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Comparison of Antibiotic-Resistant *E. coli* Isolated from Clinical and Marine Environment

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ABSTRACT
Microbiological pollution in source water has created concerns for human health, resulting in the failure of infectious illness treatment due to the presence of hazardous microorganisms and the emergence of antibiotic resistance in bacterial strains. The aim of our study was to comparatively analyze the profile of antimicrobial resistance of E. coli isolates from clinical samples and marine areas obtained from seawater and marine sediments. A total of 150 marine strains and 175 clinical strains of E. coli were isolated and characterized for their antibiotic profiling and these strains were compared by RAPD analysis. Antimicrobial susceptibility testing was performed for 11 antimicrobials, multiple antibiotic-resistantindexeswere calculated and resistant pattern among strains was analyzed in detail. The results indicated that strains from unrelated samples collected at different periods of time showed more than 80% (or) even >90% similarity. This can be critically viewed as they may be transported from hospital environment to marine through sewage stormwater input or vice versa. Their spatial and temporal variation indicated the possibility of their extended survival in the marine environment. As a consequence, circumstantial evidence of Antibiotic-Resistant Bacteria expanding over the country is already being reported. As a reason, it's important to share information about the location of ARB in natural ecosystems that haven't been constantly exposed to antibiotics. The findings can also be utilized to improve antibiotic management in Chidambaram, Parangipettai, and Cuddaloredist. by pointing to the need for better water quality management in this area.
Key Words: *Antibiotic resistance, Marine, Clinical isolates, E.coli, RAPD analysis, Environment,Pollution*

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INTRODUCTION

Antibiotic-resistant bacteria (ARB) have evolved in medical institutions, and ARB-related nosocomial infections have become a global issue. Globally, 700,000 people die from ARB each year, with the figure expected to rise to 10 million by 2050 [1]. The increase in fecal pollution in source water is a menace not only in developing countries but also in developed countries. Waterborne bacterial pathogens viz., *E.coli, Salmonella, Shigella* , and *V.cholerae* can lead to outbreaks of intestinal diseases and result in serious health implications as well as economic loss [2]. Improper management of sewage as well as industrial wastes, hospital waste, and their entry into the waterways finally pollute the coastal waters. Heavy use of antibiotics for medical and veterinary purposes (3). As well the domestic and agricultural use of pesticides and related compounds caused significant antibiotic contamination of the natural environment and consequent development of resistance in communities [4]. Few studies demonstrated a statistically significant correlation between industrial pollution and the spatial distribution of antibiotic resistance [5].

The resistance developing in one part of the country, or indeed in the world, can be disseminated readily [6]. The problem of microbial drug resistance is a major public health concern due to its global dimension and alarming magnitude, although the epidemiology of resistance can exhibit a remarkable geographical variability and a rapid temporal evolution [7]. The major resistance issues overall are, those which are related to the methicillin-resistant *Staphylococcus aureus* (MRSA), *Vancomycin resistant enterococci* (VRE), Extended-spectrum β-lactamase producing *Enterobacteriaceae,* and the multidrug-resistant *P.aeruginosa* and *Acinetobacter baumannii* [8]. Antimicrobial resistance (AMR) is one of humanity's most serious problems. In the present investigation *E. coli,* an emerging pathogen was dealt with and the influence of clinical resistance on the marine environment was evaluated.

MATERIAL AND METHODS

Collection of clinical samples

Clinical *E.coli* isolates were collected from hospitals located in Chidambaram, Parangipettai and Cuddalore areas using sterile containers and were brought to the laboratory and stored at 4°C. All the samples were brought to the laboratory immediately and analyses were made.

Marine samples of *E. coli* strains were isolated from seawater and marine sediment. Typical isolates after biochemical identification were used for antimicrobial susceptibility testing.

Antimicrobial susceptibility Testing

Antibiotic susceptibilities of the isolates were determined by disk diffusion method using Muller Hinton agar and eleven antibiotics namely Ampicillin (AMP) - 25µg, Cefuroxime (CXM) - 30µg, Amoxcillin (AMC) - 30µg, Cefpodoxime (CPD) - 10µg, Cephalexin (CN) - 30µg, Doxycycline (DO) - 30µg, Levofloxacin (LE) - 5µg, Gentamicin (GEN) - 10µg, Ciprofloxacin (CF) - 5µg, Norfloxacin (NX) - 10µg and Ofloxin (OF) - 5µg. The results were interpreted using Clinical and Laboratory Standards Institute criteria (CLSI, 2006). The multiple antibiotic resistance (MAR) index of each strain was calculated according to the method described by Krumperman (1983) using the formula: a/b, where 'a' represents the number of antibiotics to which a particular isolate was resistant and 'b' the total number of antibiotics tested.

RAPD Analysis

About 20 marine strains (1-15 – marine water isolates and 16-20 marine sediments), and 32 clinical strains were undergone composite RAPD analysis. RAPD-PCR amplification reactions were performed in 50µl volumes in 0.2 mL optical-grade PCR tubes (Tarsons, India). Each 50µl reaction volume contained 1.5U of Taq DNA Polymerase, 1.5 mM MgCl2, 100µM of dNTPs (Genei, India), 100pM of random primer (5'- GTTTCGCTCC - 3'). 2µl crude DNA was used per reaction. The RAPD-PCR cycling conditions were, initial denaturation at 94ºC for 5 min. denaturation at 94ºC for 1 min., primer annealing at 36ºC for 1 min. and extension at 72ºC for 2 min. for a total of 35 cycles, followed by a final extension at 72ºC for 10 min. RAPD was performed in a Thermal cycler (Lark Research Model L125 +, India). The PCR amplification products were visualized by running 25µl of the amplified products on 1.5% agarose gel. The gel was stained with ethidium bromide and photographed under UV illumination. Gel images were digitally captured and analyzed using the application of Quantity one software (Bio-Rad). Based on Dice co-efficient analysis of analog densitometry scans of the RAPD profiles, similarity trees were constructed according to UPGMA.

