82
Ampicillin	10	0	0	4	11.76	30	88.23
Streptomycin	10	4	11.76	14	41.17	16	47.05
Enrofloxacin	10	32	94.11	2	5.88	0	0
Co-trimaxazole	25	20	58.82	9	26.47	5	14.70
Methicillin	5	10	29.41	12	35.29	12	35.29
Vancomycin	30	30	88.23	0	0	4	11.76
Piperacillin	100	4	11.76	25	73.52	5	14.70
Imipenam	10	31	91.17	2	5.8	1	2.94
Gentamicin	10	0	0	18	52.94	16	47.05
Penicillin-G	2units/disc	0	0	2	5.8	32	94.11
Tetracycline	30	34	100	0	0	0	0
Clindamcin	2	1	2.94	7	20.58	26	76.47
Amoxicillin	10	0	0	6	17.64	28	82.35
Ciprofloxacin	5	10	29.41	22	64.70	2	5.8
Azithromycin	30	24	70.58	7	20.58	3	5.2
Cefotaxime	30	16	47.05	17	50	1	1.7

Discussion

In this study, the antimicrobial resistance of *E. coli* isolates to penicillin-G and amoxicillin were 94.11 and 82.32 per cent, respectively. This is in concurrence with the finding of Caine *et al*. (2013) [11]. Akram *et al*. (2013) [10] found that the sensitivity of enrofloxacin was 92.15 per cent which is in agreement with our findings. Adzitey *et al*. (2016) [12] reported *E. coli* in cow milk and hands of milkers and found that *E. coli* were resistant to ampicillin (22.22%), ceftriaxone (29.63%) and tetracycline (25.93%). Higher intermediate resistances were observed for ampicillin (51.85%), chloramphenicol (37.04%) and erythromycin (48.15%) and all

the isolates were susceptible to ciprofloxacin (100.00%). A high percentage of the *E. coli* was also susceptible to suphamethoxazole/trimethoprim (92.59%), gentamicin (74.07%), tetracycline (70.37%) and ceftriaxone (62.96%).

Whereas, Abike *et al.* (2015) [13] reported that *E. coli* isolated from raw cow milk, yoghurt and cheese were resistant to gentamicin (6.81%) and tetracycline (56.80%), which were higher than that of the present study. *E. coli* isolated from raw cow milk were resistant to ampicillin (34.00%), gentamicin (30.55%), trimethoprim/sulfamethoxazole (22.22%) and ciprofloxacin (16.66%) (Bonyadian *et al.,* 2014) [14]. Reuben and Owuna (2013) [15] opined that *E. coli* isolated from Nigerian fermented milk samples were resistant to tetracycline (100.00%), erythromycin (94.70%), sulphamethoxazole/trimethoprim (84.20%) and chloramphenicol (68.40%) which was contradict to our study.

Tetracycline is not commonly used for treating *E. coli* infection in humans. Interestingly, in the present study 100 per cent sensitivity was recorded for tetracycline. This shows that unnecessary use of antibiotics may be the risk for inducing resistance in the bacterial species.

In India, β lactam antibiotics like penicillin-G, ampicillin and amoxicillin as well as first generation cephalosporin like cephalothin were used commonly in both human and animal medicine for their therapeutic effect. Hence, inevitable use of antibiotic will lead to the development of antibiotic resistance. In our study, among the 34 *E. coli* isolates, 28 (82.35%) were multiple drug resistant (MDR). The chances of development of MDR strains may also be due to plasmids, transposons, mutation of existing genes or by acquisition of heterologous resistance gene. Even though the incidence of multiple drug resistant was not high in our study still it is a matter of concern, since there is a reservoir of antibiotic resistant genes within the community.

Conclusion

Antibiotic resistance pattern keep on changing for many food borne pathogens which needs to be monitored very closely to safeguard both human and animal health. Hence, regular antimicrobial susceptibility surveillance is essential. Furthermore, this study alarms the need for devising appropriate strategies to emphasize the rational use of antibiotic and also to prevent the spread of resistance in the study area. Surveillance is essential to decrease the incidence of multiple drug resistant strains of *E. coli* both in animals and humans.

