Probiotic attribution of Lactobacillus spp. Against MDR E.coli.

Agrawal Nikhil*, Ukesh Chandrikiran. Shendre Latika and Patil Sahedaorao
Dept of Microbiology,
B.S.Patel ACS College, Pimpalgaon Kale.
Shri Shivaji Science College, Amravati.

Abstract:- Infection and the therapy against it is the processes, but in therapy use of antibiotic and the development of MDR (multi drug resistance) is the common and most challenging problem in day today's life in front of medical practitioners as well as the for scientists. In the present study the MDR e.coli were isolated from the patients suffering from gastrointestinal diseases. In the present study out of total 663 samples 335 samples were positive. While out of them 264 were resistance and 23 were sensitive and remaining were intermediate. For lactobacillus isolation total 180 samples were screened out of them 84 were isolated. The Probiotic potential for these isolated lactobacillus were tested for antibiotic resistance, heat, pH and bile concentration. Out of them 9 samples shows positive results. The bacteriocin produced by these isolates were screened against MDR E.coli. Out of them 3 samples shows excellent probiotic properties.

Key words:- Enteric pathogen, E.coli, MDR, Lactobacillus, Probiotic.

Introduction:-

As the discovery of first antibiotic was the new weapon against pathogens and it was useful for the patients suffering from various diseases (Saga and Yamaguchi., 2009). But after some time, the over use and misuse of antibiotics in developing countries leads to the antibiotic resistance in the organisms (Roy, 1997; Yoneyama and Katsumata, 2006).

In the present study the enteric pathogens showing MDR are selected. From the ancients time we are using the Lactobacillus as a food source so for the betterment of human being and for society everyone is thinking to find the alternative for antibiotics. Lactobacilli possess two major advantages in that some of them are known to be probiotics and secondly, they also possess the GRAS status. (Generally Recognized as Safe) therefore we have examined the MDR pathogens isolated from our study against isolated lactobacillus spps and the resistance due to these organism was studied. To cure the human being and for society it's a need to find the alternative for antibiotics and it's a time to think as a drug towards it. (Patil *et al*, 2010; Mithun *et al*, 2015; Khandare *et al*., 2016).

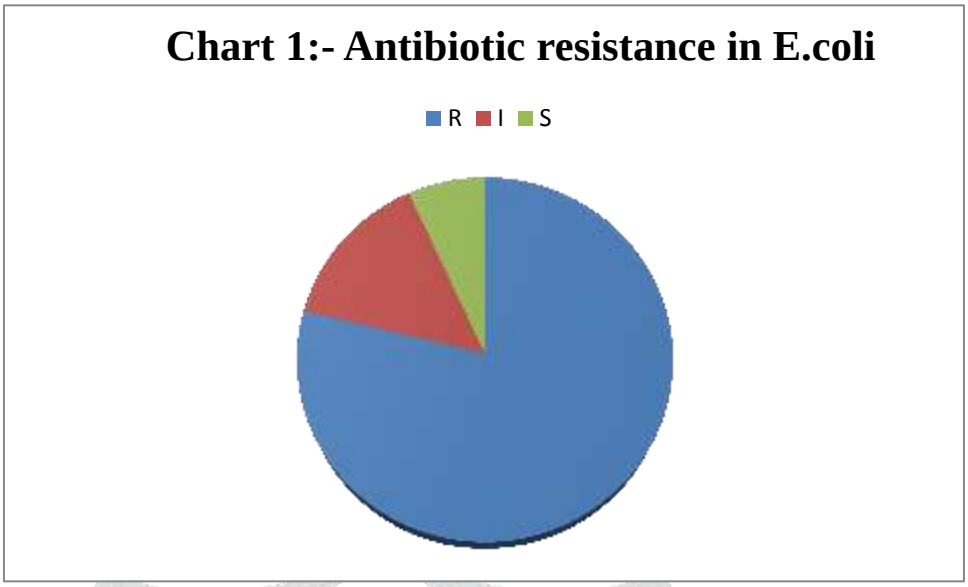
Material and Methods:-

Stool samples were collected from the hospitalized patients with enteric disease. The stool samples were collected in a sterilized container and immediately proceed for further isolation processes.(Sharma RM *et al*,1969, Ruiz J *et al* 1996). On the basis of morphological, cultural and biochemical characterization the microorganisms were screened. The selected isolates then further screened for their antimicrobial activity as per the CLSI (2014) guidelines.

The E.coli were isolated by standard convensnal method and the organism were characterized on the basis of morphological, cultural and biochemical methods. The antibiotic susceptibility were done by Kirby Bauer disk diffusion method as per the guideline of CLSI. While for isolation of Lactobacillus MRS agar were used and the organism were characterized on the basis of morphological, cultural and biochemical methods. In probiotic potential screening the antimicrobial susceptibility were done by disk diffusion method while for heat, pH and bile conc. test the Lactobacillus were survived in different temperature, pH conc. and bile salt conc. were selected.