RESULTS

Antibiotic Resistance of Clinical Strains

In the present study, 11 antibiotics belonging to 4 different classes were dealt with Ampicillin and Amoxcillin which are belong to beta-lactams whereas, cephalexin, cefuroxime, cefpodoxime respectively belong to first, second and third-generation cephalosporines which are another group of β-lactams. Among quinolones, levoflaxocin is an older generation quinolone and ciprofloxacin, norfloxacin, oflaxocin are belonged to fluroquinolones. Other two antibiotics such as Doxycycline and gentamicin are representing tetracycline group of antibiotics and aminoglycosides respectively. A total of 175 clinical *E. coli* strains were used for testing their antimicrobial susceptibility. Among them 100% resistance was observed for Ampicillin followed by Cephalexin (67.4%), Cefuroxime (59.4%), Cefpodoxime (58.2%), Ofloxin (54.2%), Amoxcillin (53.7%), Ciprofloxacin (53.1%), Doxycycline (52%), Levofloxacin (49.7%), Norfloxacin (31.4%) and Gentamycin (33.7%) (Figs. 1 and 2). The individual percentage resistance of UTI, stool and pus strains is also represented in (Table 1).

Table1: Antibiotic Resistant Pattern of *E.coli* isolated from different clinical samples

Antibiotics class	Antibiotic	No.of strains Positive out of 175 Tested	Resistance %	*E.coli* isolates from different clinical samples					
				UTI		Stool		Pus	
				No.of samples	%	No.of samples	%	No.of samples	%
β-Lactam	Ampicillin(AMP)	175	100	88	100	70	100	17	100
	Amoxcillin(AMC)	94	53.7	53	60.22	33	47.14	8	47.05
Cephalosporine	Cephalexin(CN)	118	67.4	85	96.59	30	42.85	3	17.64
	Cefuroxime(CXM)	104	59.4	70	79.5	30	42.85	4	23.52
	Cefpodoxime(CPD)	102	58.2	65	73.86	32	45.71	5	29.41

OlderQuinolone	Levofloxacin(LE)	87	49.7	40	45.45	44	62.85	3	29.41
Fluoroquinolone	Ciprofloxacin(CF)	93	53.1	45	51.13	44	62.85	4	23.52
	Norfloxacin(NX)	55	31.4	26	29.54	26	37.14	3	29.41
	Ofloxin(OF)	95	54.2	45	51.13	45	64.28	5	29.41
Tetracycline	Doxycycline(DO)	91	52	76	86.36	10	14.28	5	29.41
Aminoglycoside	Gentamicin(GEN)	59	33.7	27	30.68	28	40	4	23.52

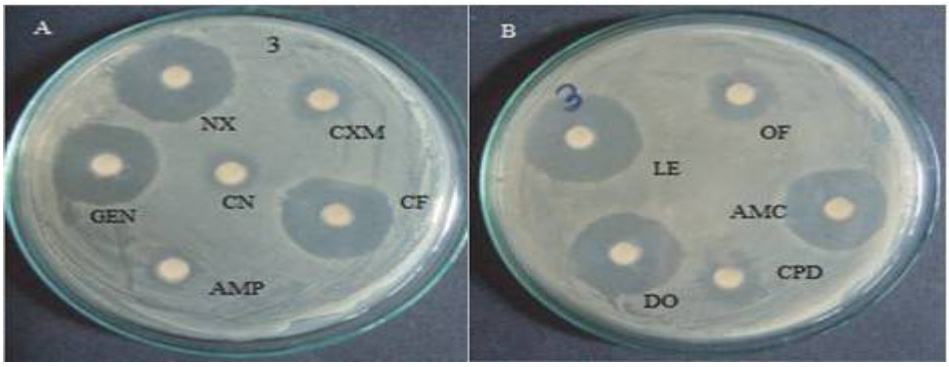

Fig 1: Clinical isolate antibacterial susceptibility testing for isolate no.3 (Plate A & B) for tested 11 antibiotics viz. Ampicillin (AMP)- 25 µg, Cefuroxime (CXM) – 30 µg, Amoxcillin (AMC) - 30 µg, Cefpodoxime (CPD) - 10 µg, Cephalexin (CN) -30 µg, Doxycycline (DO) – 30 µg, Levofloxacin (LE)- 5 µg, Gentamicin (GEN) – 10 µg, Ciprofloxacin (CF) - 5 µg, Norflocacin (NX) - 10 µg and Ofloxin (OF) - 5 µg

