EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES

http://www.ejbps.com

ISSN 2349-8870
Volume: 3
Issue: 2
371-375
Year: 2016

EVALUATION OF ANTIBIOTIC SUSCEPTIBILITY PATTERNS OF *ENTEROCOCCI* ISOLATED FROM UTI PATIENTS OF AKOLA DISTRICT.

Jyoti H. Anandani and Z. H. Khan*

Department of Biochemistry, Shri Shivaji College, Akola.

***Author for Correspondence: Dr. Z. H. Khan**
Department of Biochemistry, Shri Shivaji College, Akola.

Article Received on 19/12/2015 **Article Revised on 10/01/2016** **Article Accepted on 01/02/2016**

ABSTRACT

Enterococci, an indigenous flora of the intestinal and urinogenital tract of humans and animals are known to produce biologically active peptides termed enterocin. A total of 40 enterococcal isolates were obtained from urine specimens of UTI patients. They were further speciated and antibiogram was determined by disk diffusion method. All the obtained isolates were sensitive (100%) to penicillin. The maximum isolates had shown susceptibility to ampicillin followed by chloramphenicol, amoxicillin, gentamycin, streptomycin, vancomycin & tetracycline. Both *E. faecalis* and *E. hirae* isolates were resistant to norfloxacin (73.07% & 42.85%) respectively. Thus, the knowledge of an antibiotic susceptibility pattern is essential to formulate treatment guidelines for urinary tract infections.

KEYWORDS: Enterocin, *Enterococus*, Antibiotics, Disc diffusion technique.

INTRODUCTION

The occurrence of multi antibiotic resistant bacteria has become serious clinical obstacle worldwide. Therefore there is a need to much more research to develop new antimicrobial agents. Bacteriocins which are produced by bacteria are group of bactericidal compounds of peptide entity. The widespread occurrence of bacterial species with bacteriocin production in microbial ecosystems such as intestinal tract and epithelial surfaces has renewed interests in their bactericidal activities in recent years. Lactic acid bacteria (LAB) are perhaps the most bioprospected bacteriocin producers. Lactic acid producing bacteria from the order *Lactobacilliales* contains several genera including *Lactobaccilus, Enterococcus, Lactococcus, Streptoccus, Leuconostoc, Pediococcus* (Maryam Hassan *et al 2015*).

The genus *Enterococcus* is a hetrogenous group of bacteria which includes 20 different species. *Enterococci* are notorious as ubiquitous microbes inhabiting soil, food, water and gastrointestinal tract of humans and animals. Because of their abundance in different ecological niches, *Enterococci* are significant in environmental, food & clinical fields. The main reason for their successful survival & persistence in such microbiologically diverse environments is in their rough nature. *Enterococci* were formerly classified as “faecal” or Lancefield group D Streptococci which indicate that despite enterococcal dissemination the predominant habitat of *Enterococci* still remains the GIT of humans & animals. Good adaptation on rigorous intestinal condition gives *Enterococci* excellent opportunity for their beneficial activities. *Enterococci* are gram positive homofermentative lactic acid bacteria that can tolerate a wide variety of growth conditions like temperature of 10 to 45^{o}c, hypotonic, hypertonic, acidic or alkaline environments. Sodium azide and concentrated bile salts which inhibit or kill most micro organism are tolerated by *Enterococci*. As facultative organism, *Enterococci* grow under reduced or oxygenated conditions. Enterococcal bacteriocins i.e. enterocin have gained attention in recent years because bacteriocin producing strains can be isolated with ease from several fermented food and also from various clinical specimens like urine ,skin swab, pus and blood. Apart from humans and animals this bacteria can also be found in the soil and water while some strains of *E. faecalis* can cause opportunistic and hospital acquired infections in humans, many other strains have been used for a variety of beneficial purposes by both nature and man (Desai PJ *et al.,*. 2001).

It was shown that different species of *Enterococci* can produce a large variety of enterocins (bacteriocins) small secreted peptide which have interesting and proving ability to inhibit many kinds of food borne pathogens (Simonetta *et al.*, 1997). Enterocins have developed a great deal of interest as an approach to control food borne diseases, to be used as starter culture and bio preservative in various food products. In some cases, *Enterococci* are used as probiotics as a result of their protective effects in the gastro intestinal tract (Franz *et al.,* 1999).

The past decade has witnessed a significant increase in the prevalence of resistance to antimicrobial agents. The increase of microbial resistance to antibiotics has led to a continuing search for newer and more effective drugs. Nowadays there is a growing concern regarding the replacing of use of chemical preservation in food.