Results and Discussion:-

Out of total 663 samples 335 isolates were separated for E.coli. In the Kipkorir *et al.*, (2016) study 90.2% isolation rate of E.coli was reported in the epidemiological study in Keniya isolated from the same fecal sources. Amira M, Zakaria *et al*., (2015) from Egypt isolates 65.3% of E.coli in different stool samples. In Nigeria by Akinnibosun and Nwafor (2015) a study was made in that out of 50 samples 62.58% *E.coli* was occurred. Alikhani *et al.*, (2013) also collected the 187 stool samples and reported 21.4% positive samples for *E.coli* out of them 67.5% were MDR.



Out of 335 isolates the antimicrobial susceptibility results shows 264 were found to be resistance and 23 sensitive and remaining intermediate. In the antibiotic therapy the antibiotic from the β-lactum group was Ampicillin, in this study 95% resistance rate was observed which is very much higher than that of the study carried out by Sarshar *et al*., (2014) according to them the 36.11% resistance was reported. The lower resistance rate was reported by Rigobelo *et al*., (2006) that is 41.0%, followed by 55.6% by Akingbade *et al.,* (2014), 75% by Zakiria *et al*., (2015), while 84% resistance rate reported by two studies in 2014 and in 2016 by Ali *et al*., (2014) and Kipkorir *et al*., (2016). The Alikhani *et al*., (2013) reported 87.5% while 90.7% Manikandan and Asmath ., (2013). Our results somewhat correlates with the Tawfick *et al.,* (2016) it becomes 93.1%. The higher resistance rate than that of our findings was reported by Moini *et al.,* (2015) by 97%.

The resistance rate of Amoxyclav in this study was 90% which is higher than that of 55.3% Tawfick *et al.,* (2016), but exactly similar to the study of Deshmukh and Ukesh (2014) study reported 90.56% resistance rate, similarly the resistance rate of Cefoperazone/sulbactum was 80% in our findings while, Dshmukh and Ukesh (2014) reported less resistance than that of present finding which is 13.20%.

In the enteric pathogens Cephalosporin group of antibiotics was the primary choice of drug in medical practitioners. In this study the number of generation of this group was studied. The antibiotic resistance for Cephalexin was founded 100% while 94% resistance was found to Ali *et al.,* (2014), similarly for Cephalothine 90% resistance rate was found, while for Cephaloridine 85% resistant was identified, the lower resistance rate was reported by Rigobelo *et al.,* (2006) which is 46.1%.

The resistance rate of 51.6% for Cefpodoxime was reported by Tawfick *et al.,* (2016) which is lower than that of our findings 75%. Similarly for Cefuroxime 80% resistance was found while higher that is 100% resistance rate was reported by Egbule *et al.,* (2016) followed by lower rate that is 38.3% by Akingbade *et al.,* (2014).

The antibiotic resistance for Cefixime was 75% in our study while Egbule *et al.,* (2016) reportes higher resistance than that of our findings that is 100% while lower resistance reported by Ali *et al.,* (2014) that is 54% followed by 40.7% by Akingbade *et al.,* (2014) 37.5% by Alikhani *et al.,* (2013). 30.56% and 14% resistance rate reported by Sarshar *et al.,* (2014) and Manikandan and Asmath., (2013) respectively.

The resistance rate of Ceftriaxone was 80% which is higher than other studies 33.3% by Akingbade *et al.,* (2014), 16.67% by Sarshar *et al.,* (2014), 17.5% by Alikhani *et al.,* (2013) and 38.8% by Moini *et al.,* (2015) respectively. Similarly for Cefepime 90% resistance rate was studies in this study which is higher than the findings of Deshmukh and Ukesh (2014) study they reported 20.75% resistance rate, followed by 85% resistance for Cefpirome

The Imipenem was most effective drug found in this study with only 5% resistance rate which is much lower than that of 29% reported by Zakiria *et al.,* (2015) and correlates with 5.56% reported by Sarshar *et al.,* (2014) 100% resistance rate was reported by Egbule *et al.,* (2016) followed by 68.8%, by Moini *et al.,* (2015) for the antibiotic Gentamycin. In this study 60% resistance rate was calculated which is lower than previous studies and higher than that of 42% by Zakiria *et al* (2015), 21.4 by Rigobelo *et al.,* (2006), 32.1% by Akingbade *et al.,* (2014), 8.33% by Sarshar *et al.,* (2014), 46.1% by Tawfick *et al.,* (2016), 9.3% by Manikandan and Asmath (2013) and 27.5% by Alikhani *et al.,* (2013).