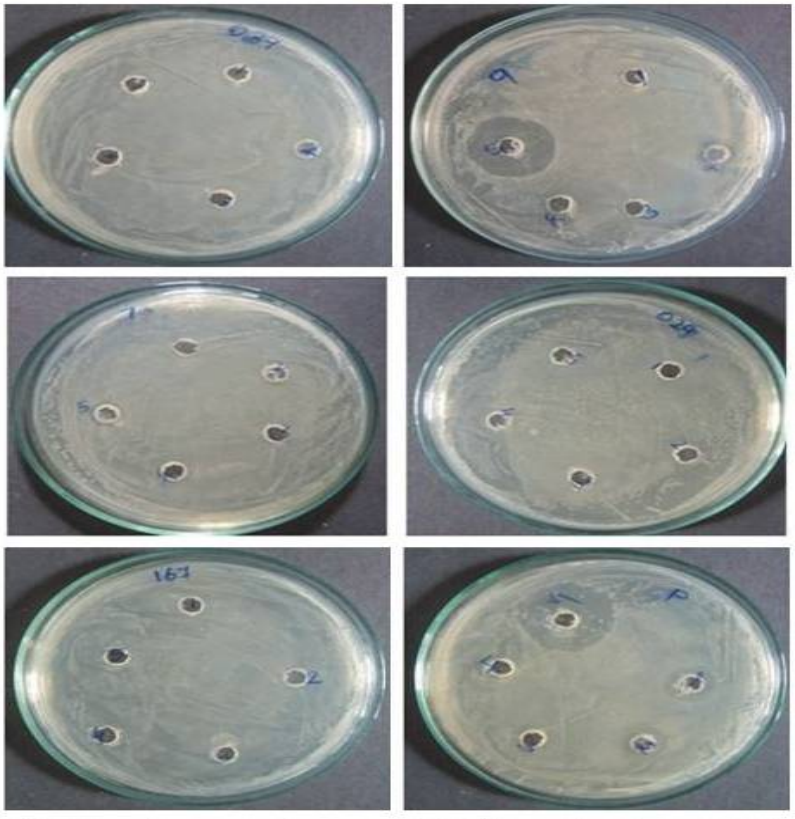

Fig 2: Multiple antibiotic resistances of *E.Coli* isolates from clinical samples (isolates 039,9,73,029,167 and P) to different antibiotics (Well No. 1-5) tested by Well Diffusion Method

In this study, MAR index for the isolates was calculated and the range was observed from 0.18-0.9. About 15.6% of the strains showed a MAR index of 0.36. Likewise 22% showed 0.46, 15.6% showed 0.55, 22%

showed 0.73 and 15.6% showed 0.9 as MAR index. The isolates with a MAR index of 0.9 were found to be resistant to 10 antibiotics (Table 2).

Table2:Antibiotic resistance pattern and multiple antibiotic resistant index of *E.coli* isolates from clinical samples

S.No	Antibiotic resistant pattern (ARP)	No. of isolates showed similar ARP	% of resistance	MAR index
1	AMP	15	8.5	-
2	AMP-CPD-CN-DO	6	3.4	0.36
3	AMP-CXN-AMC-CPD	3	1.7	0.36
4	AMP-CXN-CPP-NX	5	2.8	0.36
5	AMP-CN-DO-GEN	12	6.4	0.36
6	AMP-CXM-AMC-CPD-CN	5	2.8	0.46
7	AMP-AMC-DO-LE-GEN	3	1.7	0.46
8	AMP-CXM-LE-CF-OF	5	2.8	0.46
9	AMP-AMC-CN-GEN-OF	6	3.4	0.46
10	AMP-CN-DO-GEN-CF	3	1.7	0.46
11	AMP-CXM-CPD-CF-NX	3	1.7	0.46
12	AMP-DO-CF-NX-OF	5	2.8	0.46
13	AMP-CXM-AMC-CPD-CN-DO	8	4.5	0.55
14	AMP-CXM-AMC-DO-GEN-CF	8	4.5	0.55
15	AMP-CXM-DO-LE-CF-NX	5	2.8	0.55
16	AMP-CXM-CN-LE-GEN–OF	5	2.8	0.55
17	AMP-CXM-CPD-CN-DO-GEN	4	2.2	0.55
18	AMP-AMC-CPD-CN-LE-CF-OF	18	10.2	0.64
19	AMP-CXM-AMC-CPN-CN-DO-GEN	5	2.8	0.73
20	AMP-CXM-AMC-CPN-CN-LE-CF-OF	4	2.2	0.73
21	AMP-CXM-CPD-DO-LE-CF-NX-OF	5	2.8	0.73
22	AMP-CXM-CPD-CN-LE-CF-NX-OF	3	1.7	0.73
23	AMP-CN-DO-LE-GEN-CF-NX-OF	5	2.8	0.73
24	AMP-CXM-AMC-CPD-CN-LE-GEN-CP-OF	10	5.7	0.82
25	AMP-CXM-AMC-CPD-CN-DO-LE-CF-NX-OF	24	13.7	0.9

Regarding antibiotic resistant pattern, 8.5% of the isolates showed resistance to single antibiotic (AMP). Whereas 3.4%, 1.7%, 2.8% and 6.4% of the isolates showed resistant to 4 antibiotics with the antibiotic resistant pattern of AMP-CPD-CN-DO, AMP-CXN-AMC-CPD, AMP-CXN-CPP-NX and AMP-CN-DO-GEN respectively. Similarly different resistance pattern to 5 antibiotics of 2.8%, 1.7%, 2.8%, 1.7%, 1.7% and 2.8% was observed with patterns AMP-CXM-AMC-CPD-CN, AMP-AMC-DO- LE-GEN, AMP-CXM-LE-CF-OF, AMP-AMC-CN-GEN-OF, AMP-CN-DO-GEN-CF, AMP-CXM-CPD-CF-NX and AMP-DO-CF-NX-OF respectively. 13.7% of the isolates showed resistance to 10 antibiotics with the resistant pattern of AMP- CXM-AMC-CPD-CN-DO-LE-CF-NX-OF (Table 2).

Antibiotic Resistant Pattern of Marine Strains

In marine environment, among 150 E.coli strains 12.5% of the strains showed resistance to single antibiotic (i.e) 5.3 % to AMP, 3.3% to CPD 2.6% to CXM and 1.3% towards CN (Figs. 3 and 4, Table 3). The resistant patterns were tabulated in table 4. The MAR index for the marine isolates was calculated and the range was observed from 0.18-0.64 (Table 4).