In such cases, enterocins (antimicrobial peptides) may act as new possibility for combating infectious diseases. These enterocins are particularly active against pathogenic bacteria; such as *Listeria, Clostridium* and *Staphylococcus.* They inhibit growth of pathogenic microorganism without affecting the host and have broad spectrum of antimicrobial activity. This is a broad field of investigation where peptides and proteins have been widely used in the pharmaceutical & food industries and in medical therapy due to their huge biological activity (Moreno Foulquie M.R. et.al 2002).Considering such need, a work has been done to study the antibiotic susceptibility pattern of clinical enterococcal isolates from UTI patients.

MATERIALS AND METHODS

Isolation and Identification of bacteriocinogenic *Enterococci*

A total of 82 urine samples were collected from different origins (Table 1) like government hospitals, private hospitals and pathology laboratories and were processed. All the samples were collected in sterile broth medium and transferred immediately to laboratory for further processing. The samples were inoculated onto De Man, Rogosa & Sharpe broth for enrichment purpose and incubated at 30^{o} c for 24 hrs The enriched cultures were further analysed for isolation of relevant organism. The isolation was performed by routine microbiological isolation procedure and inoculation was performed on selective and differential media viz Enterococcus Confirmatory Agar, De Man, Rogosa & Sharpe Agar, Bile Esculin Agar. All plates were incubated at 30^{o}c for 24-48hrs.

Detection of Antibiotic Susceptibility Pattern

All the enterocin producing *Enterococci* were tested for antibiotic susceptibility pattern to screen out susceptible isolates by the Standard Kirby –Bauer Disk Diffusion technique using Muller Hinton Agar. Enterococcal isolates were propogated individually on Brain Heart Infusion Agar plates to get isolated colony. The isolated colonies of pure culture were then inoculated in 3-4ml of nutrient broth & incubated at 37^{o}c for 6hrs.The turbidity of broth culture was adjusted equivalent to 0.5 Mc Farland Turbidity Standard. Sterile swabs were used to swab the culture on Muller Hinton agar plates to get lawn growth. After inoculums has dried specific antibiotics discs were placed 2cm apart from each other with sterile forcep and was incubated for 18-24hrs at 37^{o}c .The zone of inhibition (zone size) was measured in mm as recommended by NCCLS. The antibiotics discs used were: Ampicillin (10mcg), Penicillin G (10mcg), Amoxicillin (10mcg), Ceftazidime (10mcg), Vancomycin (30mcg), Gentamicin (10mcg), Streptomycin (10mcg), Chloramphenicol (25mcg), Tetracycline (30mcg), Norfloxacin (30mcg).

RESULTS AND DISCUSSION

Recent years have witnessed increased interest in *Enterococcci* not only because of their presence in urinary tract infections but also because of the increasing resistance to many antimicrobial agents. The present study investigates that 40 isolates of *Enterococci* were isolated from urine specimens from different sources from which 59% contributing from pathology lab while 26% & 13% from government and private hospitals respectively.

Prevalence of *Enterococci* in clinical specimens can thus be attributed to their ability to grow and survive due to selective pressure of antimicrobial agents. *Enterococci* isolated from specimens in this study do not reflect true incidence of infection caused by these organisms, but definitely suggest the increased frequency of their isolation from various clinical materials(PJ Desai *et al.,*) Phenotypic and biochemical identification of isolates was carried out according to the characteristic shown in Table 2. All isolates were Cocci, Gram positive, Catalase negative and were found to be negative for hemolysin. Enteroccocus isolates were identified on the basis of their growth on Enteroccocus confirmatory agar showing typical yellow colonies and by forming Black colonies on Bile Esculin agar containing 40% bile. Sugar fermentation pattern are considered to be reliable method of distiguinshing *Enteroccocus* species (Mundt, 1986, Klein, 2003). From the sugar fermentation profiles and arginine catabolism the isolates were identified *Enteroccocus faecalis* raffinose (-), arabinose (-), mannitol (+), sorbitol (+) and arginine (+). *Enteroccocus hirae* raffinose (+), arabinose (-), mannitol (+), sorbitol (-) and arginine (+). Table 3 display the distribution and species identification of Enteroccocal isolates. The majority of isolates were *E. faecalis* 60% while *E. hirae* accounted for 40%.The resistance pattern or susceptibility of the organism isolated from urine specimen of UTI varies from region and hospital settings.