The antibiotic resistance rate of Streptomycin in present work was 55% which is somewhat higher than that 40.7% reported by Akingbade *et al.,* (2014), 32.4% by Rigobelo *et al.,* (2006) and 9% in Kipkorir *et al*., (2016). For Ciprofloxacin 75% resistance rate was reported in this study which correlates with the 74% in Ali *et al.,* (2014). Followed by 67% resistance rate reported by Egbule *et al* (2016), 55.1% by Zakiria *et al.,* (2015) 38.8% by Moini *et al.,* (2015) 24% by Tawfick *et al.,* (2016), 14% by Manikandan and Asmath (2013) and 8.33% by Sarshar *et al* (2014).

The antibiotic resistance pattern of Tetracycline recorded which is 70% in the present Work. It is lower than that of Alikhani *et al.,* (2013) reported 75% resistance and higher than 64% in Ali *et al.,* (2014), 54.3% by Akingbade *et al.,* (2014), 50% by Zakiria *et al*., (2015), 45.7% by Rigobelo *et al.,* (2006), 41.67% by Sarshar *et al.,* (2014) 16% in Kipkorir *et al.,* (2016).

Isolation of lactobacillus

Total 180 samples (Milk and curd) were screened. Out of them 84 lactobacillus were isolated.

Sr.no	Sample	Acid tolerance				Bile Tolerance				Growth at temp		Antibiotic Resistance									
		2	3	4	5	0.5	1	1.5	2	15^o	45^oc	A	CP	CIP	NA	NF	NX	V	IMP	TE	C
1.	84	-	-	+	+	+	+	-	-	+	+	R	R	R	R	R	R	S	S	R	R

Table 1:- Acid, Bile, Heart and antibiotic tolerance of Lactobacillus isolates

+=growth present, - = growth absent, S=Sensitive, R=Resistant, A=Ampicilhn, ,Cp=Cephalcxin Cip=Ciprofloxacin, Na=Nalidixic acid , Nf=Nirofurantoin, Nx=Norfloxacin, V=Vancomycin Imp= Imipenum, Te=Tetracycline c= Cholerempheniсol

In probiotic attribution all the samples were grown at the pH 4 and 5 while at 0.5 and 1% bile salt conc. the growth was observed while at both the temperature range that is 15^oc and 45^oc lactobacillus were grown. In the Antibiotic resistance test, most of the isolates were sensitive to vancomycin and Imipenum while all the other antibiotics were resistance.

In the antimicrobial susceptibility testing of enteropathogens mostly the high MDR samples were selected for antimicrobial susceptibility towards Lactobacillus Spps screened from all the tests. In that the sensitive isolates shows 6 to 20 mm of zone of inhibition.

Sr.no	code	Heat						pH						AN	CT
		60^0	70^0	80^0	90^0	100^0	121^0	2	4	6	7	8	10		
1	CMb	+	+	+	+	+	+	+	+	+	+	-	-	+	+
2	Cuc	+	+	+	+	+	+	+	+	+	+	-	-	+	+
3	Cud	+	+	+	+	+	+	+	+	+	+	-	-	+	+

+=Active, -=not active, AN= Acid neutralization, CT=Catalase,

Table 2:- Effect of different parameters on activity of crude bacteriocin

In this screening processes the isolated Lactobacillus were cultivated at different acid concentration like pH 2 to 5, out of total 84 tested isolates from cow milk, buffalo milk and from curd samples, total 6 samples were positive in the pH range 2 while 12 samples were in the pH range 3. Similarly in the bile salt tolerance in the range of 0.5% to 2% all the samples tolerate the 0.5% bile concentration while in the higher concentration there was change in scenario. In bile salt tolerance, 21 samples were positive for 1.5% bile conc. while none of the sample tolerate 2% bile concentration.

Sr.no	Probiotic isolates	*E.coli*				
		E1	E2	E3	E4	E5
1	CMa	+	-	-	+	-
2	CMb	+	+	-	+	+
3	CMc	-	+	+	-	-
4	BMa	+	-	-	+	-
5	BMb	+	+	-	+	+
6	Cua	+	-	-	-	+
7	Cub	+	+	-	-	-
8	Cuc	+	+	+	+	+
9	Cud	+	+	+	-	-

Table3:- Antimicrobial susceptibility of enteropathogens towards Lactobacillus Spps.

After performing all the above tests from all the sources total 9 lactobacillus isolates were selected. In that 3 samples were screened from cow milk (CM), 2 samples from buffalo milk (BM) and from curd (Cu) 4 samples were selected for further antimicrobial testing against some selected high MDR enteric pathogens.

Mithun *et al.,* (2015) reported that the crude bacteriocin obtained from these isolates showed maximum activity against *E.coli* whereas relatively lower activity was seen against *B.subtilis*. Upon subsequent heat treatment, the activity of the crude bacteriocin preparations diminished gradually and was completely destroyed at 100°C. This indicates that the bacteriocins produced by these isolates are heat labile and may not sustain high temperatures for long time periods.