Table 3: Antibiotics resistance percentage of marine isolates

S.no	Antibiotic tested	No. of strains positive	Percentage of resistance
1.	AMP	99	66%
2.	CXM	51	34%
3.	AMC	23	15.3%
4.	CPD	68	45.3%
5.	CN	58	38.6%
6.	DO	0	0%
7.	LE	40	26.6%
8.	GEN	29	19.3%
9.	CF	40	26.6%
10.	NX	29	19.3%
11.	OF	40	26.6%

Table 4: Antibiotic resistance pattern and percent resistant to antibiotics of *E.coli* Isolates from marine samples

S.no	Antibiotic resistant pattern of marine strains	No. of strains	Percentage of resistance	MAR index
1.	AMP	8	5.3%	-
2.	CPD	5	3.3%	-
3.	CN	2	1.3%	-
4.	CXM	4	2.6%	-
5.	AMP-CPD	4	2.6%	0.18
6.	AMP-CN	7	4.6%	0.18
7.	CXM-CPD	2	1.3%	0.18
8.	CPD-CN	16	10.6%	0.18
9.	CXM-AMC	8	5.3%	0.18
10.	AMP-CXM	24	16%	0.18
11.	AMP-CPD-CN	15	10%	0.28
12.	AMP-CXM-CPD	6	4%	0.28
13.	CXM-CPD-CN	2	1.3%	0.28
14.	AMP-CXM-CPD-CN	3	2%	0.36
15.	AMP-CXM-AMC-CPD	2	1.3%	0.36
16.	AMP-CXM-AMC-CPD-CN	2	1.3%	0.45
17.	LE-GEN-CF-NX-OF	2	1.3%	0.45
18.	AMP-LE-GEN-CF-NX-OF	17	11.3%	0.54
19.	CN-GEN-LE-CF-NX-OF	10	6.6%	0.54
20.	AMP-AMC-CPD-CN-LE-CF-OF	11	7.3%	0.64

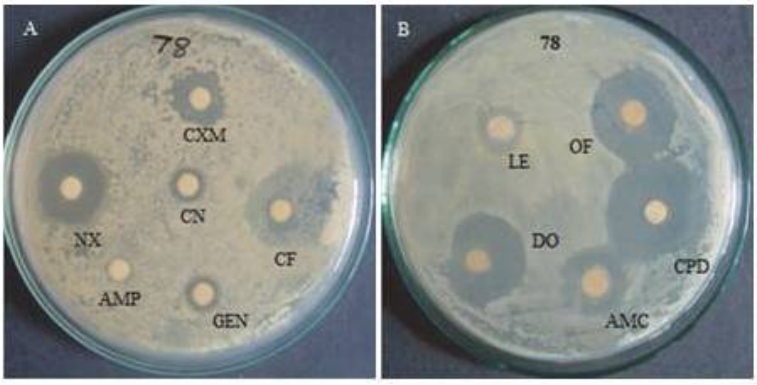

Fig 3: Marine strain antibacterial susceptibility testing for marine isolate no.78 (Plate A & B) for tested 11 antibiotics viz.. Ampicillin (AMP)- 25 µg, Cefuroxime (CXM) – 30 µg, Amoxcillin (AMC) - 30 µg, Cefpodoxime (CPD) - 10 µg, Cephalexin (CN) -30 µg, Doxycycline (DO) – 30 µg, Levofloxacin (LE)- 5 µg, Gentamicin (GEN) – 10 µg, Ciprofloxacin (CF) - 5 µg, Norflocacin (NX) - 10 µg and Ofloxin (OF) - 5 µg

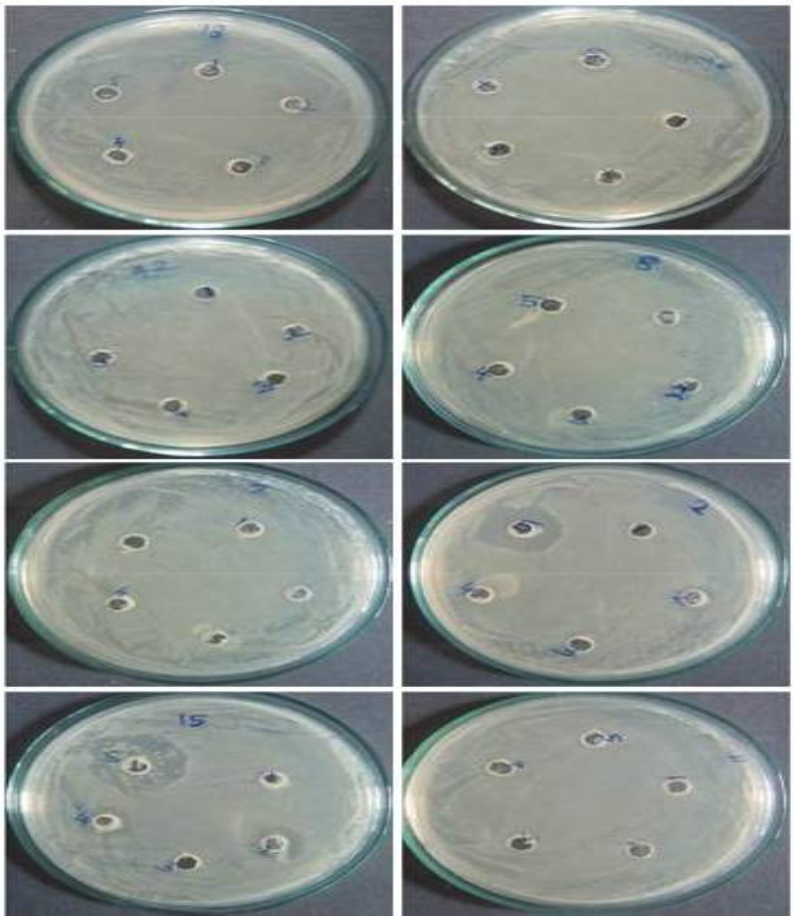

Fig 4: Multiple antibiotic resistances of *E.Coli* isolates from marine environmental samples (isolates 13, 38 ,42 ,8 ,7 ,2 ,15 and 11) to different antibiotics (Well No. 1-5) tested by Well Diffusion Method