It is important to check the absence of resistance against different antibiotics for further applications of these producer isolates and their enterocin in food or health research (application). In present study all selected 40 efficient and enterocinogenic *E.faecalis* and *E. hirae* isolates were focused to screened for drug resistance by disc diffusion method. Ten different commercially available antibiotics discs were used and antibiogram were made by following NCCL criteria (NCCLS, 1997). Drug study in present investigation represents different antibiotics groups exerting antagonism by varied mode of action Ampicillin and Penicillin (penicillin group), Ceftazidime (cephalosporin group), Vancomycin (glycopeptides group) and antibiotics responsible for inhibition of cell wall synthesis. Gentamycin, Streptomycin (aminoglycoside group), Tetracycline (Tetracycline group) and Chloramphinicaol are responsible for protein synthesis whereas Norfloxacin (Quinolone group) are responsible for inhibition of nucleic acid synthesis.

The results of antibiotic susceptibility pattern of the Enterococcal isolates tested by disc diffusion method are shown in table 4 (Graph1) and Plate 1 and 2. Antibiotic susceptibility pattern showed that, all 40 enterococcal isolates tested were susceptible to ampicillin while rest of pattern was different for both the isolates. Amoxicilin, chloramphenicol and streptomycin were significantly effective on *E. faecalis* isolates showing 84.61%, 88.46% and 84.61% respectively. Gentamycin, Ceftazidime shown 76.92% & 61.53% while vancomycin & tetracycline occupied middle position showing 53.84% & 57.69%.

The present study also reveals *E. faecalis* to be more drug resistant than *E. hirae*. All the 14 isolates of *E. hirae* were sensitive to ampicillin and penicillin G while Amoxcillin, ceftazidime and streptomycin 92.85%, 85.71% and 78.5% respectively. Norfloxacin showed 57.14 % sensitivity to *E. hirae* as compared to *E. faecalis* which was only 26.92%.

One of the most important characteristic of *Enterococci* is the evaluation of susceptibility for different antibiotics. In present study all the isolates of enterococci were susceptible to commonly used antibiotics namely ampicillin, amoxycilin, tetracycline, gentamycin along with vancomycin and streptomycin. These isolates were resistant to norfloxacin.

Abriouel *et al.*, 2006 had almost same findings for their isolated bacteriocin producing *E.faecium* strainRJ16 which was resistant to ciprofloxacin & levofloxacin & showed intermediate resistance to nitrofurantoin & erythromycin, but was sensitive to ampicillin, penicillin, streptomycin, gentamycin, refampicin, chloramphenicol, tetracycline, vancomycin & teicoplanin. Similarly Drahovska *et al.*, 2004 concluded from their study that *E.faecium*, *E. faecalis* & *E.durans* were found to be predominant species with different sensitivity to antimicrobials recovered from naturally ripened European Cheeses & dairy products.

Enterococci are considered intrinsically resistant to β lactam antibiotics (Kak and Chow, 2002). The results obtained in the present study are not in agreement with this generalization since isolates were sensitive to Penicillin. Other authors obtained similar results (Ben Omar *et al.*, 2004; Valenzuela *et al.*, 2008). Thus, careful identification followed by thorough testing based upon certain criteria must be accomplished before putting any enterocinogenic enterococci to practical use in the food industry. The study also attempts to suggest the use of enterocin as efficient and safe applicable in food and medicine as novel therapeutic agent.

Table 1: Source of Sample collection

Sr.No.	Sample Source	Sample Size
1	Government Hospitals	21
2	Private Hospitals	13
3	Pathology Laboratories	48
	Total	82

Table 2: Phenotypic and Biochemical Characterization of Enterococcus isolates.

Characteristics	Isolates	
	E. faecalis	*E. hirae*
Cell Morphology	Cocci	Cocci
Gram reaction	Gram positive	Gram positive
Colony Characters		
Size	0.2mm	0.1-0.2mm
Colony shape	Circular	Circular
Elevation	Convex	Convex
Opacity	Opaque	Opaque
Colour on Enterococcus Confirmatory Agar	Yellow colony	Yellow colony
Bile Esculin Agar	Black colony	Black colony
Catalse	Negative	Negative
Haemolysin production	Negative	Negative
CO_2 production from glucose	Positive	Positive
Hydolysis of Esculin	Positive	Positive
Growth in presence of NaCl		
4%	Positive	Positive
6.5%	Positive	Positive
Utilization of Carbohydrate		
L-arabinose	Negative	Negative
Ribose	Positive	Positive