References :-

[1] Abdel-Nasser A, El-Moghazy, Tawfick MM and El-Habibi MM (2016).Prevalence, antimicrobial susceptibilities and molecular characterization of enteric bacterial pathogens isolated from patients with infectious diarrhoea in Cairo, Egypt *Int.J.Curr.Microbiol App.Sci* 5(4): 553-564.

[2] Akingbade OA, Damola AB, Shobayo BI, Nwanze JC and Okonko IO (2014). Multi-Drug Resistant (MDR) *Escherichia coli* among Children suffering from Diarrhea infections in Abeokuta, Nigeria Science pub Researcher. 6(8);11-17.

[3] Alikhani MY, Hashemi SH, Aslani MM and Safar F (2013) Prevalence and antibiotic resistance patterns of diarrheagenic *Escherichia coli* isolated from adolescents and adults in Hamedan, Western Iran. *Iran. J. Micr*. 5(1) 42-47

[4] Amira M, Zakaria MH. Abdel AS and Selim A (2015). Multi-drug resistant (mdr) *escherichia coli* originated from clinical and environmental sources in Ismailia-Egypt. *eup j aresbio life scis.* 3(1)8-20

[5] Clinical and Laboratory Standards Institute, (2014). Performance standards for antimicrobial susceptibility testing: Twenty fourth informational supplement: Approved standards M100-S24.Clinicaland Laboratory Standards Institute, Baltimore, USA

[6] Deshmukh PM and Ukesh CS (2014).Multi drug resistance pattern of *Escherichia coli* isolated from urinary tract infected (UTI's) patients. *Int. J. of Life Sciences*, 2(1): 53-57

[7] Egbule OS, Owhe-Ureghe UB and Odih EE. (2016). Occurrence of Multidrug Resistance among E. coli O157:H7 Isolated from Stool Samples Obtained from Hospitalized Children J Prob Health, 4:3, 1-4.

[8] Khandare SS and Patil SD (2016). Encapsulation of Lactic acid bacteria in calcium alginate beads for higher bacteriocin production Int. J. of Life Sciences Vol. 4 (4): 539-546

[9] Kipkorir KC, Bett PK, Onyango PO,Onyango DM, Ayieko C and Angienda PO (2016). Epidemiology of Antimicrobial Resistance among *Escherichia coli* Strains in Trans-Nzoia County, Kenya. Journal of Microbiology and Infectious Diseases, 6 (3);107-112.

[10] Kipkorir KC, Bett PK, Onyango PO,Onyango DM, Ayieko C and Angienda PO (2016). Epidemiology of Antimicrobial Resistance among *Escherichia coli* Strains in Trans-Nzoia County, Kenya. Journal of Microbiology and Infectious Diseases, 6 (3);107-112.

[11] Manikandan C and Amsath A(2013). Antimicrobial resistance of enteric pathogens isolated from children with acute diarrhoea in Pattukkottai, Tamil Nadu, India. *Int. J. Pure Appl. Zoo.*, 1(2): 139-145,

[12] Mithun S, Dipak V and Sheela S.(2015). Screening and characterization of novel bacteriocin producing lactobacilli obtained from raw milk samples. wjpr. 4(4).

[13] Moini AS, Soltani B, Ardakani AT, Moravveji A, Erami M, Rezaei MH, and Namazi M (2015) Multidrug-Resistant *Escherichia coli* and *Klebsiella pneumoniae* Isolated From Patients in Kashan, *Iran Jundishapur. J Microbiol*. 8(10):1-8

[14] Patil MM, Pal A, T. A. and K. V. R. (2010) Isolation and Characterization of Lactic Acid Bacteria from Curd and Cucumber. *Indian J. Biotechnol. 9*; 166–172.

[15] Rigobelo EC, Gamez HJ, Marin JM, Macedo C, Ambrosin JA,and Ávila FA (2006). Virulence factors of Escherichia coli isolated from diarrheic calves Arq. Bras. Med. Vet. Zootec., 58(3), 305-310.

[16] Ruiz J, Maria-luz EZ, Julia AZ, Lorente I, Jero REZ, And Joaqui MEZ(1996). Comparison of Five Plating Media for Isolation of *Salmonella* Species from Human Stools. *J. Of clin micro,*Vol. 34, No. 3,686–688.

[17] Sadeghabadi AF, Ajami A, Fadaei R, Zandieh M, Heidari E, Sadeghi M, Ataei B, Hoseini SG (2014).Widespread antibiotic resistance of diarrheagenic Escherichia coli and Shigella species.Res Med Sci;19:S51-S55

[18] Sharma RM and Packer (1969). Evaluation of culture media for the Isolation of Salmonellae from feces. *Appl micro*, Vol. 18, No. 4, 589-595.