RAPD Analysis

In the present work above 80% similarity was obtained in 27 clusters were obtained in which 5 pairs were in combination of marine and clinical strains.(27,11; 27,1; 20,23; 19,38; 6,28). The strains of marine environment and clinical isolates showed >90% genomic similarity. This directly confirms that clinical strains are entering into marine environment and survive for limited (or) extended periods. Above 40% similarity, 13more clusters were observed, <40% similarity were considered as totally non-similar/different single isolates (52, 32; 48,45; 42,19).

RAPD analysis showed among mine water strains the percentage similarity varied from 0-93% whereas among sediment strains it varied between 0-73. %. Among clinical strains 0-90% similarity was observed. However, in clinical strains 0% similarity was represented by more number of strains. (Figs. 5 and 6 a&b).

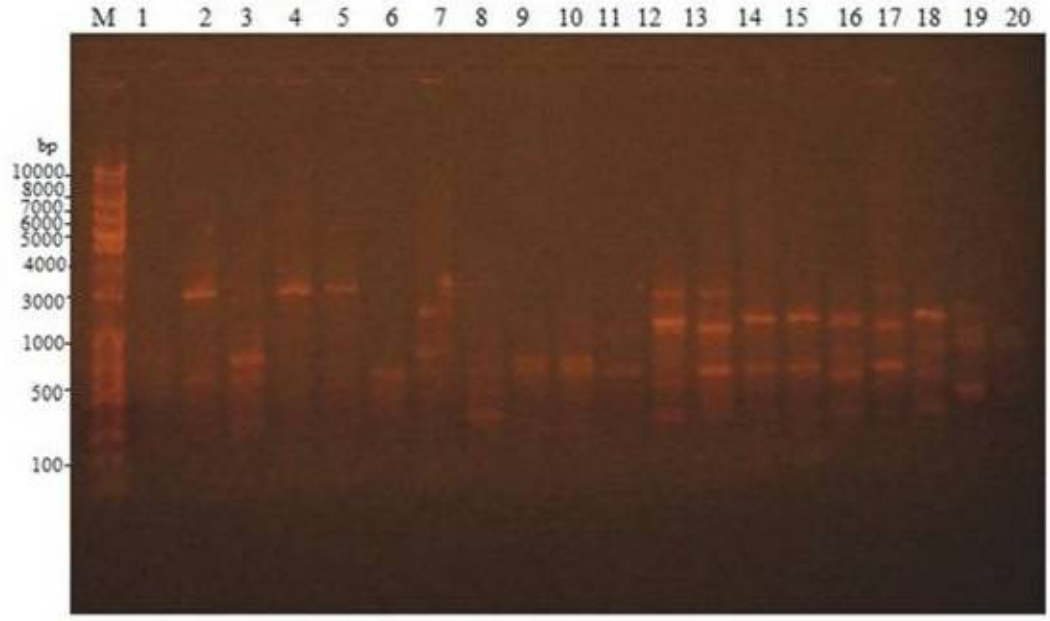

Fig 5: Showing RAPD analysis of marine isolates; Lane M: 1kb DNA Ladder; Lane 1-20: RAPD analysis of marine isolates; 1-15 (Water samples) and 16 -20 (Sediment samples)

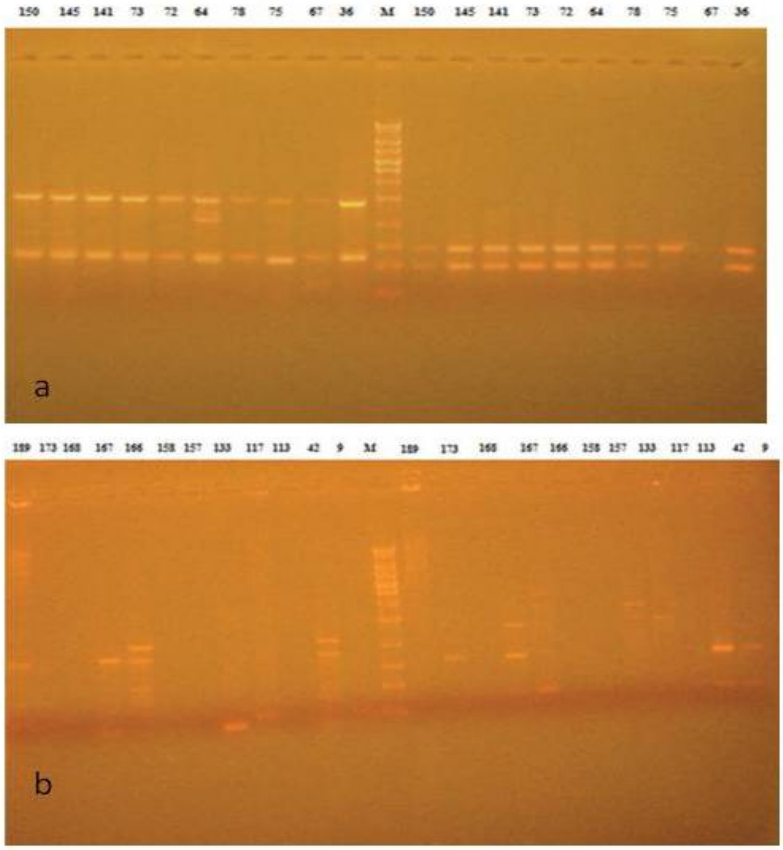

Fig 6 a & b : Showing RAPD analysis of clinical isolates; Lane M: 1kb+ 100bp DNA Ladder mix; Lane 150 - 36 (Gel A) & 189 -9 (Gel –B) RAPD analysis of clinical isolates:

DISCUSSION

In the present investigation, when the clinical strains were compared with the environmental isolates they exhibited lower percentage of resistance to all the 11 tested antibiotics. The results of environmental isolates to another study done on clinical isolates in the nearby area and they found the resistant pattern of both isolates were strongly correlated which endorsed that present results [9]. However, the clinical isolates exhibited higher resistance against most of the antibiotics tested which again supported the present results. They also found the enteric bacteria isolated from river water nearer to the industrial sites showed higher level of resistance against several antibiotics compared to other sites (i.e) environmental industrial and or human activities may impact on the microbial resistance to antibiotics of a particular environment [10]. The higher resistance of Enterobacteriaceae to rifampicin in their study coincided with the high resistance of Mycobacterium tuberculosis isolated from TB patients in this region to the same antibiotic (19.3%), (i.e) disease prevalence in a particular area and antibiotics used to cure the disease seemed to be directly influencing the nearby areas, especially aquatic environments. That might be true in the present study area also [11]. The low resistance of *E.coli* strains to norflaxacin (31.4%) and gentamicin (33.7%), in the present study, might be due to the fact they were not frequently used. Uncontrolled use of certain wide-spectrum antibiotics seemed to be responsible for the development of resistant phenotypes in the bacterial population [12, 13]. The resistance to β- lactams including cephalosporin could be due to this reason. This was observed by many researchers worldwide [14].

In the present study, the frequency of resistance to more than one antibiotic was 91.5% observed it was 85% [15]. As 81% which indicates the antibiotic resistance increases rapidly year by year [16]. In the present study, 53.1% of resistance was observed towards ciprofloxacin. In recent years, due to resistance developed to most of the β-lactam antibiotics ciprofloxacin became the first-line drug [17]. Due to this enhanced empirical use, monotherapy with the cheapest drug available and treatment interruption before complete recovery may be the reasons behind the newer patterns of drug resistance development [18].

[19] In long surveillance of *E. coli* in UTI, found an increasing trend of resistance to gentamycin, fluoroquinolones, and cephalosporin's, which was reflected in the results of the present study also. The development of resistance was faster towards fluoroquinolones and cephalosporin than gentamycin, whereas chloramphenicol resistance showed a downward trend [20]. Amikacin and nitrofurantoin were observed as the most effective antibiotics with only 10% and 28% resistant levels over a period of time when they were used.

The result obtained in the present study indicated local resistant patterns, as well as specific patient antimicrobial and microbiologic history, should be given due importance in treatment not only for the early recovery of the patient but also to prevent community-based dissemination of MAR strains.

In all these studies though the level of antibiotic resistance and density of *E.coli* and other pathogens varied, the distribution of MAR strains was observed in all waters irrespective of developed (or) underdeveloped countries [21]. This is because of the widespread use of various antibiotics for clinical, veterinary, and agriculture purposes worldwide. So environmental antibiotic concentration may exert selective pressure on environmental bacteria and may also faster the transfer of resistant genes, helping to recreate the "resistome" mixing pot of genetic AMR traits worldwide [22].

The higher level of antibiotic resistance in both clinical and marine environments reflected the abuse (or) misuse of antibiotics during the treatment of bacterial infection in the area selected (23). The highest MAR index of 0.9 and 0.64% strains in the present investigation emphasize the public health risk of coastal as well as freshwater environs under study.

In the area of the present study, cows generally graze in open fields especially in Pichavaram mangrove area as well as the coastal beaches of Parangipettai and Cuddalore [24]. It is customary in this area that cattle rearing is not restricted to farms or in restricted areas. They drink water from open water sources like a pond, stagnant water,etc., so that they may play a role in the development of resistant *E.coli* in the gut of cattle which may easily enter into the nearby estuarine waters [25].

In addition, empirical therapy recommended by health care personnel is the rule and antimicrobial susceptibility is not performed on individual patients [26, 27]. Poverty is closely linked with chronic bacterial and viral diseases [28, 29], which leads to inappropriate selection of antibiotic resistance, especially against wide spectrum antibiotics. Aquafarms are also present on the banks of Vellar, Paravanar, Uppanar as well as in the Pichavaram mangrove areas and they are indiscriminately using a variety of antibiotics which may ultimately find their way directly into the estuaries. This may have direct impact on the culture and capture fisheries of the coastal waters under study and may influence the water as well as sea food quality [30].

When ≥80% similarity is observed in RAPD profiles that are considered as the reproducibility limit of the same isolate [31]. (i.e.) when the same isolates are not used (or) when the isolates are not from the same sample that can be considered of originated from the same strain and transported from somewhere else. In the present work above 80% similarity was obtained in 27 clusters obtained in which 5 pairs were in a combination of marine and clinical strains 27,11; 27,1; 20,23; 19,38 and 6,28. The strains of the marine environment and clinical isolates showed higher genomic similarity. This directly confirms that clinical strains are entering into the marine environment and survive for limited (or) extended periods and vice versa. Above 40% similarity, 13 more clusters were observed, <40% similarity were considered as totally non- similar/different single isolates (52,32; 48,45 and 42,19).