References

1. Angulo F, Nargund V, Chiller T. Evidence of an association between use of anti-microbial agents in food animals and anti-microbial resistance among bacteria isolated from humans and the human health consequences of such resistance. J. Vet. Med. 2004; 51:374-379.
2. Phillips I, Casewell M, Cox T, De Groot B, Friis C, Jones R *et al*. Does the use of antibiotics in food animals pose a risk to human health? A critical review of published data. J. Antimicrob. Chemother. 2004; 53:28-52.
3. Disassa N, Sibhat B, Mengistu S, Muktar Y, Belina D. Prevalence and Antimicrobial Susceptibility Pattern of *E. coli* O157:H7 Isolated from Traditionally Marketed Raw CowMilk in and around Asosa Town, Western Ethiopia. Hindawi Vet. Med. Int. 2017, 7.
4. Ochman H, Lawrence JG, Groisman EA. Lateral gene transfer and the nature of bacterial innovation. Nature. 2000; 405(6784):299-304.
5. Chen YM, Wright PJ, Lee CS, Browning GF. Uropathogenic virulence factors in isolates of Escherichia coli from clinical cases of canine pyometra and feces of healthy bitches. Vet Microbio. 2003; 94:57-69.
6. CLSI. (Clinical and Laboratory Standards Institute). Performance standards for antimicrobial susceptibility testing. eighteenth informational supplement. CLSI document, 2008, M100-118.
7. NCCLS. (National Committee for Clinical Laboratory Standards), Performance Standards For Antimicrobial Disk And Dilution Susceptibility Tests For Bacteria Isolated From Animals. Approved Standard M13-A. Wayne; NCCLS, 1999.
8. Rota C, Yanguela J, Blanco D, Carraminana JJ, Arino A, Herrera A. High prevalence of multiple resistances to antibiotics in 144 *Listeria* isolates from Spanish dairy and meat products. J. Food Prot. 1996; 59:938-943.
9. Normanno G, LaSalandra G, Dambroise A, Quaglia N, Corrente M, Parisi A. Occurrence, characterization for enterotoxigenic *Staphylococcus aureus* isolated from meat and dairy product. J. Food Microbiol. 2007; 115:290-296.
10. Akram N, Chaudhary AH, Ahmed S, Ghuman MA, Nawaz G, Hussain S. Isolation of bacteria from mastitis affected bovine milk and their antibiogram. European J. Vet. Med. 2013; 2:38-46.
11. Caine L, Pekana A, Lukanji Z, Idamokoro M, Green E. Pathogenic *Escherichia coli* strains in raw milk obtained from two farms of the Eastern Cape Province, South Africa. Mendelnet, 2013, 184-187.
12. Adzitey F, Saba CKS, Gabriel AT. Antibiotic susceptibility of *Escherichia coli* isolated from milk and hands of milkers in Nyankpala community of Ghana. Curr. Res. Dairy Sci. 2016; 8:6-11.
13. Abike TO, Olufunke OA, Oriade KD. Prevalence of multiple antibiotic resistant *Escherichia coli* serotypes in cow raw milk samples and traditional dairy products in Osun State, Nigeria. Br. Microbiol. Res. J. 2015; 5:117-125.
14. Bonyadian M, Moshtaghi H, Taheri MA. Molecular characterization and antibiotic resistance of enterotoxigenic and entero-aggregative *Escherichia coli* isolated from raw milk and unpasteurized cheeses. Vet. Res. Forum : an Int. Quarterly J. 2014, 5:29-34.
15. Reuben RC, Owuna G. Antimicrobial resistance patterns of *Escherichia coli* O157:H7 from Nigerian fermented milk samples in Nasarawa State, Nigeria. Int. J. Pharmaceut. Sci. Invention. 2013; 2:38-44.