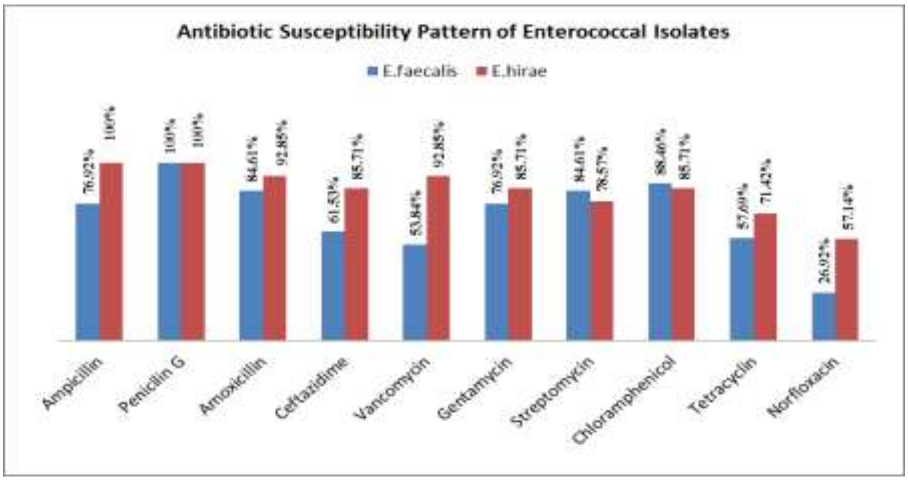
Sucrose	Positive	Positive
Mannitol	Positive	Positive
D-raffinose	Negative	Positive
Lactose	Positive	Positive
Sorbitol	Positive	Negative

Table 3: Frequency of enterocin production screened by Agar Well Diffusion Method

Identified strains	No. of producer strains/ No. of tested strains	Frequency Percentage (%)
E. faecalis	18/26	69.23
E. hirae	8/14	57.14
Total	26/40	65

Table 4: Antibiotic Susceptibility pattern of Enterococcal isolates.

Sr. No	Name of Antibiotics	E faecalis(n=26)		E hirae(n-14)	
		Resistant	Sensitive	Resistant	Sensitive
1	Ampicillin	6(23.07%)	20(76.92%)	0(0%)	14(100%)
2	Penicillin G	0(0%)	26(100%)	0(0%)	14(100%)
3	Amoxicillin	4(15.39%)	22(84.61%)	1(7.14%)	13(92.85%)
4	Ceftazidime	10(38.46%)	16(61.53%)	2(14.28%)	12(85.71%)
5	Vancomycin	2(46.15%)	14(53.84%)	1(7.14%)	13(92.85%)
6	Gentamycin	6(23.07%)	20(76.92%)	2(14.28%)	12(85.71%)
7	Straptamycin	4(15.39%)	22(84.61%)	3(21.42%)	11(78.57%)
8	Chloromphenicol	3(11.53%)	23(88.46%)	2(14.28%)	12(85.71%)
9	Tetracycline	11(42.30%)	15(57.69%)	4(28.57%)	10(71.42%)
10	Norfloxacin	19(73.07%)	07(26.92%)	6(42.85%)	8(57.14%)



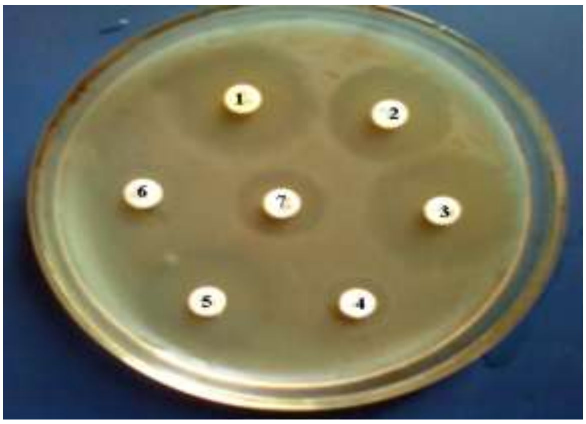


Plate 1 : Antibiotics Susceptibility Pattern
1 = Penicillin G, 2, Amoxicillin,
3 = Ampicillin, 4 = Tetracyclin,