RAPD analysis showed among marine water strains, the % similarity varied from 0-93% whereas among sediment strains it varied between 0-73.3%. Among clinical strains, 0-90% similarity was observed. However, in clinical strains 0% similarity was represented by more number of strains. When clinical and marine strains were compared the similarity varied from 0-90%. The data (Raw data not given) clearly indicated genetically the strains were highly diverse irrespective of the sample. (i.e.) even from the same source, in a single sampling, *E. coli* strains were genetically highly varied.

The same was observed E.*coli* isolated from retail meat, human stool, and clinical specimens were compared [32]. Diverse samples/different strains collected from the same type of samples at a different time (or) Vice versa showed a wide range of variation among the strains. The opposite (i.e.) unrelated samples collected at different periods of time showed more than 80% (or) even >90% similarity. This result should be critically viewed as they may be transported from the hospital environment to marine (or) through sewage (or) storm water input. Their spatial and temporal variation indicated the possibility of their extended survival in the marine environment.

CONCLUSION

As there are more chances of interaction between *E. coli* from the natural environment (soil/terrain and water), humans, and animals the resistant pattern in the clinical as well as marine strains in the present investigation clearly endorsed this fact. Thus the present study suggested the need for better water quality management in this area. In addition, the findings obtained in the present study can also be used for improving antibiotic management in this district.

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COMPETING INTEREST

The authors have declared that no competing interest exists.

REFERENCES

1. O'Neil, J. (2014). Review on antibiotic resistance. Antimicrobial resistance: Tackling a crisis for the health and wealth of nations. *Health Wealth Nations*. December, 1–16. [(accessed on 12 May 2020)]; Available online: http://www.jpiamr.eu/wp-content/uploads/2014/12/AMR-Review-Paper-Tackling-a-crisis-for-the-health-and-wealth-of-nations_1-2.pdf.
2. Gwimbi, P., George, M., & Ramphalile, M. (2019). Bacterial contamination of drinking water sources in rural villages of Mohale Basin, Lesotho: exposures through neighborhood sanitation and hygiene practices. *Environmental health and preventive medicine*, *24*(1), 33. Uhttps://doi.org/10.1186/s12199-019-0790-z
3. White, D.G., Piddock, L.J.V., Maurer, J.J., Zhao, S., Ricci, V., and Thayer, S.G. (2000). Characterization of fluoroquinolone resistance among veterinary isolates of avian *Escherichia coli. J. Antimicrobiol*., *Agents Chemother*., 44 (10) 2897- 2899.
4. Balague, C. and Garcia-Vescovi, E.(2001). Cultivation of multiple antibiotic resistancein uropathogenic *Escherichia coli* strains by aryloxoalcanoic acid compounds. *Antimicrob. Agents Chemother*., 45 . 1815-1822.
5. Goni-Urriza, M., Capdepuy, M., Arpin, C., Raymond, N., Caumette, V., and Quentin, C. (2000). Impact of an urban effluent on antibiotic resistance of riverine *Enterobacteriaceae* and *Aeromonas* spp. *Appl. Environ. Microbiol*., 66 (1) 125-132.
6. Greenwood, D. (1998). Resistance to antimicrobial agents: A personal view. *J. Med.Microbiol.,* 47 751-5.
7. McArthur, J.V., & Tuckfield, R.C.(2000). Spatial patterns in antibiotic resistance among stream bacteria: Effects of industrial pollution. *Appl. Environ.Microbiol.,* 66 (9) 3722–3726.
8. Rossolini, G.M. and Mantengoli, E. (2008). Antimicrobial resistance in Europe and its potential impact on empirical therapy. *Clin . Microbiol. Infect.* 14 (6) 2–8.
9. Lin, J., Biyela, P.T., & Puckree, T.(2004). Antibiotic resistance profiles of environmental isolates from Mhlathuze River, KwaZulu-Natal (RSA). *WaterSA*.,30(1) 23-28.