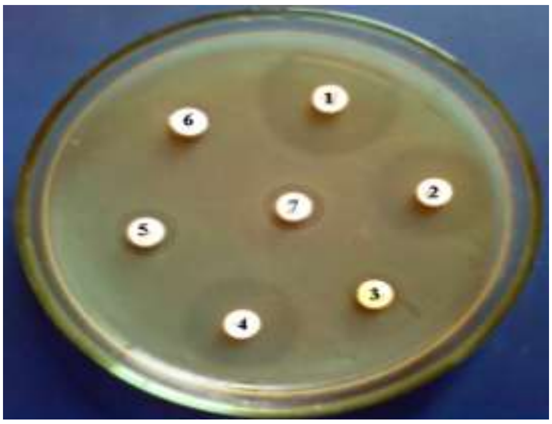


Plate 2 : Antibiotics Susceptibility Pattern
1 = Penicillin G, 2, Streptomycin,
3 = Tetracycline, 4 = Ceftazidime

5 = Vancomycin, 6 = Gentamycin,
7 = Chloramphenicol

5 = Norfloxacin, 6 = Gentamycin,
7 = Norfloxacin

CONCLUSION

Antibiotic susceptibility pattern revealed that both E.hirae and E.faecalis were susceptible to maximum antibiotics used in our study and were resistant to norfloxacin only.Thus, stating that the knowledge of antibiotic susceptibility pattern is essential to formulate treatment guidelines for UTI.

REFERENCES

1. Abriouel, H., Ben Omar, N., Lucas, R., Martinez-Canamero, M. and Galvez, A. Bacteriocin production, plasmid content and plasmid location of enterocin P structural gene in enterococci isolated from food sources. Lett Appl Microbiol., 2006; 42: 331–337.
2. Bauer A. W. Kirby, W.M. and Sheories J. C.Antibiotic susceptibility testing by standardized single disc method. An.J. Clin. Pathol, 1996; 48: 493-497.
3. Ben Omar N, Castro, A., Lucas R., Abriouel H, Yousif n. M, Franz, C. M, Holzapfel, W. H. Perez Pulido R.,Martinez-Canamero, M & Galvez A. Functional and safety aspects of Enterococci isolated from different Spanish foods. Syst Appl Microbiol., 2004; 27(1): 118-30.
4. Canzek Majhenic A.Enterococci: yin yang microbes. 2006; 56(1): 5-20.
5. Desai P.J., D.Pandit, M Mathur, A Gogate Prevalence, identification and distribution of various species of Enterococci isolated from clinical specimens with special reference of urinary tract infection in catheterized patients. Indian J. of Med. Microbiology 2001; 19(3): 132-137.
6. Drahovska H, Kocinova D, Seman M, Koncekova R, Trupl J, Turna J .Antibiotic resistance and virulence factors among clinical and food enterococci isolated in Slovakia. Folia Microbiologica, 2004; 49: 763–768.
7. Franz CAMP, Holzapfel WH, Stiles ME. Enterococci at the cross roads of food
8. safety ? Inter J Food Microbiol 1999; 47: 1-24.
9. Kak V, Chow JW. Acquired antibiotic resistances in enterococci. In: Gilmore,M.S. (ed.) The Enterococci: Pathogenesis, Molecular Biology and Antibiotic Resistance. American Societyfor Microbiology, W ashington, 2002; 355–383.
10. Klein G.Taxonomy ,ecology and antibiotic resistance of enterococci from food and the gastrointestinal tract.Int.J.Food Microbiol 2003; 88(2): 123-131
11. Maryam Hassan, Dag Anders Brede, Dzung B. Diep, Ingolf F. Nes, Farzanesh Lotfipour, Zoya Hojabri Efficient Inactivation of Multi-antibiotics Resistant nosocomial Enterococci by purified hiracin bacteriocin. Adv. Pharm Bull, 2015; 5(3): 3939-401.
12. Mundt J.O. Enterococci In:Sneath PHA,Mair NS,Sharpe ME,Holt JG (Eds),Bregy's | Manual of systematic Bacteriology.Williams and Wilkins,Baltimore 1986; 1063-1065.
13. National Committee for Clinical laboratory Standards. Performance standards for antimicrobial susceptibility testing. NCCLS.Wayne PA. Ninth informational supplement MS, 1997; 100-S12.
14. Simonetta A.C., Moragues De Velasco, L.G. and Frison L.N. Antibacterial activity of enterococce strains against vibrio cholera. Lett Appl. Microbiol. 1997; 24: 139-143
15. Valenzuela, A.S, Ben-Omar, N., Abriouel, H., Lopez, R.L., Ortega, E., Canamero, M.M., Galvez, A., Risk factors in enterococci isolated from foods in Morocco: determination of antimicrobial resistance and incidence of virulence traits. Food Chem. Toxicol. 2008; 46: 2648–2652.