10. Biyela, P.T. (2000). A study of causes of diarrhoea in the Northern KwaZulu-Natal.Honours dissertation, University of Zululand.
11. Manyi-Loh, C., Mamphweli, S., Meyer, E., & Okoh, A. (2018). Antibiotic Use in Agriculture and Its Consequential Resistance in Environmental Sources: Potential Public Health Implications. *Molecules (Basel, Switzerland)*, *23*(4),795. https://doi.org/10.3390/molecules23040795.
12. Roger, F.G., Greenwood, D., Norrbby, S.R., Whistley, R.J.(eds.)(2003) . Antibiotic and *Chemotherapy, the Problem of R*esistance. 8th Edn., Churchill Livingstone, pp: 25-47.
13. Madingan, M.T., &Martinko, J.M. (2007). Biology of Microorganisms. 11th Edn., Pearson Education. Paris, France, pp: 1047.
14. Bisht, R., Katiyar, A., Singh, R., & Mittal, P., (2009). Antibiotic resistance: A global issue of concern. *Asian J. Pharm. Clin. Res*., 2 34-39.
15. Wang, Q., Ruiyuan, Lv., Rene, E.R., Qi, Xiaoyu., Hao, Q., Du, Y., Zhao, C., Xu, Fei., and Kong, Qiang (2020). Characterization of microbial community and resistance gene (CzcA) shifts in up-flow constructed wetlands-microbial fuel cell treating Zn (II) contaminated wastewater. *Bioresource Technology*, Volume 302;122867,ISSN 0960-8524.
16. Ventola, C.L. (2015). The antibiotic resistance crisis: part 1: causes and threats. P T. Apr;40(4):277-83. PMID: 25859123; PMCID: PMC4378521.
17. Davies, J., & Davies, D.(2010). Origins and evolution of antibiotic resistance. *Microbiology and molecular biology reviews: MMBR*, *74*(3), 417–433. https://doi.org/10.1128/MMBR.00016-10
18. Seung, K. J., Keshavjee, S., & Rich, M. L.. *(2015).* Multidrug-Resistant Tuberculosis and Extensively Drug-Resistant Tuberculosis. *Cold Spring Harbor perspectives in medicine*, *5*(9), a017863. https://doi.org/10.1101/cshperspect.a017863
19. Mandal, J., Acharya, N.S., Buddhapriya, D., Parija, S.C. (2012). Antibiotic resistance pattern among common bacterial uropathogens with a special reference to ciprofloxacin-resistant*Escherichia coli. Indian J Med Res.* Nov;136(5):842-9. PMID: 23287133; PMCID: PMC3573607.
20. Fair, R. J., & Tor, Y. (2014). Antibiotics and bacterial resistance in the 21st century. *Perspectives in medicinal chemistry*, *6*, 25–64. https://doi.org/10.4137/PMC.S14459
21. Manyi-Loh, C., Mamphweli, S., Meyer, E., & Okoh, A. (2018). Antibiotic Use in Agriculture and Its Consequential Resistance in Environmental Sources: Potential Public Health Implications. *Molecules (Basel, Switzerland)*, *23*(4), 795. https://doi.org/10.3390/molecules23040795
22. Brown, M., & Balkwill, David. (2008). Antibiotic Resistance in Bacteria Isolated from the Deep Terrestrial Subsurface. *Microbial Ecology*, 57(3):484-93. DOI:10.1007/s00248-008-9431-6
23. Kraemer, S. A., Ramachandran, A., & Perron, G. G. (2019). Antibiotic Pollution in the Environment: From Microbial Ecology to Public Policy. *Microorganisms*, *7*(6), 180. https://doi.org/10.3390/ microorganisms 7060180
24. Peter, V., Emily, G., Suraj, G., Seju, K., Ayella, M.M., and Ni Zhu. (2019). Differential Drivers of Antimicrobial Resistance across the World. *Accounts of Chemical Research* 52(4), 916-924.DOI: 10.1021/acs.accounts.8b00643.
25. Selvam, V., Ravichandaran, K.K., Karunakaran, V.M., Mani, K.G., Beula, E.J., & Gnanappazham, L. (2010). Pichavaram mangrove wetlands: Situation Analysis, Chennai, 39 pp
26. Jang, J., Hur, H.G., Sadowsky, M.N., Byappanahalli, T., Yan, S. (2017). Environmental Escherichia coli: ecology and public health implications – a Review. *Journal of Applied Microbiology*. Volume 123 (3);570-581.https://doi.org/10.1111/jam.13468
27. Wise, R., Hart, T., Cars, O., Streulens, M., Helmuth, R., Huovinen, P., & M. Sprenger. (1998). Antimicrobial resistance is a major threat to public health. *BMJ.*, 317 ; 609-610.
28. Fasehun, F. (1999).The antimicrobial paradox: Essential drug's effectiveness and cost. *WHO.* 77 211-216.
29. Cizman, M. (2003). The use and resistance of antimicrobials in the community. *Int. J.Antimicrob. Agen.,* 21; 297-307.
30. Newman, M.J., Frimpong, E., Donkor, E.S., Opintan, J.A., & Asamoah-Adu, A. (2011). Resistance to antimicrobial drugs in Ghana. *Infect Drug Res.,* 4; 215-220.
31. Jessica, A., Gephart, T,M., Patrik, J.G., Henriksson, M. C.M., Beveridge, Verdegem, M., Metian, M., Lara, D. M., Lisa Deutsch. (2017). The `seafood gap' in the food-water nexus literature—issues surrounding freshwater use in seafood production chains. *Advances in Water Resources,* Volume 110 505-514, ISSN 0309-1708, https://doi.org/10.1016/j.advwatres.2017.03.025.
32. Hannah, E.L., Johnson, J.R., Angulo, F., Haddadin, B., Williamson, J., & Samore, M.H. (2009). Molecular Analysis of Antimicrobial-Susceptible and –Resistant *Escherichia coli*from Retail Meats and Human Stool and Clinical Specimens in a Rural Community Setting. *Foodborne Pathog Dis.*, 6(3) 285–295.
33. Karlowsky, J. A., Hoban, D. J., Decorby, M. R., Laing N. M., & Zhanel, G. (2006). Fluoroquinolone-resistant urinary isolates of *Escherichia coli* from outpatients are frequently multidrug-resistant: results from the North American urinary tract infection collaborative alliance-quinolone resistance study. *Antimicrob. Agents Chemother*., 50(6): 2251–2254.
34. Nishimura, E., Nishiyama, M., Nukazawa, K., & Suzuki, Y. (2021). Comparison of Antibiotic Resistance Profile of *Escherichia coli* between Pristine and Human-Impacted Sites in a River. *Antibiotics (Basel, Switzerland)*, 10(5), 575. https://doi.org/10.3390/antibiotics10050575

35. Sahoo, K.C., Tamhankar, A.J., Sahu, P.S., Klintz S.R., & Lundborg, C. S. (2012). Geographical variation in antibiotic-resistant E*scherichia coli* isolates from stool, cow-dung and drinking water. *Int. J. Environ. Res. Public Health*.9(3) ; 746–759.
36. Van der Starre, W. E., Nieuwkoop, C.V., & Paltansing. C. S.(2011). Risk factors for fluoroquinolone-resistant *Escherichia coli* in adults with community-onset febrile urinary tract infection. *J. Antimicrob. Chemother*.,66(3) ; 650–656.
37. Beceiro, A., Tomás, M., & Bou, G. (2013). Antimicrobial resistance and virulence: a successful or deleterious association in the bacterial world?. *Clinical microbiology reviews*, *26* (2), 185–230. https://doi.org/ 10.1128/ CMR.00059-12

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